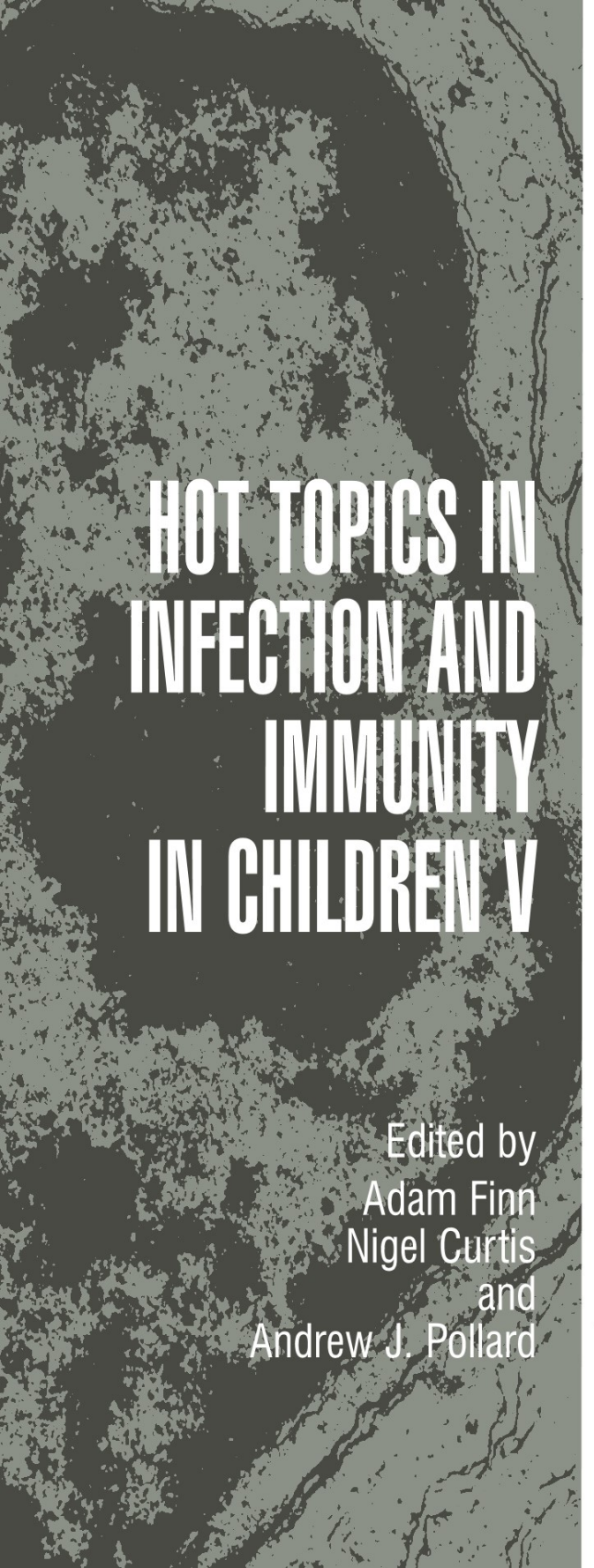

ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY

Volume 634

HOT TOPICS IN INFECTION AND IMMUNITY IN CHILDREN V

Edited by
Adam Finn
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Hot Topics in Infection and Immunity in Children V

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Preface

Each of the chapters in this book is based on a lecture given at the fifth 'Infection and Immunity in Children' course held in June 2008 at St. Catherine's College, Oxford, UK. Thus, it is the fifth book in a series which collectively provide succinct and readable updates on just about every aspect of the discipline of paediatric infectious diseases.

The sixth course was scheduled for 23–25 June 2008 with another exciting programme and renowned speakers and we expect to produce a sixth edition of this book based on that course.

Paediatric infectious diseases continue to grow and flourish in Europe with centres now being accredited for training by the European Society for Paediatric Infectious Diseases on behalf of the European Academy of Paediatrics. Plans are also taking shape for a University of Oxford Diploma Course in Paediatric Infectious Diseases, towards which participation in the Oxford IIC course, as well as other ESPID-sponsored educational activities, will give credits.

We hope this book will provide a further useful contribution to the materials available to trainees and practitioners in this important and rapidly developing field.

UK, Australia

Adam Finn
Nigel Curtis
Andrew J. Pollard

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We are indebted to all those who have contributed to the writing of manuscripts for this book. We are grateful to the staff of St. Catherine's College, Oxford, UK where the 2007 'Infection and Immunity in Children' course was held; many of the lectures of which form the basis for the chapters herein. Sue Sheaf has administered and run the course for several years now with enormous efficiency and effectiveness and we extend our sincere thanks to her on behalf of all the organizers, speakers, and delegates who have benefited from her labours. Lorraine Cantle patiently coaxed the authors and editors into action, carefully corrected and formatted the chapters and liaised with the publishers to ensure the book's efficient production and needs an extra special thanks for all her work.

We thank the European Society for Paediatric Infectious Diseases for consistent support and financial assistance for this and previous courses and for providing bursaries which have paid the costs of many young ESPID members' attendance. We also acknowledge the recognition given to the course by the Royal College of Paediatrics and Child Health.

Finally, we are grateful to several pharmaceutical industry sponsors who generously offered unrestricted educational grants towards the budget for the meeting.

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Shiga-Toxin Producing *Escherichia coli* and the Hemolytic Uremic Syndrome: What Have We Learned in the Past 25 Years?

Christina K. Ahn, Nicholas J. Holt, and Phillip I. Tarr

1 Introduction

Escherichia coli that belong to the serotype O157:H7 and produce Shiga toxins are important and challenging human pathogens. This organism can cause quite severe human enteric illnesses, including diarrhea and bloody diarrhea. Most notably, *E. coli* O157:H7 is the predominant cause of the hemolytic uremic syndrome (HUS) worldwide. HUS consists of nonimmune hemolytic anemia, thrombocytopenia, and acute renal failure, and disproportionately affects children. Both *E. coli* O157:H7 infections and HUS are relatively rare. According to 2007 estimates from the Centers for Disease Control (CDC) (McNabb, Jajosky et al. 2007), there are only *c*. 2600 cases of culture-proven *E. coli* O157:H7 infection annually in the entire United States. Based on these data, we estimate that there are about 400 cases of HUS per year, and half or more of the cases of HUS are in children less than 10 years of age. HUS is a thrombotic illness (Upadhyaya, Barwick et al. 1980; Inward, Howie et al. 1997; Tsai, Chandler et al. 2001), and it is quite likely that ischemic renal injury secondary to these thrombi leads to acute renal failure (Bellomo, Kellum et al. 2007). It is also probable that the thrombotic injury begins early in the illness, well before azotemia ensues. This is a challenge because interventions that target the infecting bacteria are probably futile, but if patients are identified in a timely manner, there is an opportunity to maintain renal perfusion as thrombi evolve. Because of the rarity of *E. coli* O157:H7 infections, their serious consequences and their epidemiological importance, it is critical to have good community-based microbiology diagnosis close to point of presentation, and to utilize syndrome profiling to identify infected patients accurately and expeditiously.

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2 History

HUS was first described in 1955 by von Gasser et al. who reported five children, all of whom died, with small-vessel renal thrombi, thrombocytopenia, and Coombs-negative hemolytic anemia (Gasser C 1955). In 1975, Kaplan et al. described simultaneous cases of HUS within families and suggested that their cause was environmental and probably infectious (Kaplan, Chesney et al. 1975). In 1983, Karmali et al. linked childhood HUS with a cytotoxin produced by *E. coli* in stool (Karmali, Steele et al. 1983). Some of these toxin-producing *E. coli* were of serotype O157:H7. One week later, a publication by Riley et al. described two outbreaks in adults with painful bloody diarrhea, which used the term hemorrhagic colitis (Riley, Remis et al. 1983). In both outbreaks, undercooked hamburgers were eaten, and many of the patients had *E. coli* O157:H7 in their stools. That same year, O'Brien and colleagues established that *E. coli* O157:H7 produced a toxin similar to that produced by *Shigella dysenteriae* serotype 1 (O'Brien, Lively et al. 1983). These studies underlie our current understanding of the pathophysiology of *E. coli* O157:H7 infections. They also form the basis for our understanding of HUS as a toxemic systemic consequence of a nonbacteremic enteric infection.

3 Epidemiology of *E. coli* O157:H7 Infections and HUS

Time of year: The most common times of year for *E. coli* O157:H7 infections and HUS to occur are the summer and autumn. It is not clear if this pattern is related to seasonal environmental or food exposures, or to seasonally varying levels of contamination from these or other vehicles of transmission.

Location E. coli: O157:H7 infections and HUS seem to be more prevalent in the Northern Hemisphere and in latitudes farther from the equator (Tarr and Hickman, 1987; Jernigan and Waldo, 1994; Slutsker, Ries et al. 1997; Cummings, Mohle-Boetani et al. 2002). However, there are notable exceptions. Buenos Aires, Argentina, although below the equator, has a high incidence of HUS cases (Lopez, Diaz et al. 1989). In countries that lack the laboratory resources to diagnose the infection, rates of *E. coli* O157:H7 infections are probably underestimated.

Rural versus urban: Rural locations have a higher incidence of diagnosed *E. coli* O157:H7 infections and HUS than do urban populations. A possible explanation for this difference is that environmental rather than food-borne exposures are responsible, which is also inferred in a recent study from rural Scotland (O'Brien, Adak et al. 2001).

Outbreaks of E. coli: Contrary to popular belief, *E. coli* O157:H7 infections are largely sporadic, or occur in small clusters, such as within households. Large, well-publicized outbreaks are the exception and not the rule. Absence

of associated cases should not discount the possibility that a patient is infected with *E. coli* O157:H7.

Vehicle of transmission: While people can certainly be infected through poorly cooked, ground beef, there are many other modes of infection that are often overlooked. In fact, in aggregate, nonbeef sources for this pathogen might be more prominent. Such nonhamburger vehicles include municipal (Swerdlow, Woodruff et al. 1992) and swimming water (Keene, McAnulty et al. 1994), deer jerky (Keene, Sazie et al. 1997), unpasteurized milk (Keene, Hedberg et al. 1997), spinach (Uhlich, Sinclair et al. 2007), salami (Tilden, Young et al. 1996), lettuce (Ackers, Mahon et al. 1998), bovine contact (Crump, Sulka et al. 2002), radish sprouts (Michino, Araki et al. 1999), unpasteurized apple cider (Besser, Lett et al. 1993), and salmon roe (Terajima, Izumiya et al. 2002). Also, the infection can be spread by person-to-person contact and via air-borne routes (Varma, Greene et al. 2003).

4 Clinical Course of *E. coli* Infection

Figure 1 illustrates the typical course of an *E. coli* O157:H7 infection. According to data accumulated from a large epidemic in Seattle, the median time between ingestion of the bacteria and onset of diarrhea was 3 days (Bell, Goldoft et al. 1994). The first day of illness is most appropriately defined as the first day that a patient has diarrhea. Other prodromal symptoms can precede or accompany the diarrhea (e.g., fatigue, headache, abdominal pain, vomiting, muscle pain, fever, etc.), but they are nonspecific, and variably experienced and reported. Furthermore, the date of onset of these symptoms is difficult to assign. For these reasons, we identify the first day of diarrhea as the first day of illness. Diarrhea caused by *E. coli* O157:H7 usually lasts for about 1 week (Ostroff, Kobayashi et al. 1989). Bloody diarrhea is noted in about 85% of bacteriologically confirmed cases, and occurs about 2–5 days into illness (Ostroff,

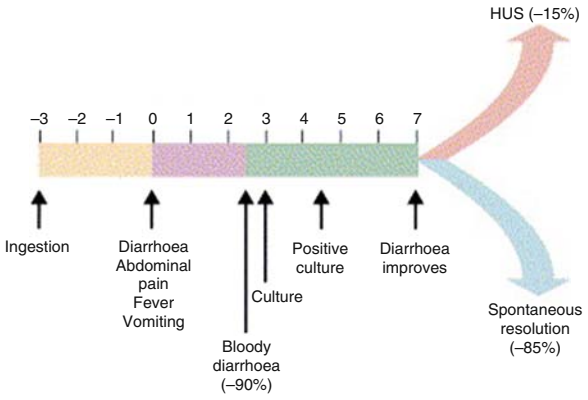

Fig. 1 Progression of *E. coli* O157:H7 infections in children (Tarr et al. 2005)

Kobayashi et al. 1989). There is a spectrum of severity, but most culture-confirmed cases are quite ill.

Some clues help distinguish children and adults infected with *E. coli* O157:H7 from those infected with other pathogens that cause bloody diarrhea. First, patients infected with *E. coli* O157:H7 generally have no fever at their initial presentation (Wong, Jelacic et al. 2000), though it is important to note that about half of all patients report having a fever earlier in the illness. Second, only half of fecal samples contain leukocytes and when leukocytes are present they are rarely abundant (Slutsker, Ries et al. 1997; Klein, Stapp et al. 2002). Furthermore, the abdominal pain experienced is usually greater than that associated with gastroenteritis, and more painful during defecation. Physicians often document tenderness on palpation of the abdomen of infected patients (Slutsker, Ries et al. 1997; Klein, Stapp et al. 2002).

5 Pathophysiology of *E. coli* O157:H7 Infections

The central virulence factor for Shiga-toxin producing *E.coli* (STEC) is its ability to produce Shiga toxin or toxins. Shiga toxin (Stx) is the main extracellular cytotoxin produced by *Shigella dysenteriae* serotype 1, but only rarely by other shigellae. Stx is classified as an A_1B_5 toxin. The A subunit, an N-glycosidase, halts protein synthesis by disrupting the large eukaryotic ribosomal subunit (Endo, Tsurugi et al. 1988), while the B subunit attaches to a glycosphingolipid on eukaryotic cell surfaces. Because of these properties, Shiga toxins induce renal cell death among other adverse effects (Karpman, Hakansson et al. 1998; Taguchi, Uchida et al. 1998). The type of toxin that is carried by *E. coli* O157:H7 might determine the virulence; most *E. coli* O157:H7 carry the *Shiga toxin 2* gene (Stx2) (Slutsker, Ries et al. 1997; Klein, Stapp et al. 2002). For reasons that are unclear, *E. coli* O157:H7 that generate both Stx1 and Stx2 are paradoxically less virulent than those that produce only Stx2.

6 Diagnostic Considerations for Pathogenic STEC

The clinical importance of making a rapid and accurate diagnosis of *E. coli* O157:H7 cannot be understated. Children who are diagnosed early in illness are at lower risk of severe consequences than those diagnosed later (Ake, Jelacic et al. 2005). Also, establishing a microbiological diagnosis can provide clinical clarity in cases that can be confusing, and avert unnecessary or risky additional diagnostic efforts.

However, the optimal diagnostic methodologies for the detection of *E. coli* O157:H7 and of other STEC are not yet delineated (Bielaszewska, Kock et al. 2007). Clearly the best way to recover *E. coli* O157:H7, in the context of current technology, is to plate all submitted stools on sorbitol–MacConkey (SMAC)

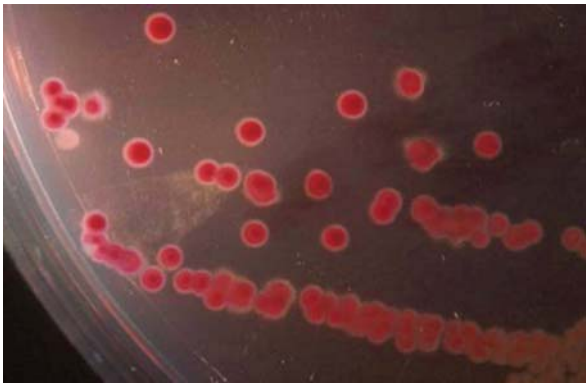

Fig. 2 *E. coli* O157:H7 on a sorbitol–MacConkey agar plate. *Arrow* indicates a distinctive colorless *E. coli* O157:H7 colony (Tarr et al. 2005)

agar (March and Ratnam 1986), which uses sorbitol as a carbon source, and not lactose. Unlike most human fecal *E. coli*, O157:H7 strains cannot ferment sorbitol rapidly and appear as colorless colonies on SMAC agar (Fig. 2).

Another test, which is available commercially, is the Stx detection assay, which is performed on a broth culture of the stool. The advantage of this technique is that a positive signal will be obtained from non-O157:H7 STEC as well as *E. coli* O157:H7. However, Stx assays do not tell the clinician if the strain producing the toxin is an *E. coli* O157:H7 or a non-O157:H7 STEC. This latter group of organisms is considerably less likely to cause HUS or to be associated with outbreaks, and the value of their detection to clinicians and disease control authorities is nowhere near as great as is the value of detecting *E. coli* O157:H7. Furthermore, for unexplained reasons, Stx assays are not always as sensitive as SMAC agar screening for the detection of *E. coli* O157:H7 (Fey, Wickert et al. 2000; Klein, Stapp et al. 2002; Carroll, Adamson et al. 2003; Park, Kim et al. 2003; Teel, Daly et al. 2007). More specifically, SMAC agar can sometimes detect *E. coli* O157:H7 when simultaneously performed toxin assays do not. Toxin assays should not be performed directly on stool as this approach lacks sensitivity (Cornick, Jelacic et al. 2002).

We believe that current best microbiological practice throughout the world is to plate all submitted stools onto SMAC agar, whether or not the medical provider requests detection of *E. coli* O157:H7. The stool should also be inoculated into a nutrient broth, and the broth should be tested the next day for Stx presence. Regrettably, resources might not permit such simultaneous culture and nonculture diagnosis. In that case, because of *E. coli* O157:H7's enduring epidemiologic predominance, its strong linkage with HUS, and its repeatedly demonstrated ability to cause outbreaks, we believe that plating of the stools on SMAC agar should take precedence over toxin assays because culture is accurate and recovers *E. coli* O157:H7 expeditiously.

We do not believe that observation of blood in the stool by microbiology staff should be used as a criterion to seek *E. coli* O157:H7, because about 15% of patients infected with *E. coli* O157:H7 will have nonbloody diarrhea (Wong,

Jelacic et al. 2000; Klein, Stapp et al. 2002), and laboratory staff often fail to perceive the red color in submitted specimens (Klein, Stapp et al. 2002). We also believe that all stools should be plated on receipt in the laboratory, 24 h a day, 7 days a week. This practice reduces the time needed to identify positive specimens that contain this pathogen and the more rapid the diagnosis, the stronger the association with a good case outcome (Ake, Jelacic et al. 2005). Finally, we believe that microbiologists should notify the requesting physician as soon as a presumptive *E. coli* O157:H7 is identified, even before confirming the organism as an *E. coli*, or determining the presence or absence of the H7 antigen. Once a sorbitol nonfermenting colony is found that reacts with a serologic reagent that detects the O157 lipopolysaccharide antigen, the provider should be alerted that a presumptive *E. coli* O157:H7 has been identified. Strains should be forwarded to the appropriate public health authorities for typing as expeditiously as possible.

7 Management of Patients with Confirmed or Suspected *E. coli* O157:H7 Infection

If a patient is suspected of having an *E. coli* O157:H7 infection, we recommend admission to hospital to mitigate infection risk in the household, to expand circulating volume, and to control pain.

Infection control: Patients infected with *E. coli* O157:H7 are highly contagious in the early diarrheal phase of the illness, and they should be placed on contact precautions and removed from the community where they can continue to spread the infection. In many hospitals, the placement of these patients on contact precautions is inconsistent. The current recommendations are to continue contact precautions until the diarrhea resolves and two consecutive stool cultures are negative for *E. coli* O157:H7 (Pediatrics 2006). Some authorities have recommended admission as a form of community infection control (Seto, Soller et al. 2007; Werber, Mason et al. 2008), and we believe that this measure is justified for both outbreak and sporadic cases (Ahn, Klein et al. 2008). Indeed, Werber et al. calculated that the number needed to isolate (NNI) in order to prevent one HUS case was 95 (95% CI 38–200), while the number needed to treat to prevent one secondary household case of meningococcal disease was appreciably more at 200 (Werber, Mason et al. 2008).

Volume expansion: Recent data suggest that circulating volume expansion early in illness with isotonic crystalloid, and not hypotonic fluids, is associated with less severe courses of HUS (Ake, Jelacic et al. 2005). The risk of developing HUS in children under age 10 years who are infected with *E. coli* O157:H7 is appreciable: over 20% of children presenting on or before day 4 of illness will develop this complication (Wong, Jelacic et al. 2000), and we believe that all such patients should be considered to be at risk. As mentioned above, we

consider the first day of diarrhea to be the first day of illness, and base decisions on that time point.

We usually administer a bolus of at least 20 mL of normal saline/kg of body weight at the first opportunity, and then maintain intravenous fluid infusion at maintenance volume. We recommend infusion of repeated boluses of normal saline if there is any suggestion of oliguria or pain (see below). Such patients should be assiduously monitored, and before administering boluses or continuing fluids, they should be evaluated for hypertension and 'central' volume overload. Peripheral edema, without signs of central overload or hypertension, should not deter fluid infusion.

The typical endpoint of intravenous fluid infusion is either a rising platelet count, which is usually not difficult to identify, or a patient whose platelet count is stable and whose symptoms have abated. This resolution rarely occurs before the fourth day of illness. Potassium can be added to the fluids if the serum potassium concentration is normal or low. Hyperkalemia is surprisingly rare as HUS ensues despite the renal insufficiency and hemolysis. Daily laboratory tests should consist of a complete blood count (CBC), electrolytes, blood urea nitrogen (BUN), and creatinine. Urinalysis should not be obtained; the daily creatinine concentration is sufficient to assess renal function. Furthermore, the urinalysis can be misleading, especially if collected from a patient with diarrhea, where contamination is likely. Urinary catheterization should be also avoided, principally to avoid infection risk in the setting of diarrhea.

We recognize that the patient who is experiencing a rising creatinine, but who is still passing urine, poses a management challenge. On the one hand, if anuria is inevitable, then fluid restriction is probably prudent. On the other hand, anuria cannot be predicted, and any fluid restriction is likely to diminish renal blood flow, increase renal ischemia (Bellomo, Kellum et al. 2007) and is ill advised. We prefer to continue the fluids, monitor the patient extremely closely, and restrict fluids for persistent hypertension or clinical signs of cardiopulmonary overload.

Pain control: Patients infected with *E. coli* O157:H7 are often in quite severe pain. Narcotics and antimotility agents are associated with increased risks of HUS (Bell, Griffin et al. 1997). We encourage liberal use of fluid boluses in attempts to address the pain because it is plausible that intestinal ischemia from the underlying thrombotic process exacerbates the pain. A benzodiazepine such as lorazepam can be used to reduce anxiety and facilitate sleep. We discourage use of nonsteroidal anti-inflammatory drugs because they can diminish renal blood flow. Acetaminophen (paracetamol) is probably not harmful, but from our observations, it is not a very effective analgesic in this infection.

Antibiotics: Patients with suspected or confirmed *E. coli* O157:H7 should not be given antibiotics because the evidence does not suggest any benefit for them and, in fact, may suggest that antibiotics increase the risk of HUS. In an analysis of the large outbreak of *E. coli* O157:H7 in Washington State in 1993, antibiotics were given early in the illness but failed to decrease the risk of HUS (Bell, Griffin et al. 1997). In another study, administration of antibiotics probably

increased risk of HUS in children infected with O157:H7 (Wong, Jelacic et al. 2000). A trend toward worse outcome has also been seen in adults infected with *E. coli* O157:H7 (Dundas, Todd et al. 2001). A possible mechanism for this observation is that antibiotics might lead to bacterial lysis, which increases the availability of Stx for systemic absorption (Grif, Dierich et al. 1998). Also, bacteriophages that contain the *stx* genes might be stimulated by the antibiotics, leading to increased Stx generation (Kimmitt, Harwood et al. 1999). Even though a flawed meta-analysis suggested that antibiotics were not associated with a higher risk of HUS (Safdar, Said et al. 2002), we are unaware of any reports that demonstrate that the rate of HUS among patients treated with antibiotics is lower than those not treated.

Platelet transfusion: Platelet transfusions should not be given to children infected with *E. coli* O157:H7 because platelets might exacerbate the thrombotic process that underlies HUS. Clinically significant hemorrhage as a consequence of HUS is very rare. However, if an invasive procedure that has a risk of bleeding is considered, platelets might be needed to prevent hemorrhage.

8 HUS and the Course of *E. coli* O157:H7 Infections

HUS is defined by nonimmune hemolytic anemia (packed cell volume $< 30\%$ and evidence of erythrocyte destruction on peripheral blood smear), thrombocytopenia (platelets $< 150 \times 10^9$/L), and azotemia (serum creatinine above the upper limit for age). HUS occurs in about 15% of culture-proven childhood *E. coli* O157:H7 infections in children less than 10 years of age (Ostroff, Kobayashi et al. 1989; Bell, Griffin et al. 1997; Rowe, Orrbine et al. 1998; Wong, Jelacic et al. 2000; Chandler, Jelacic et al. 2002). HUS almost always develops between the 5th and 13th day of illness, and the median time of its onset (defined as when children meet the above case definition) is 7–8 days (Wong, Jelacic et al. 2000; Chandler, Jelacic et al. 2002). About 60% of patients with HUS become anuric. HUS patients who continue to pass urine through day 10 of illness rarely become anuric.

E. coli O157:H7 infections follow a remarkably stereotypical course in most patients. The relation of the laboratory tests to the day of illness is most helpful in managing such infections. Figure 3 portrays the four patterns of thrombocytopenia, anemia, and azotemia that are observed in infected children.

Pattern 1 is seen in *c*. 70% of cases. Pattern 2 (hemolysis requiring transfusion) is seen in about 5% of cases; this patient required a transfusion of erythrocytes. There is no azotemia, so, strictly speaking, this is not HUS. Pattern 3 is nonanuric HUS, which did not require dialysis, and occurs in about 5–10% of cases. Pattern 4 is anuric HUS, requiring dialysis. Anuric HUS has a worse long-term prognosis than nonanuric HUS (Siegler, Milligan et al. 1991; Garg, Suri et al. 2003), and occurs in about 10–15% of infected cases.

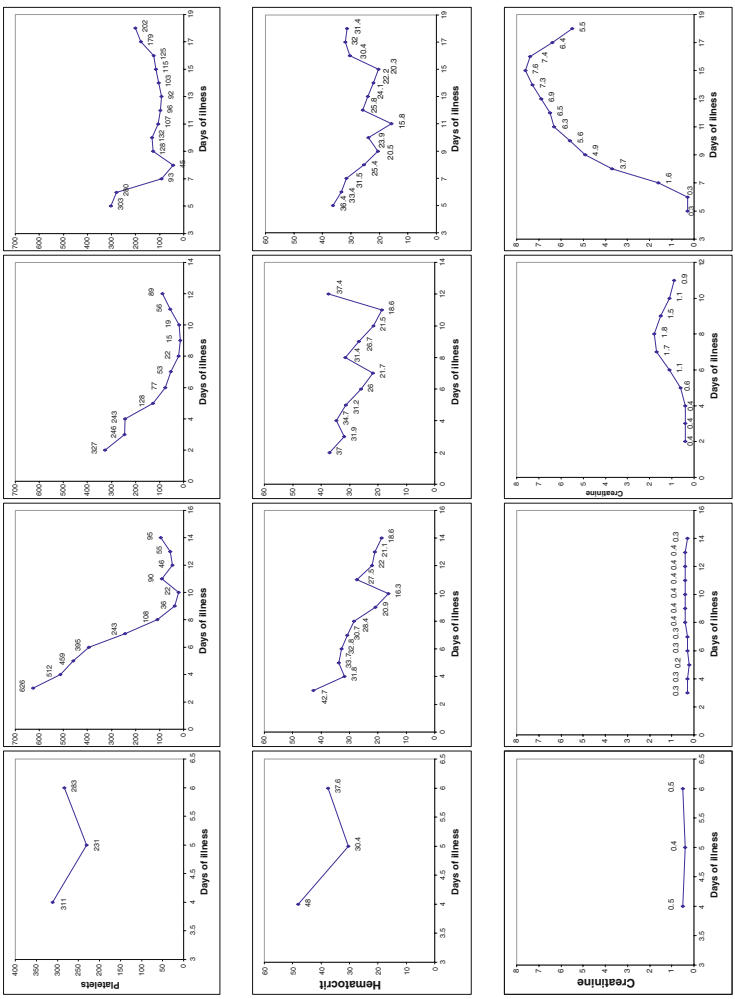

Fig. 3 Typical laboratory courses of *E. coli* O157:H7 infections. Pattern 1 represents a completely uncomplicated enteric infection. Note the temporary drop in the platelet count. Pattern 2 represents partial HUS, and portrays the profound vascular injury that can occur even in the absence of renal insufficiency. Pattern 3 is nonanuric HUS. Pattern 4 is anuric HUS

In most centers, HUS is best managed by pediatric nephrologists, and a detailed discussion of support of such patients is beyond the scope of this chapter. We direct interested readers to a recent thorough review of management of HUS (Loirat and Taylor 2003). Thrombocytopenia is generally the first abnormality to resolve in patients with HUS. Hemolysis can be prolonged, and patients can require erythrocyte transfusions well after renal recovery is underway. We find it helpful periodically to reculture inpatients so that contact precautions may be discontinued if appropriate. In any case, infection control measures should be in compliance with institutional policies, and public health guidelines and regulations.

9 Pathophysiology of HUS

Bacteremia is rare in STEC infections. It is plausible that the enteric symptoms, including the colitis, are the result of vascular injury from systemic toxemia, and not the direct effects of *E. coli* O157:H7 or its toxin on epithelial cells. Indeed, colitis in animals is induced by parenteral administration of toxin (Ritchie, Thorpe et al. 2003; Siegler, Obrig et al. 2003).

Figure 4 proposes a model for the host response to *E. coli* O157:H7 infection that leads to HUS. Tables 1 and 2 review common myths about this infection and provide some helpful hints related to management of these patients.

Stxs bind to the glycosphingolipid globotriosylceramide (Lingwood 2003), which is found on a wide variety of renal cells, including those of glomerular, endothelial, mesangial, and tubular epithelial cells (Boyd and Lingwood 1989; Takeda, Dohi et al. 1993; Lingwood 1994; Robinson, Hurley et al. 1995). Differences in organ damage might be attributed to the varying expression of this glycosphingolipid. Current data suggest that early in illness, possibly even before clinical presentation, circulating (absorbed) Stx injures the vascular endothelium, and this damage generates thrombin, which causes fibrin to be

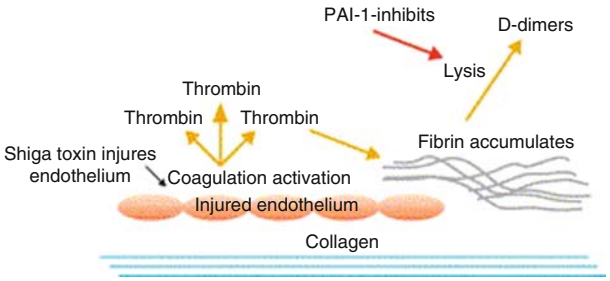

Fig. 4 Proposed model for pathological coagulation response leading to HUS (Tarr et al. 2005)

Table 1 Myths and facts about *E. coli* O157:H7 and Shiga toxin-producing *E. coli*

MYTH: As a group, non-O157:H7 STEC cause diseases that are as serious, and are at least as common, as *E. coli* O157:H7
FACT: Non-O157:H7 STEC as a group is less virulent than *E. coli* O157:H7. Individual infections are, on average, much less likely to lead to HUS or to be associated with epidemics. They are, in aggregate, about as common as *E. coli* O157:H7 or slightly less common, in most of the world (Klein, Stapp et al. 2002; Jelacic, Damrow et al. 2003; Brooks, Sowers et al. 2005). A small subset of non-O157:H7 infections will lead to HUS.
MYTH: Toxin assays will detect *E. coli* O157:H7 as well as or better than SMAC agar screening.
FACT: SMAC agar screening is more accurate and quicker at detecting *E. coli* O157:H7 than toxin assays. If you have to choose one detection method, choose SMAC agar (Fey, Wickert et al. 2000; Klein, Stapp et al. 2002; Carroll, Adamson et al. 2003; Park, Kim et al. 2003; Teel, Daly et al. 2007).
MYTH: Most cases are caused by eating poorly cooked hamburger, and epidemics are common.
FACT: Ground beef is associated with relatively fewer cases in recent years, while fresh fruits and vegetables, recreational water, and animal contact are emerging as bigger risk factors (Swerdlow, Woodruff et al. 1992; Besser, Lett et al. 1993; Keene, McAnulty et al. 1994; Tilden, Young et al. 1996; Keene, Hedberg et al. 1997; Keene, Sazie et al. 1997; Ackers, Mahon et al. 1998; Michino, Araki et al. 1999; Crump, Sulka et al. 2002; Terajima, Izumiya et al. 2002; Uhlich, Sinclair et al. 2007). Most cases are sporadic.

Table 2 Helpful hints for physicians caring for patients with confirmed or suspected *E. coli* O157:H7 infections

1.	Daily Laboratory tests: CBC, electrolytes, BUN, creatinine.
2.	Urinalysis is not helpful and could be counterproductive.
3.	Keep hydrating with isotonic crystalloid until trend in platelet counts is apparent (unequivocal rise, or stability with resolving symptoms).
4.	Obtain a CBC the day after discharge to confirm continued improvement.
5.	Give intravenous boluses with isotonic crystalloid for abdominal pain.
6.	Afebrile, bloody, painful diarrhea, especially if the blood appears after several days of nonbloody diarrhea, is suspicious for *E. coli* O157:H7 infection.
7.	If the bloody diarrhea ceases immediately after admission, patients are very unlikely to be infected with *E. coli* O157:H7.
8.	Fecal leukocytes, if present, are rarely abundant.
9.	About half of all infected patients report a fever in the days before presentation, but fever is rarely documented at presentation.
10.	Oral rehydration is not adequate.
11.	Children who continue to pass urine through day 10 of illness (day 1 is the first day of diarrhea) rarely become anuric.
12.	Platelet transfusions should not be given.
13.	Potassium can be added to the IV fluids if serum potassium is normal or low.
14.	If you are highly suspicious that a patient is infected with *E. coli* O157:H7, such as a household contact of known positive case, you should obtain a stool culture. If the child is, for whatever reason, not admitted, obtain a CBC, as this will provide a useful reference value for the platelet count if the stool is reported positive. If the count is rising, and the patient is doing well, then you might be able to avert an admission.
15.	Hospitalization of infected children at the height of their symptoms could be an important community infection control strategy.

deposited in the microvasculature. At the same time, circulating plasminogen activator inhibitor 1 (PAI-1) activity increases, which inhibits fibrinolysis. Fibrin further accumulates, and the thrombotic injury is compounded (Chandler, Jelacic et al. 2002)

Patients infected with *E. coli* O157:H7 rarely exhibit a fever once bloody diarrhea begins. In fact, before HUS develops, they do not seem to have a classic systemic inflammation response as is seen, for example, in septic shock. Despite this finding, pro-inflammatory cytokines and chemokines probably do injure host cells (Proulx, Seidman et al. 2001), but the assessment of local effects of cytokine-mediated injury in the living human is quite difficult.

Long-term sequelae: Most long-term sequelae of HUS relate to renal function. After the large outbreak in 1993, most survivors had good renal function 5 years after infection (Brandt, Joseph et al. 1998). Risk factors for long-term sequelae include an initial white blood cell count $> 20 \times 10^3/\mu L$ with neutrophilia, a high serum creatinine or urea concentration, central nervous syndrome symptoms such as coma or seizures, ischemic colitis, hypertension, anuria, and the need for dialysis (Siegler, Milligan et al. 1991; Garg, Suri et al. 2003).

Non-O157:H7 STEC: Non-O157:H7 STEC can certainly be human pathogens, and they can cause HUS (Klein, Stapp et al. 2002; Jelacic, Damrow et al. 2003; Brooks, Sowers et al. 2005). Moreover, without a toxin assay or nucleic acid hybridization testing, such organisms can be overlooked in diagnostic protocols (Bielaszewska, Kock et al. 2007). However, even when technology has been applied that would detect this group of organisms, *E. coli* O157:H7 remains the predominant human pathogenic STEC (Pai, Ahmed et al. 1988; Jelacic, Damrow et al. 2003; Manning, Madera et al. 2007; Teel, Daly et al. 2007). Non-O157:H7 STEC are quite common in food, and *E. coli* O157:H7 is rare. The infrequency with which non-O157:H7 are isolated in stool cultures compared to *E. coli* O157:H7 suggests that as a group they are considerably less pathogenic than *E. coli* O157:H7. While non-O157:H7 STEC as a subset can cause serious and even epidemic human disease, screening technology should not be directed at non-O157:H7 at the expense of recovering *E. coli* O157:H7. Optimally, clinical laboratories will seek both sets of pathogens (*E. coli* O157:H7 and non-O157: H7 STEC) in parallel.

10 Conclusions

Since the 1980s, much has been discovered about HUS; however, specific treatments do not exist. Specific therapies are unlikely to emerge, because available data suggest that the vascular lesion that leads to HUS is well underway by the time infected patients present to medical attention. Aggressive isotonic volume expansion, especially early in illness, appears associated with a diminished risk of anuria if HUS ensues. The best way to prevent HUS is to

prevent primary infections with *E. coli* O157:H7. Additional reported measures are syndromic recognition of this rather rare event, and early and accurate microbiological detection of infected patients. Early illness recognition will lead to careful monitoring and volume expansion at an earlier stage of illness, with isolation.

References

Ackers, M. L., Mahon, B. E., Leahy, E., Goode, B., Damrow, T., Hayes, P. S., Bibb, W. F., Rice, D. H., Barrett, T. J., Hutwagner, L., Griffin, P. M. and Slutsker, L. (1998). An outbreak of Escherichia coli O157:H7 infections associated with leaf lettuce consumption. *J Infect Dis* **177**(6): 1588–93.

Ahn, C. K., Klein, E. and Tarr, P. I. (2008). Isolation of patients acutely infected with Escherichia coli O157:H7: low-tech, highly effective prevention of hemolytic uremic syndrome. *Clin Infect Dis* **46**(8): 1197–9.

Ake, J. A., Jelacic, S., Ciol, M. A., Watkins, S. L., Murray, K. F., Christie, D. L., Klein, E. J. and Tarr, P. I. (2005). Relative nephroprotection during Escherichia coli O157:H7 infections: association with intravenous volume expansion. *Pediatrics* **115**(6): e673–80.

Bell, B. P., Goldoft, M., Griffin, P. M., Davis, M. A., Gordon, D. C., Tarr, P. I., Bartleson, C. A., Lewis, J. H., Barrett, T. J., Wells, J. G. and et al. (1994). A multistate outbreak of Escherichia coli O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. The Washington experience. *JAMA* **272**(17): 1349–53.

Bell, B. P., Griffin, P. M., Lozano, P., Christie, D. L., Kobayashi, J. M. and Tarr, P. I. (1997). Predictors of hemolytic uremic syndrome in children during a large outbreak of Escherichia coli O157:H7 infections. *Pediatrics* **100**(1): E12.

Bellomo, R., Kellum, J. A. and Bagshaw, S. M. (2007). Normotensive ischemic acute renal failure. *N Engl J Med* **357**(21): 2205; author reply 2205–6.

Besser, R. E., Lett, S. M., Weber, J. T., Doyle, M. P., Barrett, T. J., Wells, J. G. and Griffin, P. M. (1993). An outbreak of diarrhea and hemolytic uremic syndrome from Escherichia coli O157:H7 in fresh-pressed apple cider. *JAMA* **269**(17): 2217–20.

Bielaszewska, M., Kock, R., Friedrich, A. W., von Eiff, C., Zimmerhackl, L. B., Karch, H. and Mellmann, A. (2007). Shiga toxin-mediated hemolytic uremic syndrome: time to change the diagnostic paradigm? *PLoS ONE* **2**(10): e1024.

Boyd, B. and Lingwood, C. (1989). Verotoxin receptor glycolipid in human renal tissue. *Nephron* **51**(2): 207–10.

Brandt, J. R., Joseph, M. W., Fouser, L. S., Tarr, P. I., Zelikovic, I., McDonald, R. A., Avner, E. D., McAfee, N. G. and Watkins, S. L. (1998). Cholelithiasis following *Escherichia coli* O157:H7-associated hemolytic uremic syndrome. *Pediatr Nephrol* **12**(3): 222–5.

Brooks, J. T., Sowers, E. G., Wells, J. G., Greene, K. D., Griffin, P. M., Hoekstra, R. M. and Strockbine, N. A. (2005). Non-O157 Shiga toxin-producing Escherichia coli infections in the United States, 1983–2002. *J Infect Dis* **192**(8): 1422–9.

Carroll, K. C., Adamson, K., Korgenski, K., Croft, A., Hankemeier, R., Daly, J. and Park, C. H. (2003). Comparison of a commercial reversed passive latex agglutination assay to an enzyme immunoassay for the detection of Shiga toxin-producing *Escherichia coli*. *Eur J Clin Microbiol Infect Dis* **22**(11): 689–92.

Chandler, W. L., Jelacic, S., Boster, D. R., Ciol, M. A., Williams, G. D., Watkins, S. L., Igarashi, T. and Tarr, P. I. (2002). Prothrombotic coagulation abnormalities preceding the hemolytic-uremic syndrome. *N Engl J Med* **346**(1): 23–32.

Cornick, N. A., Jelacic, S., Ciol, M. A. and Tarr, P. I. (2002). *Escherichia coli* O157:H7 infections: discordance between filterable fecal shiga toxin and disease outcome. *J Infect Dis* **186**(1): 57–63.

Crump, J. A., Sulka, A. C., Langer, A. J., Schaben, C., Crielly, A. S., Gage, R., Baysinger, M., Moll, M., Withers, G., Toney, D. M., Hunter, S. B., Hoekstra, R. M., Wong, S. K., Griffin, P. M. and Van Gilder, T. J. (2002). An outbreak of *Escherichia coli* O157:H7 infections among visitors to a dairy farm. *N Engl J Med* **347**(8): 555–60.

Cummings, K. C., Mohle-Boetani, J. C., Werner, S. B. and Vugia, D. J. (2002). Population-based trends in pediatric hemolytic uremic syndrome in California, 1994–1999: substantial underreporting and public health implications. *Am J Epidemiol* **155**(10): 941–8.

Dundas, S., Todd, W. T., Stewart, A. I., Murdoch, P. S., Chaudhuri, A. K. and Hutchinson, S. J. (2001). The central Scotland *Escherichia coli* O157:H7 outbreak: risk factors for the hemolytic uremic syndrome and death among hospitalized patients. *Clin Infect Dis* **33**(7): 923–31.

Endo, Y., Tsurugi, K., Yutsudo, T., Takeda, Y., Ogasawara, T. and Igarashi, K. (1988). Site of action of a Vero toxin (VT2) from *Escherichia coli* O157:H7 and of Shiga toxin on eukaryotic ribosomes. RNA N-glycosidase activity of the toxins. *Eur J Biochem* **171**(1–2): 45–50.

Fey, P. D., Wickert, R. S., Rupp, M. E., Safranek, T. J. and Hinrichs, S. H. (2000). Prevalence of non-O157:H7 shiga toxin-producing *Escherichia coli* in diarrheal stool samples from Nebraska. *Emerg Infect Dis* **6**(5): 530–3.

Garg, A. X., Suri, R. S., Barrowman, N., Rehman, F., Matsell, D., Rosas-Arellano, M. P., Salvadori, M., Haynes, R. B. and Clark, W. F. (2003). Long-term renal prognosis of diarrhea-associated hemolytic uremic syndrome: a systematic review, meta-analysis, and meta-regression. *JAMA* **290**(10): 1360–70.

Gasser C, G. E. Steck A. (1955). Hamolytisch-uramishe syndrome: Bilaterale Ni.

Grif, K., Dierich, M. P., Karch, H. and Allerberger, F. (1998). Strain-specific differences in the amount of Shiga toxin released from enterohemorrhagic Escherichia coli O157 following exposure to subinhibitory concentrations of antimicrobial agents. *Eur J Clin Microbiol Infect Dis* **17**(11): 761–6.

Inward, C. D., Howie, A. J., Fitzpatrick, M. M., Rafaat, F., Milford, D. V. and Taylor, C. M. (1997). Renal histopathology in fatal cases of diarrhoea-associated haemolytic uraemic syndrome. British Association for Paediatric Nephrology. *Pediatr Nephrol* **11**(5): 556–9.

Jelacic, J. K., Damrow, T., Chen, G. S., Jelacic, S., Bielaszewska, M., Ciol, M., Carvalho, H. M., Melton-Celsa, A. R., O'Brien, A. D. and Tarr, P. I. (2003). Shiga toxin-producing *Escherichia coli* in Montana: bacterial genotypes and clinical profiles. *J Infect Dis* **188**(5): 719–29.

Jernigan, S. M. and Waldo, F. B. (1994). Racial incidence of hemolytic uremic syndrome. *Pediatr Nephrol* **8**(5): 545–7.

Kaplan, B. S., Chesney, R. W. and Drummond, K. N. (1975). Hemolytic uremic syndrome in families. *N Engl J Med* **292**(21): 1090–3.

Karmali, M. A., Steele, B. T., Petric, M. and Lim, C. (1983). Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. *Lancet* **1**(8325): 619–20.

Karpman, D., Hakansson, A., Perez, M. T., Isaksson, C., Carlemalm, E., Caprioli, A. and Svanborg, C. (1998). Apoptosis of renal cortical cells in the hemolytic-uremic syndrome: in vivo and in vitro studies. *Infect Immun* **66**(2): 636–44.

Keene, W. E., Hedberg, K., Herriott, D. E., Hancock, D. D., McKay, R. W., Barrett, T. J. and Fleming, D. W. (1997). A prolonged outbreak of *Escherichia coli* O157:H7 infections caused by commercially distributed raw milk. *J Infect Dis* **176**(3): 815–8.

Keene, W. E., McAnulty, J. M., Hoesly, F. C., Williams, L. P., Jr., Hedberg, K., Oxman, G. L., Barrett, T. J., Pfaller, M. A. and Fleming, D. W. (1994). A swimming-associated outbreak

of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and Shigella sonnei. *N Engl J Med* **331**(9): 579–84.

Keene, W. E., Sazie, E., Kok, J., Rice, D. H., Hancock, D. D., Balan, V. K., Zhao, T. and Doyle, M. P. (1997). An outbreak of *Escherichia coli* O157:H7 infections traced to jerky made from deer meat. *JAMA* **277**(15): 1229–31.

Kimmitt, P. T., Harwood, C. R. and Barer, M. R. (1999). Induction of type 2 Shiga toxin synthesis in Escherichia coli O157 by 4-quinolones. *Lancet* **353**(9164): 1588–9.

Klein, E. J., Stapp, J. R., Clausen, C. R., Boster, D. R., Wells, J. G., Qin, X., Swerdlow, D. L. and Tarr, P. I. (2002). Shiga toxin-producing *Escherichia coli* in children with diarrhea: a prospective point-of-care study. *J Pediatr* **141**(2): 172–7.

Lingwood, C. A. (1994). Verotoxin-binding in human renal sections. *Nephron* **66**(1): 21–8.

Lingwood, C. A. (2003). Shiga toxin receptor glycolipid binding. Pathology and utility. *Methods Mol Med* **73**: 165–86.

Loirat, C. and Taylor, C. M. (2003). Hemolytic Uremic Syndromes. *Pediatric Nephrology*. E. D. Avner, W. E. Harmon and P. Niaudet, Lippincott Williams and Wilkins: 887–915.

Lopez, E. L., Diaz, M., Grinstein, S., Devoto, S., Mendilaharzu, F., Murray, B. E., Ashkenazi, S., Rubeglio, E., Woloj, M., Vasquez, M. and et al. (1989). Hemolytic uremic syndrome and diarrhea in Argentine children: the role of Shiga-like toxins. *J Infect Dis* **160**(3): 469–75.

Manning, S. D., Madera, R. T., Schneider, W., Dietrich, S. E., Khalife, W., Brown, W., Whittam, T. S., Somsel, P. and Rudrik, J. T. (2007). Surveillance for Shiga toxin-producing Escherichia coli, Michigan, 2001–2005. *Emerg Infect Dis* **13**(2): 318–21.

March, S. B. and Ratnam, S. (1986). Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *J Clin Microbiol* **23**(5): 869–72.

McNabb, S. J., Jajosky, R. A., Hall-Baker, P. A., Adams, D. A., Sharp, P., Anderson, W. J., Javier, A. J., Jones, G. J., Nitschke, D. A., Worshams, C. A. and Richard, R. A., Jr. (2007). Summary of notifiable diseases — United States, 2005. *MMWR Morb Mortal Wkly Rep* **54**(53): 1–92.

Michino, H., Araki, K., Minami, S., Takaya, S., Sakai, N., Miyazaki, M., Ono, A. and Yanagawa, H. (1999). Massive outbreak of *Escherichia coli* O157:H7 infection in schoolchildren in Sakai City, Japan, associated with consumption of white radish sprouts. *Am J Epidemiol* **150**(8): 787–96.

O'Brien, A. O., Lively, T. A., Chen, M. E., Rothman, S. W. and Formal, S. B. (1983). *Escherichia coli* O157:H7 strains associated with haemorrhagic colitis in the United States produce a Shigella dysenteriae 1 (SHIGA) like cytotoxin. *Lancet* **1**(8326 Pt 1): 702.

O'Brien, S. J., Adak, G. K. and Gilham, C. (2001). Contact with farming environment as a major risk factor for Shiga toxin (Vero cytotoxin)-producing *Escherichia coli* O157 infection in humans. *Emerg Infect Dis* **7**(6): 1049–51.

Ostroff, S. M., Kobayashi, J. M. and Lewis, J. H. (1989). Infections with *Escherichia coli* O157:H7 in Washington State. The first year of statewide disease surveillance. *JAMA* **262**(3): 355–9.

Pai, C. H., Ahmed, N., Lior, H., Johnson, W. M., Sims, H. V. and Woods, D. E. (1988). Epidemiology of sporadic diarrhea due to verocytotoxin-producing Escherichia coli: a two-year prospective study. *J Infect Dis* **157**(5): 1054–7.

Park, C. H., Kim, H. J., Hixon, D. L. and Bubert, A. (2003). Evaluation of the duopath verotoxin test for detection of shiga toxins in cultures of human stools. *J Clin Microbiol* **41**(6): 2650–3.

Pediatrics, A. A. O. (2006). Isolation Precautions. In: Pickering LK, ed. Red Book: 2006 Report of the Committee on Infectious diseases. *Red Book*. Elk Grove Village, IL: American Academy of Pediatrics: 154–160.

Proulx, F., Seidman, E. G. and Karpman, D. (2001). Pathogenesis of Shiga toxin-associated hemolytic uremic syndrome. *Pediatr Res* **50**(2): 163–71.

Riley, L. W., Remis, R. S., Helgerson, S. D., McGee, H. B., Wells, J. G., Davis, B. R., Hebert, R. J., Olcott, E. S., Johnson, L. M., Hargrett, N. T., Blake, P. A. and Cohen, M. L. (1983). Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N Engl J Med* **308**(12): 681–5.

Ritchie, J. M., Thorpe, C. M., Rogers, A. B. and Waldor, M. K. (2003). Critical roles for stx2, eae, and tir in enterohemorrhagic Escherichia coli-induced diarrhea and intestinal inflammation in infant rabbits. *Infect Immun* **71**(12): 7129–39.

Robinson, L. A., Hurley, R. M., Lingwood, C. and Matsell, D. G. (1995). *Escherichia coli* verotoxin binding to human paediatric glomerular mesangial cells. *Pediatr Nephrol* **9**(6): 700–4.

Rowe, P. C., Orrbine, E., Lior, H., Wells, G. A., Yetisir, E., Clulow, M. and McLaine, P. N. (1998). Risk of hemolytic uremic syndrome after sporadic *Escherichia coli* O157:H7 infection: results of a Canadian collaborative study. Investigators of the Canadian Pediatric Kidney Disease Research Center. *J Pediatr* **132**(5): 777–82.

Safdar, N., Said, A., Gangnon, R. E. and Maki, D. G. (2002). Risk of hemolytic uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 enteritis: a meta-analysis. *JAMA* **288**(8): 996–1001.

Seto, E. Y., Soller, J. A. and Colford, J. M., Jr. (2007). Strategies to reduce person-to-person transmission during widespread *Escherichia coli* O157:H7 outbreak. *Emerg Infect Dis* **13**(6): 860–6.

Siegler, R. L., Milligan, M. K., Burningham, T. H., Christofferson, R. D., Chang, S. Y. and Jorde, L. B. (1991). Long-term outcome and prognostic indicators in the hemolytic-uremic syndrome. *J Pediatr* **118**(2): 195–200.

Siegler, R. L., Obrig, T. G., Pysher, T. J., Tesh, V. L., Denkers, N. D. and Taylor, F. B. (2003). Response to Shiga toxin 1 and 2 in a baboon model of hemolytic uremic syndrome. *Pediatr Nephrol* **18**(2): 92–6.

Slutsker, L., Ries, A. A., Greene, K. D., Wells, J. G., Hutwagner, L. and Griffin, P. M. (1997). *Escherichia coli* O157:H7 diarrhea in the United States: clinical and epidemiologic features. *Ann Intern Med* **126**(7): 505–13.

Swerdlow, D. L., Woodruff, B. A., Brady, R. C., Griffin, P. M., Tippen, S., Donnell, H. D., Jr., Geldreich, E., Payne, B. J., Meyer, A., Jr., Wells, J. G. and et al. (1992). A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. *Ann Intern Med* **117**(10): 812–9.

Taguchi, T., Uchida, H., Kiyokawa, N., Mori, T., Sato, N., Horie, H., Takeda, T. and Fujimoto, J. (1998). Verotoxins induce apoptosis in human renal tubular epithelium derived cells. *Kidney Int* **53**(6): 1681–8.

Takeda, T., Dohi, S., Igarashi, T., Yamanaka, T., Yoshiya, K. and Kobayashi, N. (1993). Impairment by verotoxin of tubular function contributes to the renal damage seen in haemolytic uraemic syndrome. *J Infect* **27**(3): 339–41.

Tarr, P. I. and Hickman, R. O. (1987). Hemolytic uremic syndrome epidemiology: a population-based study in King County, Washington, 1971 to 1980. *Pediatrics* **80**(1): 41–5.

Tarr, P. I., Gordon, C. A. and Chandler, W. L. (2005). Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. *Lancet* **365**(9464): 1073–86.

Teel, L. D., Daly, J. A., Jerris, R. C., Maul, D., Svanas, G., O'Brien, A. D. and Park, C. H. (2007). Rapid detection of Shiga toxin-producing *Escherichia coli* by optical immunoassay. *J Clin Microbiol* **45**(10): 3377–80.

Terajima, J., Izumiya, H., Tamura, K. and Watanabe, H. (2002). [PulseNet Japan–network system for the utilization of epidemiological information and the results of pulsed-field gel electrophoresis]. *Nippon Rinsho* **60**(6): 1070–6.

Tilden, J., Jr., Young, W., McNamara, A. M., Custer, C., Boesel, B., Lambert-Fair, M. A., Majkowski, J., Vugia, D., Werner, S. B., Hollingsworth, J. and Morris, J. G., Jr. (1996). A new route of transmission for Escherichia coli: infection from dry fermented salami. *Am J Public Health* **86**(8): 1142–5.

Tsai, H. M., Chandler, W. L., Sarode, R., Hoffman, R., Jelacic, S., Habeeb, R. L., Watkins, S. L., Wong, C. S., Williams, G. D. and Tarr, P. I. (2001). von Willebrand factor and von Willebrand factor-cleaving metalloprotease activity in *Escherichia coli* O157:H7-associated hemolytic uremic syndrome. *Pediatr Res* **49**(5): 653–9.

Uhlich, G. A., Sinclair, J. R., Warren, N. G., Chmielecki, W. A. and Fratamico, P. (2007). Characterization of Shiga toxin-producing *Escherichia coli* associated with two multi-state foodborne outbreaks that occurred in 2006. *Appl Environ Microbiol*.

Upadhyaya, K., Barwick, K., Fishaut, M., Kashgarian, M. and Siegel, N. J. (1980). The importance of nonrenal involvement in hemolytic-uremic syndrome. *Pediatrics* **65**(1): 115–20.

Varma, J. K., Greene, K. D., Reller, M. E., DeLong, S. M., Trottier, J., Nowicki, S. F., DiOrio, M., Koch, E. M., Bannerman, T. L., York, S. T., Lambert-Fair, M. A., Wells, J. G. and Mead, P. S. (2003). An outbreak of *Escherichia coli* O157 infection following exposure to a contaminated building. *JAMA* **290**(20): 2709–12.

Werber, D., Mason, B. W., Evans, M. R. and Salmon, R. L. (2008). Preventing household transmission of Shiga toxin-producing Escherichia coli O157 infection- promptly separating siblings might be the key. *Clin Infect Dis*.

Wong, C. S., Jelacic, S., Habeeb, R. L., Watkins, S. L. and Tarr, P. I. (2000). The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. *N Engl J Med* **342**(26): 1930–6.

Global Epidemiology of Pneumococcal Disease—New Prospects for Vaccine Control

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1 Overview of the Pneumococcus and Pneumococcal Disease

Streptococcus pneumoniae, or 'the pneumococcus', is a Gram positive, encapsulated diplococcus. Based on differences in the composition of its polysaccharide capsule, about 90 serotypes are identified. However, the majority of pneumococcal disease in infants is associated with a small number of these serotypes, which may vary by region. Globally, about 20 serotypes are associated with >80% of invasive pneumococcal disease occurring in all age groups, and only 10–12 serogroups (representing 12–14 serotypes) are responsible for the vast majority of pediatric invasive and mucosal acute otitis media (AOM) disease worldwide (WHO, 2007; Hausdorff et al., 2008).

Pneumococcus is an exclusively human pathogen, usually carried harmlessly and often asymptomatically in the nasopharynx, especially by children. Transmission occurs by direct contact with respiratory secretions or inhalation of respiratory aerosols from carriers or from individuals with pneumococcal disease. Initially, this can result in nasopharyngeal carriage of pneumococcus. Near the time of acquisition or subsequently, the bacterium may also spread to other locations of the body to cause disease.

The risk of disease is highest among young children, older adults, smokers, and persons with certain chronic illnesses (Robinson et al., 2001). Data from the active bacterial core surveillance (ABCs) of the Centers for Disease Control and Prevention show that incidence per 100,000 of invasive pneumococcal disease (IPD) in 1998 was highest among children younger than 2 years (166.9) and adults aged 65 years or older (59.7) (Robinson et al., 2001). A study of community-acquired pneumonia conducted in Finland showed a similar pattern, affecting the extremes of age, with age-specific incidence per 100,000 inhabitants of 3600 among children less than 5-years-old and 3420 among adults more than 75-years-old (Jokinen et al., 1993). These numbers highlight

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the importance of having vaccines that are effective both in infancy and in the elderly.

In 2006, Melegaro et al. estimated the burden of pneumococcal disease in England and Wales (Melegaro et al., 2006) using on a combination of database analyses and assumptions based on mathematical modeling. They estimated that 26% of community-acquired pneumonia and 23% of AOM are due to the pneumococcus. If such estimates are extrapolated to the population of the European Union under 5 years of age, the predicted approximate annual number of pneumococcal cases are: AOM = 2.1 million, pneumonia = 62,000, sepsis = 4700, and meningitis = 1800.

2 Pneumonia

Pneumonia is the largest infectious cause of child death worldwide. Although pneumonia may be caused by viruses and/or bacteria, a major cause is pneumococcal infection, which is estimated to be responsible for between 1 and 2 million child deaths annually, mostly in developing countries (UNICEF and WHO, 2006). Currently, pneumococcal disease is considered a major priority for several organizations devoted to improving the health of children in impoverished countries, including the Global Alliance for Vaccines and Immunization (GAVI), the Bill and Melinda Gates Foundation and the World Health Organization (WHO). In industrialized countries, pneumococcal pneumonia is responsible for relatively few deaths in children, although there is a high morbidity (UNICEF and WHO, 2006). In addition, complicated pneumonia rates—specifically empyema, or the presence of pus in the pleural cavity, a serious complication of pneumonia often requiring surgical chest drainage—are reported to have risen dramatically in some studies in Spain (Calbo et al., 2006), the US (Byington et al., 2006), the UK (Spencer et al., 2006), and Taiwan (Hsieh et al., 2004) in recent years. While the reasons underlying these rises are unclear, in each study apart from the one done in Taiwan, serotype 1 was reported to be the dominant serotype.

3 Invasive Pneumococcal Disease

Invasive pneumococcal disease (IPD) is defined as the isolation of *S. pneumoniae* from normally sterile body fluids. Several manifestations of disease may be included in this definition, such as meningitis, sepsis, bacteremic pneumonia, and occult bacteremia. The apparent incidence of IPD varies considerably around the world and may largely depend on local blood culture practices including the willingness to perform blood cultures in the outpatient setting (Table 1). For example, the rates of IPD seen in outpatient blood cultures in Kenya, Chile, and Argentina are detected only because healthcare staff in these

Table 1 Incidence of invasive pneumococcal disease per 100,000, hospitalized and outpatient cases, < 2 years-old (pre- PCV-7 (*Prevenar*^{TM}) U.S. (Whitney et al., 2003), Chile (Lagos et al., 2002), Argentina (Tregnaghi et al., 2006)) and < 5 years-old (Kenya (Berkley et al., 2005; Brent et al., 2006))

IPD cases/100,000	US	Chile	Argentina	Kenya
Hospitalized only	56.8	44.6	78.6	111
Outpatient only	132.7	34.5	128.2	~486
Total IPD	189.5	79.1	206.8	597

countries performed outpatient blood cultures in a study setting. Normally nearly all of these cases would go undetected. Thus much IPD, some self-limiting and some more severe, goes undiagnosed, unreported, and untreated, particularly in settings with suboptimal access to facilities with adequate laboratory capacity and antibiotics (WHO, 2007).

Variations in the local epidemiology of pneumococcal serotypes may influence the burden of disease. There is evidence that individual serotypes differ in their abilities to activate, deposit, and/or degrade complement, to resist phagocytosis, and to elicit immune responses (Hausdorff, Feikin and Klugman, 2005).

Certain serotypes are much more likely to be associated with nasopharyngeal colonization than to cause invasive disease. Compared with transient or infrequent colonizers, some serotypes carried at high rates by young children may rapidly elicit age-associated natural immunity to invasive disease. Other serotypes seem to be of particular importance as causes of disease in very young infants, in older children, in immunocompromised individuals, or in elderly people. Some serotypes, such as 1 and 5, seem to be associated with particular disease syndromes, such as complicated pneumonias in children or with higher rates of hospitalization in children, or are consistently responsible for outbreaks in certain populations (Hausdorff, Feikin, and Klugman, 2005).

The frequency with which certain serotypes are isolated from the nasopharynx seems to correlate approximately with their likelihood of becoming resistant to antibiotics, consistent with the notion that the site of selection for antibiotic-resistant strains is the nasopharynx. These serotypes are largely represented in PCV-7, in particular 6B, 9V, 14, 19F, 23F, and the vaccine-related types 6A and 19A. In contrast, serotypes 1 and 5, which are not carried at high frequency or for long duration, are rarely antibiotic resistant (Hausdorff, Feikin, and Klugman, 2005).

In the past, multiple pneumococcal outbreaks have been described. Nowadays, outbreaks have become rare, possibly because of increased availability of antibiotics and improvements in socioeconomic conditions, such as crowding, and perhaps due to an apparently secular trend toward predominance of PCV-7 types in recent years (Feikin and Klugman, 2002). Nevertheless, certain serotypes, such as 1 and 5, have been observed to predominate as causes of IPD in certain populations for a few years, and then decrease to much lower levels. In Sweden, a 10-fold increase in type 1 occurred between 1992 and 1997, representing 10% of

all pediatric cases at its peak, and declined thereafter until 2001 (Hedlund et al., 2003; Henriques et al., 2001). In Ghana, a progressive increase in the incidence of pneumococcal meningitis was observed between 2000 and 2003. The case-fatality rate was 44.4%; the majority of pneumococcal isolates was antibiotic sensitive and expressed the serotype 1 capsule (76%) (Leimkugel et al., 2005). Lagos et al. reported that the annual incidence of IPD due to serotypes 1 and 5, in children aged between 36 and 59 months in Chile, exhibited high variability during the period 1994–2004, with peaks of serotype 1 occurring in 1995, 1999, and 2004 and of serotype 5 in 1995 and 2003 (Lagos et al., 2006).

The absolute incidence of IPD also changes with age. Studies performed in Denmark, Germany, England and Wales, Slovenia, and the US have shown that the absolute incidence of IPD caused by serotypes represented in PCV-7, together or individually, is highest in the first 2 years of life, but then drops by 70% or more in the next few years, eventually reaching levels in older children that are 2–3% of those seen in the youngest infants. The incidence of disease caused by serotypes 1 and 5 also peaks in the first year of life, mostly due to infection and subsequent disease within the first few months and falls substantially by the second year. Then, in sharp contrast to the pattern seen with the PCV-7 types, their incidence remains constant or even slightly increases over the next several years. These observations suggest that, after the first year of life, only gradual immune maturation to these serotypes occurs, perhaps due to the lack of prolonged nasopharyngeal colonization (Hausdorff, Feikin, and Klugman, 2005).

While all major types of pneumococcus can cause all disease manifestations, some are more likely to cause certain syndromes. In Spain, a dramatic increase in the incidence of parapneumonic empyema among children has been observed in the last decade. With the use of polymerase chain reaction (PCR) techniques, pneumococcus has been identified as the causative pathogen in 80% of the cases. It is remarkable that serotype 1 was identified in 48% of these cases, followed by serotype 7F (14%), serotype 3 (11%), serotypes 5 and 14 (8% each) (Obando et al., 2007). However, the authors concluded that these findings were probably not related to the introduction of PCV-7. Interestingly, this pattern of serotype distribution for empyema cases has also been observed in other countries, such as the US and the UK (Byington et al., 2006; Eastham et al., 2004).

4 Acute Otitis Media

Acute otitis media (AOM) is the rapid onset of signs and symptoms of acute infection within the middle ear. The most common manifestations of the disease are fever, otalgia, otorrhea, and the recent onset of irritability, anorexia, vomiting, or diarrhea. The tympanic membrane is bulging, opaque, inflamed, and has limited or no mobility on pneumatic otoscopy, all of which indicate middle-ear effusion (Bluestone and Klein, 2007).

AOM is one of the most frequently reported childhood diseases. In the first two years of life, more than 75% of all children get AOM. It is the most frequent bacterial infection of children and the primary reason for visits to and prescription of antibiotics by pediatricians and thus a major driver of antimicrobial resistance (Caceres Udina et al., 2004; McCaig and Hughes, 1995; Riquelme et al., 2004).

To add to the high impact of AOM on healthcare utilization and costs, the disease may lead to recurrent episodes, otitis media with effusion (no acute symptoms but persistent fluid in middle ear), and the need for tympanostomy tube placement. Moreover, complications of AOM, although rare, have been described. They include hearing loss that potentially may affect child development, perforation of the tympanic membrane, mastoiditis, petrositis, labyrinthitis, facial paralysis, and even meningitis, encephalitis, and brain abscess (Bluestone and Klein, 2007).

Numerous studies have demonstrated that pneumococcus and nontypeable (unencapsulated) *Haemophilus influenzae* (NTHi) are the two major bacterial causes of AOM (Block et al., 2004; Eskola et al., 2001; Leibovitz, Jacobs and Dagan, 2004; Prymula et al., 2006; Rosenblut et al., 2001), and that they are not distinguishable from one another based on clinical symptoms alone (Leibovitz et al., 2003). The proportion of AOM caused by pneumococcus ranges from 26% (Israel) to 62% (Czech Republic and Slovakia) and that of AOM due to NTHi ranges from 22% (Czech Republic and Slovakia) to 56% (US).

5 Pneumococcal Vaccines

Pneumococcal conjugate vaccines are targeted at the polysaccharide capsule of the bacteria. The polysaccharide capsule is a good vaccine antigen because it is the defining phenotype of the pneumococcus; not only is it a target of the mature human immune response, but it also influences the epidemiology of the infection, the transmission of the pathogen, and the virulence of the disease (Hausdorff et al., 2008).

Currently, only one pneumococcal conjugate vaccine formulation is registered, a heptavalent conjugate vaccine formulation (PCV-7: Wyeth: *Prevnar*™/*Prevenar*™) containing conjugates against serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F linked to a diphtheria toxin variant, CRM_{197}. In addition, PCV-7 has a high level of cross-protection against serotype 6A. A 10-valent pneumococcal nontypeable *H. influenzae* protein D conjugate vaccine formulation (PHiD-CV, GlaxoSmithKline Biologicals) with the same pneumococcal serotypes as PCV-7 plus 1, 5, and 7F and also with evidence of extensive cross-reactivity against 6A and expected efficacy against nontypeable *H. influenzae* (Prymula et al., 2006) has recently been submitted for licensure in several regions. Finally, there is a 13-valent conjugate formulation (PCV-13 with all conjugates linked to CRM_{197}

Table 2 Pediatric pneumococcal vaccines licensed or in advanced development

Vaccine formulation and manufacturer	Serotypes	Carrier protein	Status
PCV-7 Pneumococcal conjugate vaccine 7 valent *Prevnar*™/ *Prevenar*™ Wyeth	4, 6B, 9V, 14, 18C, 19F, 23F	Diphtheria Toxoid Variant (CRM197)	Licensed US 2000, Europe 2001 and many other countries since. Universal mass vaccination programs in North America, Western Europe, starting elsewhere
PHiD-CV Pneumococcal Nontypeable *H. influenzae* protein D conjugate vaccine	PCV-7 + 1, 5, 7F	Mainly nontypeable *H. influenzae* protein D Also diphtheria and tetanus toxoids	Submitted for licensure EU and worldwide end 2007
PCV-13 *Prevenar13*™ Wyeth	PCV-10 + 3, 6A,19A	Diphtheria Toxoid Variant (CRM197)	In Phase II/III To be submitted for licensure 2009

and with same serotypes as PHiD-CV plus 3, 6A, and 19A) under development by Wyeth (Table 2).

While the proportion of serotypes in children potentially covered by PCV-7 varies markedly from region to region and even from country to country; in most places, at least 50% of invasive isolates from children aged <5 years are represented by PCV-7. In Western Europe, at least two-thirds of all isolates are covered by PCV-7 and in the USA, Canada, Australia, New Zealand, and several Pacific Rim countries PCV-7 covers more than 80% of isolates. In virtually all countries studied more than two-thirds of all invasive isolates are represented by the ten serotypes in PHiD-CV and in Western Europe and parts of South America more than 80% of isolates are covered by PHiD-CV. PCV-13 raises the coverage level for IPD to more than 80% in the remaining regions, with the possible exceptions of the Middle East and South Asia. However, most data from the latter two regions come from small studies concentrating on meningitis isolates, which may not be representative of the spectrum of the entirety of pediatric IPD (Hausdorff et al., 2008).

6 The Power of Pneumococcal Conjugate Vaccines I: Effect of PCV-7 on Rates of IPD in the US after Introduction in 2000

The incidence of IPD due to vaccine serotypes has decreased substantially after the introduction of PCV-7 in the US in vaccinated children as well as all other age groups, indicating that, in addition to direct protection, pneumococcal transmission was interrupted as a result of the reduction in carriage in the vaccinated pediatric population (Hausdorff et al., 2008).

Kyaw et al. reported that in the < 2-year-old age group, the incidence rate of IPD caused by penicillin–nonsusceptible pneumococci decreased by 81% (95% confidence interval, 80–82%), from a peak of 70.3 per 100,000 in 1999 to 13.1 per 100,000 in 2004. In addition, the rates of penicillin–nonsusceptible disease among adults 65 years of age or older decreased from 16.4 per 100,000 in 1999 to 8.4 per 100,000 in 2004—a reduction of 49% (95% confidence interval, 46–51%) (Kyaw et al., 2006).

Another report using data from the ABCs in the US shows that the majority (69%) of the 29,599 projected vaccine-type (VT) IPD cases prevented nationally by PCV-7 in 2003 compared with 1998–1999 cases were prevented through indirect effects of the vaccine. An estimated 9140 cases of VT IPD were directly prevented by vaccinating children aged <5 years with PCV-7; an additional 20,459 cases of VT IPD were prevented through indirect effects of the vaccine across all ages (MMWR, 2005). More recently, Hicks et al. reported that the annual incidence of disease due to nonvaccine serotypes (NVT) increased from an average of 16.3 cases per 100,000 population during prevaccine years (1998–1999) to 19.9 cases per 100,000 population in 2004 for children aged <5 years ($P = 0.01$) and from 27.0 cases per 100,000 population during prevaccine years to 29.8 cases per 100,000 population in 2004 for adults aged > 65 years ($P = 0.05$). Significant increases in the incidences of disease due to serotypes 3, 15, 19A, 22F, and 33F were observed among children during this period ($P < 0.05$ for each serotype); serotype 19A has become the predominant cause of invasive disease in US children. The incidence of disease due to these serotypes also increased among elderly persons (Hicks et al., 2007). Whether these changes in NVT disease incidence are causally related to PCV-7 introduction, or changes in antibiotic usage, or are secular trends, or some combination of these remains unclear. So far, it is unknown whether similar rises may be seen in other countries.

7 The Power of Pneumococcal Conjugate Vaccines II: Effect of the 11-Valent PHiD-Conjugate Vaccine on AOM in a Phase III Efficacy Trial

In a double blind, randomized (1:1) study, comparing an 11-valent *H. influenzae* protein D (PHiD)–conjugate vaccine (also including serotype 3) with *Havrix*™ (hepatitis A vaccine), 4968 infants received either one or the other vaccine at the ages of 3, 4, 5, and 12–15 months and were followed-up until the end of the second year of life. Middle-ear fluid was obtained for bacteriological culture and serotyping in children who presented at the otorhinolarynologist (ENT) with abnormal tympanic membrane or presence of middle-ear effusion, plus two predefined clinical symptoms. The primary endpoint was protective efficacy against the first episode of AOM caused by vaccine pneumococcal serotypes. From 2 weeks after the third dose to 24–27 months of age, 333 clinical episodes of

AOM were recorded in the protein D conjugate group ($n = 2455$) and 499 in the control group ($n = 2452$), giving a significant (33.6% (95% CI 20.8–44.3)) reduction in the overall incidence of ENT-confirmed clinical AOM episodes. Vaccine efficacy was shown for episodes of AOM caused by pneumococcal vaccine serotypes (52.6% (5.0–65.5) for the first episode and 57.6% (41.4–69.3) for any episode). Efficacy was also shown against episodes of AOM caused by NTHi (35.3% (1.8–57.4)). The vaccine reduced frequency of infection from vaccine-related cross-reactive pneumococcal serotypes by 65.5%, but did not significantly change the number of episodes caused by other NVTs. The results confirmed that using the nontypeable *H. influenzae*-derived protein D as a carrier protein for pneumococcal polysaccharides not only allowed protection against pneumococcal otitis, but also against AOM due to NTHi (Prymula et al., 2006).

8 Pneumococcal Vaccines for Adults

The pneumococcal vaccine for adults that is currently available contains polysaccharide capsules of the 23 most prevalent serotypes. It elicits functional (i.e., opsonophagocytic) serum antibodies in adults and older children. Its efficacy against invasive disease in adults and against nonbacteremic pneumonia in young adults (South African miners) is also generally accepted. However, there is no convincing evidence that it prevents nonbacteremic pneumonia in the elderly (Ortqvist et al., 1998). In addition, the vaccine induces no long-lasting immunity and it is not widely used except in a few countries (such as the US), and it does not induce immune responses to most of the serotypes in young children. It is known that chemically linking (conjugating) polysaccharides to protein carriers makes them stimulate immune responses in young children and induce immune memory. However, the question remains whether conjugates will protect against nonbacteremic pneumonia in elderly populations.

Several pneumococcal conjugate vaccines based on the pediatric formulations described here are under development.

9 Conclusions

Pneumococcal disease remains a major cause of morbidity and mortality in children and adults on a global level, but the prospects of vaccine control of pneumococcal disease, both invasive and mucosal, have never been better. The success of conjugate vaccines to date has stimulated the development of a second generation of vaccines. There is now potential for prevention with conjugate vaccines, within the same disease syndromes, not only of pneumococcal and other encapsulated bacterial infections but also of other bacterial pathogens such as nontypeable *H. influenzae*.

References

Berkley JA, Lowe BS, Mwangi I, Williams T, Bauni E, Mwarumba S, Ngetsa C, Slack MP, Njenga S, Hart CA, Maitland K, English M, Marsh K, Scott JA. Bacteremia among children admitted to a rural hospital in Kenya. N Engl J Med. 2005, 352:39–47.

Block SL, Hedrick J, Harrison CJ, Tyler R, Smith A, Findlay R, Keegan E. Community-wide vaccination with the heptavalent pneumococcal conjugate significantly alters the microbiology of acute otitis media. Pediatr Infect Dis J. 2004, 23:829–833.

Bluestone C. and Klein J. Otitis Media in Infants and Children. BC Decker, Inc. Hamilton, Ontario, 2007.

Brent AJ, Ahmed I, Ndiritu M, Lewa P, Ngetsa C, Lowe B, Bauni E, English M, Berkley JA, Scott JA. Incidence of clinically significant bacteraemia in children who present to hospital in Kenya: community-based observational study. Lancet. 2006, 367:482–488.

Byington CL, Korgenski K, Daly J, Ampofo K, Pavia A, Mason EO. Impact of the pneumococcal conjugate vaccine on pneumococcal parapneumonic empyema. Pediatr Infect Dis J. 2006, 25:250–254.

Caceres Udina MJ, varez Martinez JA, rgente del CJ, Chumilla Valderas MA, Fernandez AE, Garrido RA, Sanchez GF, Garcia-Marcos L. [Incidence, air pollution and risk factors of acute otitis media in the first year of life: a prospective study]. An Pediatr (Barc). 2004, 60:133–138.

Calbo E, Diaz A, Canadell E, Fabrega J, Uriz S, Xercavins M, Morera MA, Cuchi E, Rodriguez-Carballeira M, Garau J. Invasive pneumococcal disease among children in a health district of Barcelona: early impact of pneumococcal conjugate vaccine. Clin Microbiol Infect. 2006, 12:867–872.

Direct and indirect effects of routine vaccination of children with 7-valent pneumococcal conjugate vaccine on incidence of invasive pneumococcal disease–United States, 1998–2003. MMWR Morb Mortal Wkly Rep. 2005, 54:893–897.

Eastham KM, Freeman R, Kearns AM, Eltringham G, Clark J, Leeming J, Spencer DA. Clinical features, aetiology and outcome of empyema in children in the north east of England. Thorax. 2004, 59:522–525.

Eskola J, Kilpi T, Palmu A, Jokinen J, Haapakoski J, Herva E, Takala A, Kayhty H, Karma P, Kohberger R, Siber G, Makela PH. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. N Engl J Med. 2001, 344:403–409.

Feikin DR and Klugman KP. Historical changes in pneumococcal serogroup distribution: implications for the era of pneumococcal conjugate vaccines. Clin Infect Dis. 2002, 35:547–555.

Hausdorff WP, Brueggemann AB, Hackell JG, Scott JA. Pneumococcal Serotype Epidemiology. In: Siber G et al. Pneumococcal Vaccines: the Impact of Conjugate Vaccine. ASM Press. Washington, DC. 2008, 139–160.

Hausdorff WP, Feikin DR, Klugman KP. Epidemiological differences among pneumococcal serotypes. The Lancet Infectious Diseases. 2005, 5:83–93.

Hedlund J, Sorberg M, Henriques NB, Kronvall G. Capsular types and antibiotic susceptibility of invasive Streptococcus pneumoniae among children in Sweden. Scand J Infect Dis. 2003, 35:452–458.

Henriques NB, Kalin M, Ortqvist A, Akerlund T, Liljequist BO, Hedlund J, Svenson SB, Zhou J, Spratt BG, Normark S, Kallenius G. Dynamics of penicillin-susceptible clones in invasive pneumococcal disease. J Infect Dis. 2001, 184:861–869.

Hicks LA, Harrison LH, Flannery B, Hadler JL, Schaffner W, Craig AS, Jackson D, Thomas A, Beall B, Lynfield R, Reingold A, Farley MM, Whitney CG. Incidence of pneumococcal disease due to non-pneumococcal conjugate vaccine (PCV7) serotypes in the United States during the era of widespread PCV7 vaccination, 1998–2004. J Infect Dis. 2007, 196:1346–1354.

Hsieh YC, Hsueh PR, Lu CY, Lee PI, Lee CY, Huang LM. Clinical manifestations and molecular epidemiology of necrotizing pneumonia and empyema caused by Streptococcus pneumoniae in children in Taiwan. Clin Infect Dis. 2004, 38:830–835.

Jokinen C, Heiskanen L, Juvonen H, Kallinen S, Karkola K, Korppi M, Kurki S, Ronnberg P, Seppa A, Soimakallio S, Sten M, Tanska S, Tarkiainen A, Tukiainen H, Pyorala K, Makela PH. Incidence of Community-Acquired Pneumonia in the Population of Four Municipalities in Eastern Finland. Am J Epidemiol. 1993, 137:977–988.

Kyaw MH, Lynfield R, Schaffner W, Craig AS, Hadler J, Reingold A, Thomas AR, Harrison LH, Bennett NM, Farley MM, Facklam RR, Jorgensen JH, Besser J, Zell ER, Schuchat A, Whitney CG. Effect of introduction of the pneumococcal conjugate vaccine on drug-resistant Streptococcus pneumoniae. N Engl J Med. 2006, 354:1455–1463.

Lagos R, Munoz A, San Martin O, Heitmann I, Loyola H, Levine MM. Secular variations in age incidence (Inc) and serotypes (St) causing invasive pneumococcal disease (IPD) in children 0 to 59 months of age (MoA) in the Metropolitan Region (MR), Chile. Proceedings of the 5th International Symposium on Pneumococci & Pneumococcal Diseases. 2006, 164.

Lagos R, Munoz A, Valenzuela MT, Heitmann I, Levine MM. Population-based surveillance for hospitalized and ambulatory pediatric invasive pneumococcal disease in Santiago, Chile. Pediatr Infect Dis J. 2002, 21:1115–1123.

Leibovitz E, Jacobs MR, Dagan R. Haemophilus influenzae: a significant pathogen in acute otitis media. Pediatr Infect Dis J. 2004, 23:1142–1152.

Leibovitz E, Satran R, Piglansky L, Raiz S, Press J, Leiberman A, Dagan R. Can acute otitis media caused by Haemophilus influenzae be distinguished from that caused by Streptococcus pneumoniae? Pediatr Infect Dis J. 2003, 22:509–515.

Leimkugel J, Adams FA, Gagneux S, Pfluger V, Flierl C, Awine E, Naegeli M, Dangy JP, Smith T, Hodgson A, Pluschke G. An outbreak of serotype 1 Streptococcus pneumoniae meningitis in northern Ghana with features that are characteristic of Neisseria meningitidis meningitis epidemics. J Infect Dis. 2005, 192:192–199.

McCaig LF and Hughes JM. Trends in antimicrobial drug prescribing among office-based physicians in the United States. JAMA. 1995, 273:214–219.

Melegaro A, Edmunds WJ, Pebody R, Miller E, George R. The current burden of pneumococcal disease in England and Wales. J. Infect. 2006, 52:37–48.

Obando I, Arroyo LA, Sanchez-Tatay D, Tarrago D, Moreno D, Hausdorff WP, Brueggemann AB. Molecular epidemiology of paediatric invasive pneumococcal disease in southern Spain after the introduction of heptavalent pneumococcal conjugate vaccine. Clin Microbiol Infect. 2007, 13:347–348.

Ortqvist A, Hedlund J, Burman LA, Elbel E, Hofer M, Leinonen M, Lindblad I, Sundelof B, Kalin M. Randomised trial of 23-valent pneumococcal capsular polysaccharide vaccine in prevention of pneumonia in middle-aged and elderly people. Swedish Pneumococcal Vaccination Study Group. Lancet. 1998, 351:399–403.

Pneumococcal conjugate vaccine for childhood immunization–WHO position paper. Wkly Epidemiol Rec. 2007, 82:93–104.

Prymula R, Peeters P, Chrobok V, Kriz P, Novakova E, Kaliskova E, Kohl I, Lommel P, Poolman J, Prieels JP, Schuerman L. Pneumococcal capsular polysaccharides conjugated to protein D for prevention of acute otitis media caused by both Streptococcus pneumoniae and non-typable Haemophilus influenzae: a randomised double-blind efficacy study. Lancet. 2006, 367:740–748.

Riquelme PM, Rincon VP, Garcia RL, Tusset CJ, Bravo TR, Fernandez PC. [Acute otitis media in a pediatric primary care unit]. An Pediatr (Barc). 2004, 61:408–412.

Robinson KA, Baughman W, Rothrock G, Barrett NL, Pass M, Lexau C, Damaske B, Stefonek K, Barnes B, Patterson J, Zell ER, Schuchat A, Whitney CG, for the Active Bacterial Core Surveillance /Emerging Infections Program Network. Epidemiology of Invasive Streptococcus pneumoniae Infections in the United States, 1995–1998: Opportunities for Prevention in the Conjugate Vaccine Era. JAMA. 2001, 285:1729–1735.

Rosenblut A, Santolaya ME, Gonzalez P, Corbalan V, Avendano LF, Martinez MA, Hormazabal JC. Bacterial and viral etiology of acute otitis media in Chilean children. Pediatr Infect Dis J. 2001, 20:501–507.

Spencer DA, Iqbal SM, Hasan A, Hamilton L. Empyema thoracis is still increasing in UK children. BMJ. 2006, 332:1333.

Tregnaghi M, Ceballos A, Ruttimann R, Ussher J, Tregnaghi P, Peeters P, Hausdorff WP. Active epidemiologic surveillance of pneumonia and invasive pneumococcal disease in ambulatory and hospitalized infants in Cordoba, Argentina. Pediatr Infect Dis J. 2006, 25:370–372.

UNICEF and WHO. Pneumonia: the forgotten killer of children. United Nations Children's Fund. New York, 2006.

Whitney CG, Farley MM, Hadler J, Harrison LH, Bennett NM, Lynfield R, Reingold A, Cieslak PR, Pilishvili T, Jackson D, Facklam RR, Jorgensen JH, Schuchat A. Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. N Engl J Med. 2003, 348:1737–1746.

Current Concepts of the Pathogenesis of RSV Bronchiolitis

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1 Introduction

The respiratory syncytial virus (RSV) is a member of the *Pneumovirus* genus within the family *Paramyxoviridae*. The RSV has a 15-kb-long, single-stranded, negative-sense RNA genome, encoding 11 genes. These include the immunodominant attachment-(G) and fusion (F) proteins at the surface of the virion and the matrix (M) protein and the immunomodulatory non-structural (NS1/NS2) proteins.

RSV bronchiolitis is the leading cause of infant hospitalization during the winter season. It is not known why RSV infections do not occur during the summer. There is evidence that the incidence of RSV infections is increased during periods of low ultraviolet B radiance (Yusuf et al., 2007). Practically all children are infected with RSV before the age of 2 years, but only a minority develop severe disease requiring hospital admission. It is estimated that 10% of infants hospitalized for RSV require mechanical ventilation (Bont and Kimpen, 2002; Simoes, 1999; Smyth and Openshaw, 2006). With good management, mortality rates are close to zero. Risk factors for severe course of disease are preterm birth with or without chronic lung disease, congenital heart disease, and neonatal status. More recently, Down's syndrome was discovered as an independent risk factor for severe RSV bronchiolitis (Bloemers et al., 2007). As many as 10% of children with Down's syndrome are hospitalized for RSV bronchiolitis. Post-bronchiolitis wheeze develops in 50% of children hospitalized for RSV bronchiolitis. Although clinical symptoms may resemble asthma, there is no association between RSV bronchiolitis and allergy (Stein et al., 1999; Bont et al., 2000a). Moreover, post-bronchiolitis wheeze subsides during school age.

There is no effective treatment available for RSV bronchiolitis. Antiviral treatment, bronchodilators, and glucocorticosteroids do not alter the course of disease (van Woensel and Kimpen, 2000). To date, no safe and effective vaccine

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is available. Prevention or attenuation of RSV disease in high-risk children can be achieved using palivizumab, a humanized monoclonal antibody against the RSV F-protein. Monthly intramuscular administration of palivizumab in preterm children and children with congenital heart disease results in a 50% reduction of hospitalization for RSV bronchiolitis (Feltes et al., 2003; The IMpact-RSV study group, 1998). However, the majority of hospitalized children do not have a known risk factor for RSV bronchiolitis. Identifying children with increased risk for severe RSV infection could aid targeting prophylactic strategies, including the administration of antibodies. In addition, delineating pre-existing determinants of severe RSV infection may contribute to our understanding of the pathogenesis of disease. This chapter reviews currently available evidence about the pathogenesis of RSV bronchiolitis. Better knowledge of the mechanisms underlying RSV bronchiolitis is required for the development of effective treatment strategies and a safe and effective vaccine.

2 Genetic Determinants of RSV Bronchiolitis and Post-bronchiolitis Wheeze

There may be different reasons to perform genetic studies in children with RSV bronchiolitis. Identification of genetic risk factors might contribute to more accurate identification of children at high risk for RSV bronchiolitis. Although the heritability of RSV bronchiolitis is not known, it is probably modest compared to, for example, allergy. Therefore, it is unlikely that individual genetic risk assessment will be of direct clinical relevance. More important is the contribution of genetic studies to our understanding of the pathogenesis of RSV bronchiolitis. In the absence of a clear aetiology, genetic studies can provide insight in the underlying mechanisms of RSV bronchiolitis. In particular, pathway analysis of immunological genes may be indicative of relevant immunological mechanisms underlying the pathogenesis of RSV bronchiolitis.

A large number of studies have attempted to identify genetic risk factors for severe RSV bronchiolitis (Table 1). These studies have not attempted to identify

Table 1 Genetic polymorphisms associated with risk of RSV bronchiolitis

Innate immunity	Adaptive immunity
TLR-4	IL-4
CD14	IL-4Ra
IL-8	IL-10
Surfactants A, B, D	FCER1A
CCR5	
VDR	
JUN	
IFNA5	
NOS2A	

genes facilitating infection itself, since virtually all children are infected with the virus. Practically all genetic studies have focused on single nucleotide polymorphisms (SNP) of genes involved in the immune and pulmonary systems. First, asthma genes have been studied in relation to RSV bronchiolitis. Hull described a functional SNP of the IL-8 gene as the first gene related to the risk of RSV bronchiolitis (Hull et al., 2000). Second, SNPs implicated in the pathogenesis of allergy have been studied in relation to RSV. Several studies identified the IL4/IL4R pathway to be related to the risk of RSV bronchiolitis (Choi et al., 2002; Hoebee et al., 2003; Puthothu et al., 2006b). Third, genes from the innate immune system have been studied in relation to the risk of RSV bronchiolitis. Particular attention has been paid to the potential role of the CD14/Toll-like receptor (TLR) 4 complex (Tal et al., 2004; Puthothu et al., 2006a; Inoue et al., 2007). *TLR4* was a likely candidate gene, since this receptor was identified as a putative receptor for RSV. Tal was the first to associate the Asp299Gly and Thr399Ile TLR4 polymorphism with an increased risk of RSV bronchiolitis. For preterm infants, odds ratios of 69:99 for severe RSV bronchiolitis was described for the Asp299Gly and Thr399Ile TLR SNPs, respectively (Awomoyi et al., 2007). Confirmation of these odds ratios is required before they can be used for clinical purposes, such as targeting RSV prophylaxis. Our group recently published the most extensive genetic study reported to date (Janssen et al., 2007). A candidate gene approach was used to select 220 genes from five categories (adaptive immunity, mucosal immunity, innate immunity, chemotaxis, and allergic asthma). In a group of 470 children, 922 parents and 1030 controls, 347 genetic polymorphisms were studied using the Illumina technology. Most genetic polymorphisms identified to be related to RSV bronchiolitis were in genes coding for proteins involved in the innate immune system. Interestingly, most genetic studies have highlighted the innate immune system as a potentially important player in the pathogenesis of RSV bronchiolitis.

Two studies have analysed genetic determinants of post-bronchiolitis wheeze. In a large follow-up study, the IL-8 polymorphism, which had also been associated with risk of RSV bronchiolitis, appeared to predict wheeze during later childhood (Goetghebuer et al., 2004). More recently, we analysed a number of SNPs in relation to recurrent wheeze at age 3 and 6 following RSV bronchiolitis (Ermers et al., 2007). We established an association between SNPs in the 'allergy genes' for IL-13 and IL4 with recurrent wheeze at age 6, but not at age 3. This study suggests that early and late wheeze following RSV bronchiolitis may be distinct entities.

Comparing genetic studies has important limitations. Different case definitions may be used: age range, previous history of airway morbidity, and severity of RSV bronchiolitis are criteria which are not uniformly applied by different research groups. Given genetic differences between racial groups, comparing studies performed in different places can be problematic. In fact, this may partially explain why many positive findings in the literature still await confirmation. Finally, statistical analysis becomes increasingly complex as more genes are studied simultaneously. Use of differing statistical techniques may

confuse things further. While acknowledging these limitations, it is clear that genetic studies have raised several fascinating new hypotheses, which may eventually lead to a better understanding of the pathogenesis of RSV bronchiolitis.

3 Pre-existent Airway Morbidity

Lung function at birth has been associated with subsequent development of lower respiratory tract illness. Several prospective studies have now supported the concept that childhood wheeze is preceded by lung function abnormalities and, probably, airway hyperresponsiveness. These include the Tucson birth cohort study, the Perth birth cohort and the Norwegian ORAACLE study (Haland et al., 2006; Martinez et al., 1988). Increased airway resistance before any airway symptoms occur is predictive of later recurrent episodes of wheeze and asthma. However, it is of interest that this well-accepted phenomenon has not been demonstrated for RSV bronchiolitis. There is no evidence that the mechanism underlying RSV bronchiolitis differs from other wheezing diseases during infancy. Hence, it can be anticipated that the relationship between infant lung function and RSV bronchiolitis will be confirmed in future studies.

4 Immature Immune Responses

Severe RSV bronchiolitis is a typical disease of infancy. During later childhood and adulthood RSV reinfection occurs frequently, but no severe disease develops. It is tempting to speculate that disease severity is modified by the presence of neutralizing antibodies or specific cytotoxic T cells. However, this explanation is not sufficient. Severe RSV infection can occur in children with high titers of RSV-specific antibodies. In addition, there is no conclusive evidence that specific T cells provide any protection against reinfection. Our research group has speculated that an immature, weak immune response during infancy is a key factor in the pathogenesis of severe RSV infection (Bont and Kimpen, 2002). There is clear evidence that both the innate and the adaptive immune systems are immature at birth (Adkins et al., 2004; Levy, 2007). Neonatal T cells do not mount adult-like cytokine responses in vitro. Although recent studies show that these T cells can be brought to adult-like responses given the right conditions, they are still considered immature. This is most pronounced for Th1 cells, required for cell-mediated immune responses. Studies in patients with severe RSV bronchiolitis show a profound decrease in local interferon (IFN)γ production, which is associated with disease severity (Bont et al., 2001). In the peripheral blood, several investigators have reported similar findings (Aberle et al., 1999; Bendelja et al., 2000; Renzi et al., 1999). It can be hypothesized that a weak cell-mediated immune response allows abundant viral replication and

severe RSV bronchiolitis. The COAST birth cohort study has shown that low IFNγ in cord blood is predictive of infant wheeze and viral respiratory tract infection during the first year of life (Copenhaver et al., 2004; Gern et al., 2006). However, for RSV bronchiolitis this concept has not been proven.

The innate immune system was originally thought to be at full strength at birth. However, recent evidence clearly demonstrates immaturity of the innate immune system in the healthy neonate. Although the relevance of this finding is not yet fully understood, it can be speculated that immaturity of the innate immune system facilitates severe disease in the case of RSV infection. Apoptosis of neutrophilic granulocytes in cord blood is delayed (Molloy et al., 2005; Koenig et al., 2005). Since the neutrophil is the dominant cell type in the airways during RSV infection, prolonged granulocyte survival could contribute to enhanced RSV-induced pathology. In addition, practically all TLR systems are functionally impaired in healthy neonates (Levy et al., 2004; Levy, 2005; Levy et al., 2006). Immature TLR7 and 9-mediated interferon production by plasmacytoid dendritic cells (pDCs) may allow persistence of RSV in the airways (Schlender et al., 2005). Immature TLR3 responses may facilitate increased IL-13-mediated mucus production during RSV infection (Rudd et al., 2006). Taken together, immaturity of both adaptive and innate immune responses may interfere with the development of protective antiviral immune responses.

5 Immunopathogenesis

The immunopathogenesis of RSV bronchiolitis is still poorly understood. The dual role of the adaptive immune response has been subject of ongoing controversy (Graham et al., 2000). There is evidence that T cells enhance disease. In particular, murine studies show that specific T cells are required for pathology and disease severity. Original studies by Graham showed that T-cell depletion before infection prevents disease without decreasing viral titers (Graham et al., 1988; Graham et al., 1991). At the same time, a study by Cannon showed that adoptively transferred CTLs enhance disease upon challenge in irradiated mice (Cannon et al., 1988). The relevance of these animal studies for RSV infection in infants is not clear. The most important evidence that human RSV infection is an immune-mediated disease resulted from the disastrous vaccine trial in the 1960s. In this trial, a formalin-inactivated viral vaccine did not prevent subsequent natural infection. Moreover, the course of disease in cases of subsequent natural infection was more severe, even resulting in mortality. However, it is not known whether the immunopathogenesis of vaccine-enhanced disease and natural RSV bronchiolitis are similar. In fact, later studies also suggested that CD8 + T cells can protect against RSV-induced disease, in both mice and humans (Peebles Jr. and Graham, 2005; Chang and Braciale, 2002). Severe RSV infection in infants is characterized by low levels of cell-mediated immune responses, including IL-12 and IFNγ production (Bont et al., 2001; Bont et al., 2000b). During RSV infection,

the number of RSV-specific T cells in the airways of infants is extremely low (unpublished data). Taken together, it appears that there are coexisting protective and disease-enhancing adaptive immune responses. Current research attempts to unravel this apparent conundrum.

Both pDC and myeloid dendritic cells (mDC) have been suggested to play protective roles in the pathogenesis of RSV infection (Wang et al., 2006). Dendritic cells (DCs) can be productively infected with RSV. In addition, increased frequency of both cell types is found during RSV infection of both human and experimentally infected mice. *In vitro* RSV infection of pDC results in decreased TLR7 and TLR9-mediated production of type I interferons (Schlender et al., 2005; Schlender et al., 2000). This suppressive effect on pDCs is primarily mediated by the non-structural (NS) proteins 1 and 2. Studies in the murine model clearly suggest that pDCs are protective against disease by regulating T-cell-induced pathology. Depletion of pDCs resulted in delayed viral clearance and enhanced pathology. Myeloid DCs are the most potent antigen-presenting cells in the airways. Similar to pDCs, RSV infection of mDCs results in functional impairment of mDCs. Productive RSV infection of mDCs decreases the capacity to activate T cells by a soluble mediator (de Graaff et al., 2005). These data imply that both types of DCs provide protection against disease during RSV infection and that RSV has powerful mechanisms to interfere with immune responses initiated by DCs.

6 Parallel Versus Serial Hypothesis

The pathogenesis of RSV bronchiolitis can be described by two alternative potential mechanisms (Fig. 1). The parallel hypothesis presumes pre-existent susceptibility for chronic airway morbidity. In this case, RSV infection is the first indication of long-term respiratory morbidity. The parallel hypothesis is supported by the relation between RSV infection and genetic factors. Indirect evidence of a relationship between pre-existent immature immune responses or abnormal lung function further supports the parallel hypothesis. The serial

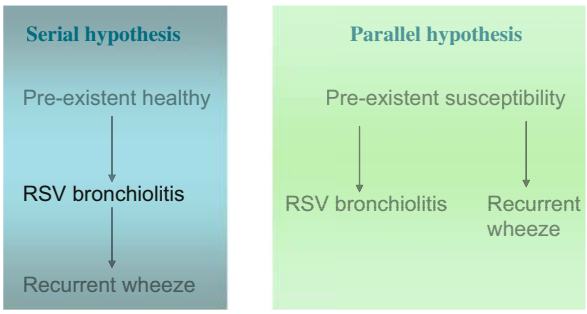

Fig. 1 Serial versus parallel hypothesis

hypothesis presumes pre-existent health and that RSV infection entirely causes disease. The serial hypothesis is supported by the epidemiological evidence that the majority of children with severe RSV infection are apparently healthy term babies until infection with RSV occurs. *In vitro* and *in vivo* studies clearly show that RSV has a strong cytopathic effect on airway epithelium (Garofalo et al., 1996; Johnson et al., 2007). Animal models suggest that an augmented immune response contributes to RSV-induced inflammation of the airways and destruction of the epithelium (Peebles Jr. and Graham, 2005; Openshaw, 1995). According to the serial hypothesis, post-bronchiolitis wheeze is the direct consequence of RSV infection. Evidence provided in this review shows that the mechanisms proposed by both these alternative hypotheses may coexist. However, the relative contribution of pre-existent factors determining susceptibility and RSV-mediated pathology to disease severity is not yet clear.

7 Conclusions

RSV bronchiolitis is the most common cause of airway morbidity during infancy in the winter season. Susceptibility to severe disease is determined by genetic factors, abnormal lung function at birth, and immature immune responses. The immunopathogenesis of RSV bronchiolitis is still poorly understood, and the controversial role of T cells remains intriguing. Probably, the nature of the T-cell response depends on the innate immune response initiated by the virus. Recent genetic and immunological studies have implicated the innate immune system, including DCs, as a key player in the pathogenesis of RSV infection.

References

Aberle, J. H., Aberle, S. W., Dworzak, M. N., Mandl, C. W., Rebhandl, W., Vollnhofer, G., Kundi, M., and Popow-Kraupp, T. (1999). Reduced Interferon-gamma expression in peripheral blood mononuclear cells of infants with severe respiratory syncytial virus disease. Am. J. Respir. Crit. Care Med. 160, 1263–1268.

Adkins, B., Leclerc, C., and Marshall-Clarke, S. (2004). Neonatal adaptive immunity comes of age. Nat. Rev. Immunol. 4, 553–564.

Awomoyi, A. A., Rallabhandi, P., Pollin, T. I., Lorenz, E., Sztein, M. B., Boukhvalova, M. S., Hemming, V. G., Blanco, J. C., and Vogel, S. N. (2007). Association of TLR4 Polymorphisms with Symptomatic Respiratory Syncytial Virus Infection in High-Risk Infants and Young Children. J. Immunol. 179, 3171–3177.

Bendelja, K., Gagro, A., Bace, A., Lokar-Kolbas, R., Krsulovic-Hresic, V., Drazenovic, V., Mlinaric-Galinovic, G., and Rabatic, S. (2000). Predominant type-2 response in infants with respiratory syncytial virus (RSV) infection demonstrated by cytokine flow cytometry. Clin. Exp. Immunol. 121, 332–338.

Bloemers, B., Furth v, A. M., Weijerman, M., Broers, C., Gemke, R. J. B. J., Strengers, J. L. M., Kimpen, J. L. L., and Bont, L. (2007). Increased incidence of respiratory syncytial virus lower respiratory hospitalisation in children with Down syndrome. Pediatrics. 121(4), e1076–1081.

Bont, L., Aalderen, v. W. M. C., and Kimpen, J. L. L. (2000). Long-term consequences of respiratory syncytial virus (RSV) bronchiolitis. Paediatr. Respir. Rev. 1, 221–227.

Bont, L., Heijnen, C. J., Kavelaars, A., van Aalderen, W. M., Brus, F., Draaisma, J. M., Pekelharing-Berghuis, M., Diemen-Steenvoorde, R. A., and Kimpen, J. L. (2001). Local interferon-gamma levels during respiratory syncytial virus lower respiratory tract infection are associated with disease severity. J. Infect. Dis. 184, 355–358.

Bont, L., Kavelaars, A., Heijnen, C. J., van Vught, A. J., and Kimpen, J. L. (2000b). Monocyte interleukin-12 production is inversely related to duration of respiratory failure in respiratory syncytial virus bronchiolitis. J. Infect. Dis. 181, 1772–1775.

Bont, L. and Kimpen, J. L. (2002). Immunological mechanisms of severe respiratory syncytial virus bronchiolitis. Intensive Care Med. 28, 616–621.

Cannon, M. J., Openshaw, P. J. M., and Askonas, B. A. (1988). Cytotoxic T cells clear virus but augment lung pathology in mice infected with respiratory syncytial virus. J. Exp. Med. 168, 1163–1168.

Chang, J. and Braciale, T. J. (2002). Respiratory syncytial virus infection suppresses lung CD8+ T-cell effector activity and peripheral CD8+ T-cell memory in the respiratory tract. Nat. Med. 8, 54–60.

Choi, E. H., Lee, H. J., Yoo, T., and Chanock, S. J. (2002). A common haplotype of interleukin-4 gene IL4 is associated with severe respiratory syncytial virus disease in Korean children. J. Infect. Dis. 186, 1207–1211.

Copenhaver, C. C., Gern, J. E., Li, Z., Shult, P. A., Rosenthal, L. A., Mikus, L. D., Kirk, C. J., Roberg, K. A., Anderson, E. L., Tisler, C. J., DaSilva, D. F., Hiemke, H. J., Gentile, K., Gangnon, R. E., and Lemanske, R. F., Jr. (2004). Cytokine response patterns, exposure to viruses, and respiratory infections in the first year of life. Am J Respir. Crit Care Med. 170, 175–180.

de Graaff, P. M., de Jong, E. C., van Capel, T. M., van Dijk, M. E., Roholl, P. J., Boes, J., Luytjes, W., Kimpen, J. L., and van Bleek, G. M. (2005). Respiratory syncytial virus infection of monocyte-derived dendritic cells decreases their capacity to activate CD4 T cells. J. Immunol. 175, 5904–5911.

Ermers, M. J., Hoebee, B., Hodemaekers, H. M., Kimman, T. G., Kimpen, J. L., and Bont, L. (2007). IL-13 genetic polymorphism identifies children with late wheezing after respiratory syncytial virus infection. J. Allergy Clin. Immunol. 119(5), 1086–1091.

Feltes, T. F., Cabalka, A. K., Meissner, H. C., Piazza, F. M., Carlin, D. A., Top, F. H., Jr., Connor, E. M., and Sondheimer, H. M. (2003). Palivizumab prophylaxis reduces hospitalization due to respiratory syncytial virus in young children with hemodynamically significant congenital heart disease. J. Pediatr. 143, 532–540.

Garofalo, R. P., Mei, F., Espejeo, R., Ye, G., Haeberle, H., Baron, S., Ogra, P. L., and Reyes, V. E. (1996). Respiratory syncytial virus infection of human respiratory epithelial cells up-regulates class 1 MHC Expression through the induction of IFN-beta and IL-1-alpha. J. Immunol. 157, 2506–2513.

Gern, J. E., Brooks, G. D., Meyer, P., Chang, A., Shen, K., Evans, M. D., Tisler, C., Dasilva, D., Roberg, K. A., Mikus, L. D., Rosenthal, L. A., Kirk, C. J., Shult, P. A., Bhattacharya, A., Li, Z., Gangnon, R., and Lemanske, R. F., Jr. (2006). Bidirectional interactions between viral respiratory illnesses and cytokine responses in the first year of life. J Allergy Clin. Immunol. 117, 72–78.

Goetghebuer, T., Isles, K., Moore, C., Thomson, A., Kwiatkowski, D., and Hull, J. (2004). Genetic predisposition to wheeze following respiratory syncytial virus bronchiolitis. Clin. Exp. Allergy 34, 801–803.

Graham, B. S., Bunton, L. A., Wright, P. F., and Karzon, D. T. (1991). Role of T lymphocyte subsets in the pathogenesis of primary infection and rechallenge with respiratory syncytial virus in mice. J. Clin. Invest. 88, 1026–1033.

Graham, B. S., Johnson, T. R., and Peebles, R. S. (2000). Immune-mediated disease pathogenesis in respiratory syncytial virus infection. Immunopharmacology 48, 237–247.

Graham, B. S., Perkins, M. D., Wright, P. F., and Karzon, D. T. (1988). Primary respiratory syncytial virus infection in mice. J. Med. Virol. 26, 153–162.

Haland, G., Carlsen, K. C., Sandvik, L., Devulapalli, C. S., Munthe-Kaas, M. C., Pettersen, M., and Carlsen, K. H. (2006). Reduced lung function at birth and the risk of asthma at 10 years of age. N. Engl. J. Med. 355, 1682–1689.

Hoebee, B., Rietveld, E., Bont, L., Oosten, M., Hodemaekers, H. M., Nagelkerke, N. J., Neijens, H. J., Kimpen, J. L., and Kimman, T. G. (2003). Association of severe respiratory syncytial virus bronchiolitis with interleukin-4 and interleukin-4 receptor alpha polymorphisms. J. Infect. Dis. 187, 2–11.

Hull, J., Thomson, A., and Kwiatkowski, D. (2000). Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. Thorax 55, 1023–1027.

Inoue, Y., Shimojo, N., Suzuki, Y., Campos Alberto, E. J., Yamaide, A., Suzuki, S., Arima, T., Matsuura, T., Tomiita, M., Aoyagi, M., Hoshioka, A., Honda, A., Hata, A., and Kohno, Y. (2007). CD14 -550 C/T, which is related to the serum level of soluble CD14, is associated with the development of respiratory syncytial virus bronchiolitis in the Japanese population. J. Infect. Dis. 195, 1618–1624.

Janssen, R., Bont, L., Siezen, C. L., Hodemaekers, H. M., Ermers, M. J., Doornbos, G., van't, S. R., Wijmenga, C., Goeman, J. J., Kimpen, J. L., van Houwelingen, H. C., Kimman, T. G., and Hoebee, B. (2007). Genetic susceptibility to respiratory syncytial virus bronchiolitis is predominantly associated with innate immune genes. J. Infect. Dis. 196, 826–834.

Johnson, J. E., Gonzales, R. A., Olson, S. J., Wright, P. F., and Graham, B. S. (2007). The histopathology of fatal untreated human respiratory syncytial virus infection. Mod. Pathol. 20, 108–119.

Koenig, J. M., Stegner, J. J., Schmeck, A. C., Saxonhouse, M. A., and Kenigsberg, L. E. (2005). Neonatal neutrophils with prolonged survival exhibit enhanced inflammatory and cytotoxic responsiveness. Pediatr. Res. 57, 424–429.

Levy, O. (2005). Innate immunity of the human newborn: distinct cytokine responses to LPS and other Toll-like receptor agonists. J. Endotoxin. Res. 11, 113–116.

Levy, O. (2007). Innate immunity of the newborn: basic mechanisms and clinical correlates. Nat. Rev. Immunol. 7, 379–390.

Levy, O., Coughlin, M., Cronstein, B. N., Roy, R. M., Desai, A., and Wessels, M. R. (2006). The adenosine system selectively inhibits TLR-mediated TNF-alpha production in the human newborn. J. Immunol. 177, 1956–1966.

Levy, O., Zarember, K. A., Roy, R. M., Cywes, C., Godowski, P. J., and Wessels, M. R. (2004). Selective impairment of TLR-mediated innate immunity in human newborns: neonatal blood plasma reduces monocyte TNF-alpha induction by bacterial lipopeptides, lipopolysaccharide, and imiquimod, but preserves the response to R-848. J. Immunol. 173, 4627–4634.

Martinez, F. D., Morgan, W. J., Wright, A. L., Holberg, C. J., and Taussig, L. M. (1988). Diminished lung function as a predisposing factor for wheezing respiratory illness in infants. N. Engl. J. Med. 319, 1112–1117.

Molloy, E. J., O'Neill, A. J., Grantham, J. J., Sheridan-Pereira, M., Fitzpatrick, J. M., Webb, D. W., and Watson, R. W. (2005). Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor have differential effects on neonatal and adult neutrophil survival and function. Pediatr. Res. 57, 806–812.

Openshaw, P. J. M. (1995). Immunopathological mechanisms in respiratory syncytial virus disease. Springer Semin Immunopathol. 17, 187–201.

Peebles, R. S., Jr. and Graham, B. S. (2005). Pathogenesis of respiratory syncytial virus infection in the murine model. Proc. Am. Thorac. Soc. 2, 110–115.

Puthothu, B., Forster, J., Heinzmann, A., and Krueger, M. (2006a). TLR-4 and CD14 polymorphisms in respiratory syncytial virus associated disease. Dis. Markers 22, 303–308.

Puthothu, B., Krueger, M., Forster, J., and Heinzmann, A. (2006b). Association between severe respiratory syncytial virus infection and IL13/IL4 haplotypes. J. Infect. Dis. 193, 438–441.

Renzi, P. M., Turgeon, J. P., Marcotte, J. E., Drblik, S. P., Berube, D., Gagnon, M. F., and Spier, S. (1999). Reduced interferon-gamma production in infants with bronchiolitis and asthma. Am. J. Respir. Crit Care Med. 159, 1417–1422.

Rudd, B. D., Smit, J. J., Flavell, R. A., Alexopoulou, L., Schaller, M. A., Gruber, A., Berlin, A. A., and Lukacs, N. W. (2006). Deletion of TLR3 alters the pulmonary immune environment and mucus production during respiratory syncytial virus infection. J. Immunol. 176, 1937–1942.

Schlender, J., Bossert, B., Buchholz, U., and Conzelmann, K. K. (2000). Bovine respiratory syncytial virus nonstructural proteins NS1 and NS2 cooperatively antagonize alpha/beta interferon-induced antiviral response. J. Virol. 74, 8234–8242.

Schlender, J., Hornung, V., Finke, S., Gunthner-Biller, M., Marozin, S., Brzozka, K., Moghim, S., Endres, S., Hartmann, G., and Conzelmann, K. K. (2005). Inhibition of toll-like receptor 7- and 9-mediated alpha/beta interferon production in human plasmacytoid dendritic cells by respiratory syncytial virus and measles virus. J. Virol. 79, 5507–5515.

Simoes, E. A. F. (1999). Respiratory syncytial virus infection. Lancet 354, 847–852.

Smyth, R. L. and Openshaw, P. J. (2006). Bronchiolitis. Lancet 368, 312–322.

Stein, R. T., Sherill, D., Morgan, W. J., Holberg, C. J., Halonen, M., Taussig, L. M., Wright, A. L., and Martinez, F. D. (1999). Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13. Lancet 354, 541–545.

Tal, G., Mandelberg, A., Dalal, I., Cesar, K., Somekh, E., Tal, A., Oron, A., Itskovich, S., Ballin, A., Houri, S., Beigelman, A., Lider, O., Rechavi, G., and Amariglio, N. (2004). Association between common Toll-like receptor 4 mutations and severe respiratory syncytial virus disease. J. Infect. Dis. 189, 2057–2063.

The IMpact-RSV study group (1998). Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. Pediatrics 102, 531–537.

van Woensel, J. and Kimpen, J. (2000). Therapy for respiratory tract infections caused by respiratory syncytial virus. Eur. J. Pediatr. 159, 391–398.

Wang, H., Peters, N., and Schwarze, J. (2006). Plasmacytoid dendritic cells limit viral replication, pulmonary inflammation, and airway hyperresponsiveness in respiratory syncytial virus infection. J. Immunol. 177, 6263–6270.

Yusuf, S., Piedimonte, G., Auais, A., Demmler, G., Krishnan, S., VAN, C. P., Singleton, R., Broor, S., Parveen, S., Avendano, L., Parra, J., Chavez-Bueno, S., DE Sierra, T. M., Simoes, E. A., Shaha, S., and Welliver, R. (2007). The relationship of meteorological conditions to the epidemic activity of respiratory syncytial virus. Epidemiol. Infect. 135, 1077–1090.

How Can We Eradicate Pertussis

James D. Cherry

1 Introduction

In comparison with other vaccine-preventable diseases, pertussis presents many unique challenges (see Cherry and Heininger, in press). Pertussis occurs in all age groups and immunity following natural disease or vaccination is not long lasting.

2 *Bordetella pertussis:* The Organism and Its Pathogenesis

Since its first isolation in 1906 *Bordetella pertussis* has fascinated microbiologists because it contains many toxins and surface proteins (adhesins), antibody to which might be important in protection (see Mattoo and Cherry, 2005). In general, steps in bacteria pathogenesis involve the following: entry into the host and attachment; evasion or disruption of host defenses; development of damage at the infection site; and establishment of systematic disease by dissemination of organisms or their products. Presented in Table 1 are selected toxins and surface proteins of *B. pertussis*.

Pertussis toxin (PT) is a classic A–B toxin with the smaller A subunit being the toxin (an ADP- ribosylating toxin) and a larger B subunit being an adhesin (see Mattoo and Cherry 2005). In the past it was suggested that pertussis was similar to diphtheria in regard to pathogenesis. In diphtheria, neutralizing toxin, as occurs with active immunization with diphtheria toxoid, prevents disease. This suggests that pertussis might be controlled by toxoiding PT in a vaccine. However, this is not the case in spite of the fact that many people still believe this theory. Pertussis is a much more complicated disease. The severity of pertussis suggests that other toxins and adhesins are of more importance in pathogenesis. For example, we know that PT does not cause the cough in pertussis since an identical cough occurs with infection with *B. parapertussis*

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Table 1 Selected virulence factors of *Bordetella pertussis*

Toxins
Pertussis toxin (PT)
Adenylate cyclase toxin (ACT)
Dermonecrotic Toxin (DNT)
Tracheal cytotoxin (TCT)
Lipopolysaccharide (LPS)
Adhesins
Filamentous hemagglutinin (FHA)
Pertactin (PRN), BrkA, Vag8, Tracheal colonization factor
Fimbriae (FIM)

and this organism does not liberate PT. During infection PT causes leukocytosis with absolute lymphocytosis and it contributes to severity of disease as indicated by the fact that pertussis due to *B. pertussis* is more severe than pertussis due to the *B. parapertussis*. Adenylate cyclase toxin intoxicates neutrophils and this allows attachment to continue. Dermonecritic toxin and tracheal cytotoxin, particularly the latter, cause damage to ciliated epithelial cells and therefore contribute to the disease process. However, since the cough continues long after repair of ciliated epithelial cells occurs it is likely that there is another toxin or toxins which as yet are unknown that are responsible for the cough in *B. pertussis* infections.

Lipopolysaccharide is a universal component of Gram negative bacilli and its major significance is that it is a component of whole cell pertussis vaccines (DTP vaccines) and the cause of a number of reactions following DTP vaccine administration.

As noted above, the B-subunit of PT is an adhesin and antibody to this contributes to protection. From mouse model studies it was suggested in the past that filamentous hemagglutinin (FHA) was the most important adhesin. However, this is unlikely to be so because FHA is secreted from the cell so that rather than functioning as an adhesin it seems to function more as a decoy for antibody. There are a group of outer-membrane proteins of which pertactin (PRN) is the one about which the most is known. As will be discussed later, antibody to PRN is most important in protection. Many Gram negative bacteria use fimbriae for attachment. In *B. pertussis* fimbriae play a minor role in the initial attachment but play a role in continued attachment once infection has occurred.

3 Clinical Disease

Typical (classic) pertussis is a three-stage illness (catarrhal, paroxysmal, and convalescent) that lasts 4–12 weeks (see Cherry and Heininger, in press). The specific manifestations include: paroxysmal cough, lack of fever, no systemic

illness, coryza but no significant pharyngitis, posttussive vomiting, posttussive whoop, and absolute lymphocytosis. Absolute lymphocytosis occurs in most primary cases. Once a person has received vaccine or had an infection, lymphocytosis does not occur with subsequent infections. This absolute lymphocytosis is due to PT and once a person has been primed, they recall antibody to PT so rapidly that absolute lymphocytosis does not occur. Many textbooks descriptions of pertussis refer only to classic pertussis. However, in a study in Germany by our group, of children with coughs who were cultured, regardless of whether the physicians thought the children had *B. pertussis* infections, it was found that 38% coughed for 28 days or less and 17% coughed for 21 days or less (see Heininger et al. 1997). These were mild cases and these types of cases are frequently overlooked as being pertussis but are important as they are still contagious to others.

3.1 Pertussis in Young Infants

Pertussis in young infants may be particularly severe and results in significant mortality. Infants, who die, frequently have pulmonary hypertension which is not responsive to conventional treatment measures. These infants have extremely high white blood cell counts with absolute lymphocytosis (>50,000 WBC/mm^3) (see Cherry and Heininger, in press). In the present era studies have indicated the source of pertussis in young infants is a family member in about 75% of cases, mothers being the most common source (see Bisgard et al. 2004). Also of note is that the majority of the family member-source patients are adults, so that immunization programs that target adolescents but omit adults will not be successful.

3.2 Adults

A significant proportion of adults who have pertussis have typical, classic disease with paroxysms, posttussive apnea, posttussive vomiting, and whoop (see Schmitt-Grohè et al. 1995; Cherry and Heininger, in press). Adults also have unique sweating episodes between coughing paroxysms. Complications in adults include: sinusitis, otitis media, urinary incontinence, pneumonia, weight loss, rib fracture, and fainting. Although these adults with typical illness should be easy to diagnose, physicians who care for adults are not familiar with pertussis and therefore the diagnosis is frequently missed. A prominent manifestation of adult pertussis is a 'choking sensation'. An important feature in the diagnosis is that the cough is nonproductive. A larger proportion of adult pertussis patients have disease that is difficult to diagnose clinically. In a study by our group performed in UCLA students with prolonged cough those with pertussis had similar symptoms to those without pertussis (see Mink et al.

1992). The only exceptions were that patients without pertussis were more likely to be prescribed antibiotics at the time of the first clinic visit and their coughs were more likely to be productive.

4 Laboratory Diagnosis of *B. pertussis* Infection

4.1 Culture

The prevailing opinion is that culture has low sensitivity in the diagnosis of pertussis. However, in children within 2 weeks of the onset of cough the sensitivity is ~80% (see Cherry and Heininger, in press). To be successful a specimen for culture must come directly from the ciliated epithelial cells of the nasal pharynx. The main error when using nasal pharyngeal swabs is not inserting the swab so that it touches the ciliated epithelial cells. Nasal aspirates using a feeding tube, wall suction, and a DeLee trap are the best way to attain specimens for culture or for polymerase chain reaction (PCR) (see Hallander et al. 1993). The main reason for culture failure, when the clinical specimen is obtained properly, is collection of the specimen too late in the illness. This is particularly important in adolescents and adults since they rarely seek care until the third or the fourth week of illness – a time when a culture or PCR is unlikely to be positive.

4.2 Direct Detection of B. pertussis

In the past, direct fluorescent antibody testing (DFA) was commonly used. This test lacks sensitivity and specificity (see Cherry and Heininger, in press). Today, PCR on nasopharyngeal secretions is the primary mode of diagnosing *B. pertussis* infection in many laboratories throughout the world. PCR is clearly more sensitive than culture and is particularly useful when a patient has received prior antibiotic treatment, although false positive results are a major problem. These usually occur not only as a result of contamination in the PCR laboratory but also because of the presence of *B. pertussis* DNA in the air in treatment rooms in which patients with pertussis were present. The major shortcoming of PCR in regard to diagnosing pertussis in adolescents and adults is the fact that they tend to seek care relatively late in their illness so that the likelihood of a positive test is relatively small.

4.3 Serologic Diagnosis of B. pertussis *Infection*

In a study in Germany undertaken by our group, 64 adults had laboratory evidence of *B. pertussis* infection, 57 (89%) of whom were diagnosed serologically (see Schmitt-Grohè et al. 1995). In the various efficacy trials carried out in

the early 1990s, the demonstration of a significant antibody titer change using various *B. pertussis* antigens with ELISA had high precision. Since adolescents and adults often present late in their illness, demonstration of a titer rise may not be possible. However, it has been clearly demonstrated that pertussis can be accurately diagnosed in adolescents and adults by the demonstration of a high single-serum titer to PT. Cutoff values can be set so that both sensitivity and specificity are high (see Mink et al. 1992).

5 Epidemiology

The epidemiology of reported pertussis is different from the epidemiology *B. pertussis* infection. (see Cherry 2005; Cherry 2006).

5.1 Reported Pertussis

Presented in Table 2 is the mean annual number of pertussis cases per 100,000 populations by an European country between 1998 and 2002 (see Celentano et al. 2005). As can be seen, considerable variation is noted. For example, in Switzerland and the three bordering countries (Germany, Italy, and Austria) the rates of reported pertussis varied by as much as 70-fold. In Switzerland, the rate was 123.9 per 100,000; in Germany, it was 10.1 per 100,000; in Italy, it was 6.1 per 100,000; and in Austria, 1.8 per 100,000. Since immunization practices in the four countries are somewhat similar and since there is close proximity of the

Table 2 Mean annual number of pertussis cases per 100,000 population by country, 1998–2000

Country	Total	<1 year
Switzerland	123.9	1039.9
Norway	57.1	172.5
The Netherlands	32.7	117.8
Sweden	22.3	71.2
Germany	10.1	32.7
Italy	6.1	104.1
Ireland	4.5	–
Malta	3.7	4.0
Iceland	3.4	155.0
Austria	1.8	–
Spain	0.7	23.0
Greece	0.5	8.8
England, Wales, and Northern Ireland	0.5	30.9
Portugal	0.1	6.2
Denmark	–	253.1

Table 3 Possible reasons for the resurgence of reported pertussis

1. Genetic changes in *B. pertussis*
2. Lessened potency of pertussis vaccines
3. Waning of vaccine-induced immunity
4. Greater awareness of pertussis
5. The general availability of better laboratory tests

countries, it is likely that these differences are explained by the rigorousness of the retrospective surveillance programs and do not represent a unique epidemiology by country.

In the prevaccine era in the United States, pertussis was a universally present disease with cyclic peaks every 2–5 years (see Cherry 2005; Cherry 2006; Cherry and Heininger, in press). In this era, reported cases occurred almost exclusively in unvaccinated children (>93% of cases occurred in children less than 10 years of age). In the 1970s when pertussis had been brought under control and there were only 1000–2000 cases per year reported, 50% of the cases were noted in infants. This is in contrast to the prevaccine era when only about 10% of the reported cases were in infants. In recent years, 65% of reported cases are in persons greater than 10 years of age. In the United States, immunization changed the reported pertussis rate from 157 per 100,000 in the prevaccine era to less than 1 per 100,000 in the 1970s. Since 1984, there has been a modest increase in reported pertussis (from 1 to 9 cases per 100,000). Importantly, in the vaccine era, the cyclic peaks of reported pertussis still occur at 2–5 year intervals. Possible reasons for the resurgence of reported pertussis in the United States and in a number of other countries are listed in Table 3. Of most importance relating to this increase is greater awareness of pertussis resulting from numerous publications in the 1990s relating to the vaccine-efficacy studies. Also contributing to this resurgence is, in some areas, the general availability of better laboratory tests. Although genetic changes in *B. pertussis* have been noted to occur, there is no evidence that these genetic changes have led to an increase in vaccine failures. Waning vaccine-induced immunity has always occurred so this should not cause an increase in reported pertussis. However, many current vaccines are of lower potency than DTP vaccines of the past and, therefore, waning immunity could have contributed to the problem. This is probably the case particularly in relation to outbreaks in middle school children since in general acellular pertussis vaccines (DTaP vaccines) are not as good as good whole cell vaccines (DTP vaccines).

5.2 *Epidemiology of* B. pertussis *Infections*

Despite the fact that reported pertussis is only the 'tip of the iceberg', it is clear that the cyclic disease pattern occurs and that this pattern has continued in the vaccine era. When considering the success of measles immunization in the

Table 4 Epidemiology of *B. pertussis* infections

Issues
1. Percentage of prolonged cough illnesses in adolescents and adults due to *B. pertussis* infections
2. Rate of *B. pertussis* infections in adolescents and adults
3. Rate of *B. pertussis* cough illnesses in adolescents and adults

United States, it can be noted that as the number of reported cases decreased interepidemic period lengthened (see Mink et al. 1992; Cherry and Heininger, in press). This indicates that there is both control of the disease and curtailment of the circulation of the organism. In contrast, with pertussis, since the interepidemic period has not changed, the organism is still circulating in a similar fashion to that in the prevaccine era. This observation led our group and many others to study adolescents and adults as the potential reservoir for *B. pertussis* outbreaks. When evaluating the epidemiology of *B. pertussis* infections, three categories are considered and these are presented as issues in Table 4.

During the last 20 years numerous studies have been carried out involving adolescents and adults with prolonged cough illnesses. The main method which has helped with the success of these studies is the use of a single-serum serology measuring IgA or IgG antibody titers to PT. These studies revealed that about 13% of adolescents and adults with prolonged cough have serologic evidence of infection with *B. pertussis*. However, since about 10% of adults do not produce an antibody response to PT, it is likely that in up to 20% of adults with prolonged cough illnesses, the illness is due to *B. pertussis* infection.

In attempts to find out what population rates of *B. pertussis* infections are, sera that have been gathered usually for other purposes from the same subjects over time have been analyzed for titer rises to PT. The results of these studies suggest that between 1–6.6% of adolescents and adults are infected yearly with *B. pertussis*. Of particular note, in one study by our group during which sera were collected from adults who were older than 65 years, at 4-monthly intervals for a period of 3 years, the rate was 3% (Hodder et al. 2000). Although these rates seem quite high, they include both symptomatic and asymptomatic cases.

In a number of the studies of prolonged cough illnesses, attempts were made to calculate actual rates of *B. pertussis* illnesses in adolescents and adults. Most of these studies, however, were hampered by a lack of definitive measures of the population base. However, one study designed specifically for the purpose found a rate of 500 per 100,000 or about a million cases a year in the United States (see Strebel et al. 2001). In conjunction with an adult vaccine efficacy study carried out in eight centers in the United States, in which about 1400 controls were followed for approximately 2 years and 6 months, the rate was 370 per 100,000 population (see Ward et al. 2005). Finally, in the study of older

adults mentioned previously, in addition to obtaining sera every 4 months, clinical data of cough illnesses were collected (see Hodder et al. 2000). If all the cough illnesses in the period when the titer rises occurred were due to *B. pertussis* the rate could be as high as 1.5% or 3.3 million cases per year in the United States.

To summarize the above, it is clear that *B. pertussis* infections in adolescents and adults are very common and endemic in the present vaccine era. This, however, is not a new finding because, in Germany in the early 1990s, when pertussis was epidemic because there was no routine vaccination program for pertussis in place, it was found that infections in adolescents and adults were common and frequently the primary cases in families. Rates of reported pertussis were 40–100 times lower than actual illness rates and asymptomatic infections are 4–22 times more common than symptomatic infections. Symptomatic adolescents and adults are the major source of infection for unvaccinated children.

6 Vaccines

Because of considerable international concern relating to reactions (both real reactions and temporally related events), acellular pertussis vaccines (DTaP vaccines), which had endotoxin removed, were developed in the late 1970s. In Japan, where a successful immunization program was in place, concern about reactions led to the discontinuation of pertussis immunization in infants and then its reinstitution at 2 years of age. This resulted in a cohort of unvaccinated children and the subsequent occurrence of epidemic pertussis resulting in a number of deaths. Because of this, new vaccines were developed in Japan which, with minimal study, were put into routine use in Japan in 1981. However, since data were not available from Japan regarding infant immunization, further studies were done in Europe and Africa in the 1980s and predominantly in the early 1990s. A total of nine efficacy studies were performed using pertussis vaccines containing one component (PT toxoid), two components (PT toxoid + FHA), three components (PT toxoid, FHA, and PRN), and four components (PT toxoid, FHA, PRN and FIM). A WHO Pertussis Case Definition committee was convened in Geneva on 11 January 1991. The decision of this committee, of which I was a member, suggested that a universal case definition should include laboratory confirmation plus more than 21 days of paroxysmal cough. I disagreed with this decision because requiring 21 days of paroxysmal cough allows vaccines that modify disease but do not prevent it to look as good as vaccines that are better. This clearly turns out to be the case. Specifically in an initial trial in Sweden in the late 1980s with a PT toxoid vaccine, 80% of the cases would fail to fulfill the WHO case definition (see Cherry and Olin 1999; Cherry and Heininger, in press).

Even though there were eight efficacy trials done in the early 1990s, only two of these were done in such a way that serologic correlates of protection could be examined (see Cherry et al. 1998; Storsaeter et al. 1998). Both of these studies (ours in Erlangen, Germany and one in Stockholm, Sweden) had similar results. Both suggested that antibody to PRN in modest levels was most important for protection and that antibody to FIM and, to a lesser extent, PT were also important for protection. When one examines the results of studies where mild disease as well as severe disease was analyzed, it is clear that the efficacy of vaccines that contain PRN is roughly 30% better than vaccines without PRN.

In recent years, two vaccines (Tdap vaccines) have become available for adolescent and adult immunization. These vaccines are presented in comparison with their childhood vaccine counterparts in Table 5. Relating to these two vaccines, two important questions should be asked. First, do these vaccines work? The second is for how long will they protect? In regard to Boostrix, our group carried out an efficacy study with the pertussis component of this vaccine in eight centers in the United States and found the efficacy to be 89% (see Ward et al. 2005). In regard to ADACEL, a similar study has not been performed. However, two population-based studies have been done in which all 14-year-olds were vaccinated in Canada's Northwest Territories and also in Newfoundland, and both resulted in a dramatic decrease in reported pertussis. Clearly, both vaccines have high levels of efficacy. In regard to duration of protection, we can predict that it will be considerably long lasting. Specifically we know that in children, three doses of DTaP vaccine in the first year of life gives protection for 5 years. In adults following one dose, the titers are considerably higher than those achieved in children following three doses and the decay curves of the important antigens (all except PT) decline in such a way that reasonable efficacy can be predicted for a 10-year period.

Table 5 Antigenic components of selected diphtheria, tetanus, and acellular pertussis vaccines

	Infanrix® Infants/ Children†	Boostrix™ Adolescents‡	DAPTACEL® Infants/ Children†	ADACEL ™ Adolescents & Adults‡
PT (μg)	25	8	10	2.5
FHA (μg)	25	8	5	5
PRN (μg)	8	2.5	3	3
FIM 2+3 (μg)	–	–	5	5
D (Lf)	25	2.5	15	2
T (Lf)	10	5	5	5

† 6 weeks to <7 years of age.
‡ Boostrix US license for ages 10–18 years.
‡ ADACEL US license for ages 11–64 years.
www.fda.gov, accessed 12 June 2005

7 Conclusions

B. pertussis illness is endemic in adolescents and adults of all ages so immunization of adolescents alone will not interrupt transmission of *B. pertussis* to infants. It is my belief that a Tdap immunization program starting in preadolescents with vaccine given to adults at 10-year intervals will control both adolescent and adult pertussis as well as the transmission of *B. pertussis* to unimmunized infants. This approach might also eliminate the circulation of *B. pertussis*. This suggestion is based on observations related to the impact of diphtheria immunizations in the 1930s and 1940s in the United States, when both adults and children were immunized. This resulted in curtailment of both the disease and the circulation of the organism.

The number one priority today should be good public health for adults as well as children. We need to find a way to ensure that all adults get the immunizations they need, including protection against *B. pertussis* infection.

References

Bisgard, K. M., Pascual, F. B., Ehresmann, K. R., et al. 2004. Infant pertussis: who was the source? Pediatr. Infect. Dis. J. 23:985–989.

Celentano, L.P., Massari, M., Paramatti, D., et al. 2005. Resurgence of pertussis in Europe. Pediatr. Infect. Dis. J. 9:761–765.

Cherry, J. D., Gornbein J., Heininger, U., et al. 1998. A search for serologic correlates of immunity to *Bordetella pertussis* cough illnesses. Vaccine 16:1901–1906.

Cherry, J. D., Olin, P. 1999. Commentaries: The science and fiction of pertussis vaccines. Pediatrics 104:1381–1384.

Cherry, J.D. 2005. The epidemiology of pertussis: A comparison of the epidemiology of the disease pertussis with the epidemiology of *bordetella pertussis* infection. Pediatr. Infect. Dis. J. 115:1422–1427.

Cherry, J.D. 2006. Epidemiology of Pertussis. Pediatr. Infect. Dis. J. 25:361–362.

Cherry, J.D., Heininger, U. In press. Pertussis and Other *Bordetella* Infections. In Textbook of Pediatric Infectious Diseases (6th ed.), eds. R. D. Feigin, J. D. Cherry, G. Demmler, and S. Kaplan. Philadelphia: W.B. Saunders Co.

Hallander, H. O., Reizenstein, I., Renemar, B., et al. 1993. Comparison of nasopharyngeal aspirates with swabs for culture of *Bordetella pertussis*. J. Clin. Microbiol. 31:50–52.

Heininger, U., Klich, K., Stehr, K., et al. 1997. Clinical findings in *Bordetella pertussis* infections: Results of a prospective multicenter surveillance study. Pediatrics 100: e10. <www.pediatrics.org/cgi/content/full/100/6/e10>

Hodder, S. L., Cherry, J. D., Mortimer, E. A., et al. 2000. Antibody responses to *Bordetella pertussis* antigens and clinical correlations in elderly community residents. Clin. Infect. Dis. 31:7–14.

Mattoo, S., Cherry, J. D. 2005. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to *Bordetella pertussis* and other Bordetella subspecies. Clin. Microbiol. Rev. 18:326–382.

Mink, C. A. M., Cherry, J. D., Christenson, P., et al. 1992. A search for *Bordetella pertussis* infection in university students. Clin. Infect. Dis. 14:464–471.

Schmitt-Grohè, S., Cherry, J. D., Heininger, U., et al. 1995. Pertussis in German adults. Clin. Infect. Dis. 27:860–866.

Storsaeter, J., Hallander, H. O., Gustafsson, L., et al. 1998. Levels of anti-pertussis antibodies related to protection after household exposure to *Bordetella pertussis*. Vaccine 16:1907–1916.

Strebel, P., Nordin, J., Edwards, K., et al. 2001. Population-based incidence of pertussis among adolescents and adults, Minnesota, 1995–1996. J. Infect. Dis. 183:1353–1359.

Ward, J. I., Cherry, J. D., Chang, S., et al. 2005. Efficacy of an acellular pertussis vaccine among adolescents and adults. New Engl. J. Med. 353:1555–1563.

Recognition and Management of Encephalitis in Children

James D. Cherry

1 Introduction

Encephalitis is a frightening disease for children and parents, and frequently results in long-term neurologic disability and death. There has always been much confusion relating to terminology. Encephalitis is inflammation of the brain, and meningoencephalitis is inflammation of both the brain and meninges (Cherry, Shields, and Bronstein, in press). The term emcephalopathy refers to an illness with neurologic findings suggestive of encephalitis but without inflammation of the brain. Encephalitis is often classified as primary or post-infectious/parainfectious. In primary encephalitis the illness is due to the direct invasion and replication of an infectious agent within the brain, whereas post-infectious/parainfectious occurs after, or in combination with, another non-central nervous system illness or following the administration of a vaccine or other product.

2 History

Rabies was recognized in ancient times in Europe and Asia. In AD 100, Celsus noted the relationship between animal rabies and human disease (Johnson, 1959). In the twentieth century, epizootics of encephalitis in equine animals were noted as was their temporal relationship with human illness (Meyer et al., 1960). Meningoencephalitis was noted as a complication of mumps about 100 years ago (Enders, 1959). With the development of clinical virology more than 400 zoonotic arthropod-borne viruses were discovered as well as numerous nonpolio enteroviruses (Berge, 1975).

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3 Etiology

In spite of major technical advances during the last 25 years, the cause of the majority of sporadic cases of encephalitis remains unexplained. For example, in the recent California encephalitis project the etiology was found in only 38% of 334 cases (Glaser et al., 2003). In this study, etiologic agents were grouped in the following categories: viruses spread person to person; viruses spread to people by mosquitoes or ticks; viruses spread to people by warm-blooded mammals, bacteria, and other (Tables 1–4).

Herpes group viruses Herpes simplex virus type 1 (HSV-1) is the most common cause of sporadic fatal encephalitis (Cherry, Shields and Bronstein, in press; Whitley and Kimberlin, 2005). It is most often due to reactivation of latent infection but it can be related to a primary infection or reinfection by a second HSV-1 strain. It occurs in all age groups. In neonates, herpes encephalitis is most often due to disseminated HSV-2 infection acquired during the birth

Table 1 Etiologic agents in encephalitis

Viruses: spread person to person	
HSV 1 and 2	Adenoviruses
VZV	Rubella
EBV	Coronaviruses
CMV	Mumps
HHV6	Measles
HHV7	Variola
Enteroviruses	Hepatitis A, B, C
Reoviruses	Human parvovirus B19
Influenza A and B virus	Rotavirus
RSV	BK and JC
Parainfluenza 1–3	

Table 2 Etiologic viruses in encephalitis spread by warm-blooded mammals

Rabies	Vesicular stomatitis
Herpes	Equine morbillivirus (Hendra)
Lymphocytic choriomeningitis virus	Nipah
Encephalomyocarditis	Monkeypox

Table 3 Bacterial agents which cause infections that may have an encephalitic component

Bacterial meningitides often have an encephalitic component
Spirochetal infections
Brucella sp
Actinomyces and *Nocardia*
Bartonella henselae
Listeria monocytogenes

Table 4 Other categories which may be associated with encephalitis

Chlamydia psittaci and *pneumoniae*
Rickettsial infections
Mycoplasma pneumoniae and *hominis*
Fungal meningitides often have an encephalitic component
Protozoal: *Plasmodinum sp, Trypanosoma sp, Naegleria sp, Acanthamoeba sp, Balamuthia mandrillaris* and *Toxoplasma gondii*
Helminths: *Trichinella spiralis, Schistosoma sp, Strongyloides stercoralis, Baylisascaris procyonis*
Drug: trimethroprim

process. Encephalitis in older children is almost always due to HSV-1. HSV-2 rarely causes encephalitis outside of the newborn period except in immunocompromised persons. However, aseptic meningitis due to HSV-2 is a not an infrequent illness associated with recurrent genital HSV-2 infection.

Varicella (chicken pox) is complicated by varicella-zoster virus (VZV) encephalitis in ~0.3 per 1000 cases (Cherry, Shields, and Bronstein, in press). Primary infection with VZV results in latent infection in dorsal root ganglia. Reactivation of virus leads to zoster, and between 0.5% and 5% of patients with zoster may have encephalitis. Other herpes group viruses (EBV, CMV, HHV-6, and HHV-7) may occasionally cause encephalitis. There is a particular risk of encephalitis with these viruses in immunocompromised patients due to reactivation of latent virus. In primary infection with EBV resulting in infectious mononucleosis, about 1% of cases will develop encephalitis during the course of the illness. Encephalitis is uncommon in primary CMV infection except in congenital infections. HHV-6 is a common cause of febrile convulsions in infants but it is an uncommon cause of encephalitis.

Enteroviruses Nonpolio enteroviruses are a leading viral cause of neurologic disease in children and they are a major cause of encephalitis (Cherry, Shields, and Bronstein, in press). In recent years, enterovirus 71 has been epidemic in Southeast Asia. In addition to hand, foot, and mouth syndrome, severe neurologic events including meningitis, meningoencephalitis, encephalitis, cerebellitis, and a polio-like syndrome have been observed. Brainstem encephalitis has caused numerous fatalities (Huang et al., 1999).

Influenza viruses Encephalitis is a manifestation of influenza A and B viral infections (Cherry, Shields, and Bronstein, in press). Its occurrence is irregular as it seems to be related to specific, circulating strains. Other central nervous system (CNS) manifestations associated with influenza viral infections include Reye's syndrome, acute necrotizing encephalopathy, and myelitis.

Measles, Mumps, and Rubella Encephalitis due to measles, mumps, and rubella is rare in countries with successful universal childhood immunization programs. Encephalitis can be expected in association with these infections at the following rates: measles, 0.74 per 1000; mumps, 3 per 1000; and rubella, 0.1–0.2 per 1000 (Cherry, Shields, and Bronstein, in press).

Viruses—Spread to People by Mosquitoes or Ticks Arboviruses are the most important cause of severe encephalitis worldwide. The occurrence of specific arboviruses is both seasonal and highly geographic (Cherry, Shields, and Bronstein, in press). In general, the incidence of neuroinvasive disease and sequalae of infection and death increases with age. Today, the most important cause of epidemic encephalitis worldwide is Japanese encephalitis virus with 10,000 deaths and 35,000–50,000 cases per year (Solomon et al., 2003) (Table 2).

The most important infection in this category is rabies (World Health Organization, 1978). With known exposure and postexposure prophylaxis, this is a preventable disease but more than 20,000 cases and deaths occur worldwide every year. Nipah virus, a paramyxovirus, is a new wide-scale epizoonotic encephalitis virus with direct animal-to-human rather than vectorial transmission. It is spread from infected pigs to humans (Chaudhuri and Kennedy, 2002).

Meningitis is the primary CNS manifestation of the agents, presented in Table 3. However, an encephalitic component is often a part of the infection (Cherry, Shields, and Bronstein, in press). Spirochetal infections are a more common cause of CNS disease, and specifically encephalitis, than is generally realized. Encephalopathy but not encephalitis is a relatively common complication of pertussis.

As noted in Table 4, a number of other infectious agents as well as a number of drugs are occasionally related to encephalitis. Of this group, *Mycoplasma pneumoniae* is of particular importance (Bitnum et al., 2003). In one study in Finland, 4.8% of patients hospitalized with *M. pneumoniae* infections had CNS manifestations (Pönkä, 1980).

4 Postimmunization Neurological Disease

Historically, a variety of neurologic events including encephalitis have occurred in some recipients of antiserums that were prepared in animals (Cherry, Shields, and Bronstein, in press). Also, encephalitis was an important complication of smallpox vaccination (0.5 per 100,000 vaccine doses). Encephalitis may also be a rare complication of measles immunization (<1 case per million vaccines). Encephalitis was also a relatively frequent complication of some mumps vaccines.

5 Postinfectious Encephalitis

Postinfectious or parainfectious encephalitis is an illness which occurs after a demonstrated or presumed viral infection. It is thought to be immune mediated rather than a direct effect of a virus in nerve cells (Cherry, Shields, and Bronstein in press). It is my opinion, however, that while immune mechanisms

may play a role in the pathogenesis, the process is often stimulated by the direct presence of the antigen in the nervous system. This is an important distinction when treatment with corticosteroids is considered.

Most postinfectious encephalitides which are immune mediated are subacute in onset. Acute disseminated encephalomyelitis (ADEM) is subacute at onset and is characterized by optic neuritis, myelitis, ataxia, hemiparesis, cranial nerve palsies, and multifocal white-matter lesions (Dale, 2003).

6 Chronic Encephalitic or Encephalopathic Illnesses (Slow Infections)

Slow infections are due to a number of viruses and prion diseases. Viral diseases include: progressive multifocal leukoencephalopathy (JC, SV40 and BK viruses), SSPE (measles virus), and acquired immunodeficiency syndrome (HIV-1 and HIV-2) (Cherry, Shields, and Bronstein, in press). Prion diseases, called transmissible spongiform encephalopathes, are related to similar encephalopathies in cows (mad cow disease) (Prusiner and Hsiao, 1994).

7 Encephalitis Epidemiology

Because of many different causes of encephalitis no unified epidemiologic pattern exists. In general, most cases in temperate climates occur in the summer or fall reflecting arboviral and enteroviral etiologies. Arboviruses occur in localized outbreaks and in epidemics with boundaries determined by the range of particular vectors and the prevalence of natural reservoir animals.

Arboviruses are zoonoses in which humans are infected accidentally by an arthropod vector. Most commonly, mosquitoes or other insects acquire arboviruses by biting infected birds. Encephalitis in horses and mules may be the first indication of incipient trouble in a geographic area.

8 Clinical Manifestations

Children with encephalitis may demonstrate evidence of diffuse disease, such as behavioral or personality changes and decreased consciousness and generalized seizures or localized features, such as focal seizures, hemiparesis, movement disorders, cranial nerve defects, and ataxia.

Some children may appear to be mildly affected initially but suddenly lapse into coma followed by sudden death. In others, the illness may be ushered in by high fever, violent convulsions interspersed with bizarre movement, and hallucinations alternating with brief periods of clarity. These children may recover with relatively few sequelae.

Most commonly, the initial manifestations suggest an acute systemic illness with fever, headache, or in infants, screaming spells, abdominal distress, nausea, and vomiting. With rising temperature, CNS manifestations are noted: mental dullness progressing to stupor; bizarre movements; convulsions; nuchal rigidity; and focal signs, which may be stationary, progressing, or fluctuating.

Specific forms of encephalitis or complicating manifestations of encephalitis include Guillain-Barré syndrome and related syndromes, acute transverse myelitis, acute hemiplegia, brainstem encephalitis, and acute cerebellar ataxia.

9 Differential Diagnosis

The differential diagnosis of neurologic illness compatible with encephalitis is broad (Table 5).

In a patient with encephalopathy or possible encephalitis, a careful history and physical examination is the cornerstone of the evaluation (Cherry, Shields, and Bronstein, in press). Following assessment for increased intracranial pressure, a lumbar puncture with a complete examination and culture and antigen detection tests of the cerebrospinal fluid should be done as soon as possible. Neuroimaging and an electroencephalogram should also be undertaken. Computed tomography (CT) with and without contrast should be done and followed up with MRI which is better for finding subtle changes.

10 Specific Diagnosis

The examination of the cerebrospinal fluid (CSF) is central to making a specific diagnosis (Cherry, Shields, and Bronstein, in press). In addition to the examination of the fluid for cells, protein, and sugar, cultures should be obtained for viruses, conventional and unusual bacteria, and fungi. Stained smears should be examined for usual pathogens as well as for parasites. PCR should be performed to identify herpes group viruses and enteroviruses. Viral

Table 5 Differential diagnosis

1)	Metabolic diseases
2)	Toxic disorders
3)	Mass lesions
4)	Subarachnoid hemorrhage
5)	Embolic lesions
6)	Acute demyelinating disorders
7)	Status epilepticus
8)	Infectious diseases
9)	Postinfectious diseases
10)	Acute confusional migraine

cultures and PCR of blood, throat, and stool specimens should be obtained. Serologic studies in encephalitis can be very useful but most often they are done inappropriately. The demonstration of a significant titer rise to an infectious agent is most important. This requires the collection of both an acute-phase and a convalescent-phase specimen. In general, these specimens should be studied at the same time. Except for a few specific IgM antibody tests, the examination of an acute-phase specimen alone results in misdiagnoses. Particularly problematic in this regard is the examination of enteroviral antibody panels. Also problematic is the examination of the CSF for antibodies to infectious agents.

11 Treatment

In general, specific treatment should be given for identified agents (Cherry, Shields, and Bronstein in press). However, because early treatment is critical, most patients should be treated empirically with intravenous (IV) acyclovir until HSV encephalitis is ruled out. Acyclovir should also be used for VZV and probably EBV infections and ganciclovir should be used for CMV infections. Pleconaril, if it again becomes available, should be used for enteroviral infections. If clinical characteristics and nasopharyngeal antigen tests are indicative of influenza, oseltamivir treatment should be considered. Antibacterial, antifungal, and antiparasitic agents should be used for specific infections. Aspects of general management are presented in Table 6.

A suggested treatment for status epilepticus is IV lorazepam (0.1–0.2 mg/kg up to 4 mg), which should be tried twice. If this fails, use IV phenytoin (18–20 mg/kg, maximum 1000 mg, given over 20 min).

Cerebral edema is a significant problem in encephalitis. It can be managed with dexamethasone, 0.1–0.2 mg/kg IV as an initial dose followed by 0.05–0.1 mg/kg IV every 4–6 h. In situations of active viral infections an alternative method would be: mannitol IV as a 20% solution in a dose of 0.25–1 g/kg over a 30–60 min period. This can be repeated every 8–12 h.

Table 6 General treatment is nonspecific and empiric, aimed at maintaining life and supporting each involved organ system

1) Antibiotics and antivirals
2) ICU care
3) Repeat CT and MRI
4) Monitor ICP
5) Watch for syndrome of inappropriate antidiuretic hormone secretion
6) Monitor fluids and electrolytes
7) Treat status epilepticus vigorously

12 Prognosis

The prognosis in all encephalitides is guarded with respect to both immediate outcome and sequelae. Most important are patient age and etiologic agent. Prehospital events are also predictive of outcome.

13 Prevention

Many encephalitides (measles, mumps, rubella, varicella) are preventable by universal immunization of children. The control of insect vectors is most important for arboviral agents.

References

Berge, T. O. 1975. ed. International Catalogue of Arboviruses including Certain Other Viruses of Vertebrates. 2nd ed. Atlanta, U.S. Dept. of Health, Education and Welfare, Public Health Service, No. (CDC) 75–8301.

Bitnum, A., Ford-Jones, E., Blaser, S., et al. 2003. *Mycoplasma pneumoniae* encephalitis. Sem. Pediatr. Infect. Dis. 14:96–107.

Chaudhuri, A., and Kennedy, P. G. E. 2002. Diagnosis and treatment of viral encephalitis. Postgrad. Med. J. 78:575–583.

Cherry, J. D., Shields W. D., and Bronstein, D. In press. Encephalitis and meningoencephalitis. In Textbook of Pediatric Infectious Diseases (6th ed.), eds. R. D. Feigin, J. D. Cherry, G. Demmler, and S. Kaplan. Philadelphia: W. B. Saunders Co.

Dale, R. C. 2003. Acute disseminated encephalomyelitis. Sem. Pediatr. Infect. Dis. 14:90–95.

Enders, J. F. 1959. Mumps. In Viral and Rickettsial Infections of Man (3rd ed.), eds. T. M. Rivers and F. L Horsfall, Jr., pp. 780–789. Philadelphia: J. B. Lippincott.

Glaser, C. A., Gilliam, S., Schnurr, D., et al. 2003. In search of encephalitis etiologies: diagnostic challenges in the California Encephalitis Project, 1998–2000. Clin. Infect. Dis. 36:731–742.

Huang, C. C., Liu, C. C., Chang, Y. C., et al. 1999. Neurologic complications in children with enterovirus 71 infection. N. Engl. J. Med. 341:936–942.

Johnson, H. N. 1959. Rabies. In Viral and Rickettsial Infections of Man (3rd ed.), eds. T. M Rivers and F. J. Horsfall, Jr., pp. 405–431. Philadelphia: J. B. Lippincott

Meyer, H. M., Jr., Johnson, R. T., Crawford, I. P., et al. 1960. Central nervous system syndromes of "viral" etiology: A study of 713 cases. Am. J. Med. 2:334–347.

Pönkä, A. 1980. Central nervous system manifestations associated with serologically verified *Mycoplasma pneumoniae* infection. Scand. J. Infect. Dis. 12:175–184.

Prusiner, S. B., and Hsiao, K. K. 1994. Human prion diseases. Ann. Neurol. 35:385–395.

Solomon, T., Ni, H., Beasley, D. W. C., et al. 2003. Origin and evolution of Japanese encephalitis virus in Southeast Asia. J. Virol. 77:3091–3098.

Whitley, R. J., Kimberlin, D. W. 2005. Herpes simplex encephalitis: children and adolescents. Sem. Ped. Infect. Dis. 16:17–23.

World Health Organization. 1978. World Health Statistics Annual. Vol. 11, Infectious Diseases: Cases and Deaths. Geneva, World Health Organization.

Microbiology and Management of Pleural Empyema

Julia Clark

1 Introduction

Empyema, the presence of pus in the pleural space, reflects a terminal stage of infection within this space. In children, this is usually secondary to pneumonia, although it may also arise from contiguous spread from another source such as subdiaphragmatic infection, lung, retropharyngeal, or paravertebral abscess, mediastinal lymph node, trauma, foreign body, or thoracic surgery. Differential diagnoses include pleural effusion caused by cardiac or renal failure, malignancy, particularly lymphoma, or connective tissue disease. Chest X-ray changes suggestive of effusion may also be caused by congenital abnormalities.

Cough, dyspnoea, and fever with clinical signs such as decreased or absent breath sounds, reduced chest expansion, and dullness to percussion are suggestive. Chest radiograph is the first initial helpful imaging. Ultrasound is extremely useful in detecting fluid, the size of effusion, and presence or absence of loculation. Computed tomography (CT) scanning adds little further diagnostic help in most paediatric empyema, although it maybe helpful in unusual or complicated disease or before surgery (Balfour-Lynn et al., 2005).

In order to aid understanding of the treatment options used in empyema, it is worth reviewing the natural history of pleural infection. This progresses from an exudative stage, day 1–3, where an inflammatory process associated with the underlying pneumonia produces a paraneumonic effusion. On ultrasound, this appears as anechogenic without loculation. Between day 4 and day 14, leucocytes and fibroblasts collect, loculations form, and these can be seen on ultrasound as fibrin strands. After 2 weeks, the inflammatory process starts to organize with the fibroblasts on the pleural surface producing an 'elastic peel'. Ultrasound may reveal a complex image with rind, multiple loculations and possible entrapped lung (Singh et al., 2002, Light, 2006). Biochemical staging has been widely used within adult practice with markers such as a low pH and

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glucose, high white cell count, and lactate dehydrogenase (Light, 2006), but these are not widely used to inform diagnosis or management in paediatrics (Balfour-Lynn et al., 2005).

2 Epidemiology and Aetiology

2.1 Epidemiology

In the pre-antibiotic era, empyema secondary to pneumonia was relatively common, with both *Staphylococcus aureus* and *Streptococcus pneumoniae* implicated. With the advent of antibiotics and prompt treatment of bacterial pneumonia, empyema became relatively rare (Behrman and Vaughan, 1987). However, in the early 1990s, increased rates of empyema began to be reported both from the USA and the UK (Hardie et al. 1996, Rees et al., 1997, Thompson et al. 1999, Byington et al. 2002). Even so, this remains a relatively rare disease with quoted incidence rates varying between 1 and 14 per 100,000 children (Byington et al., 2002, Byington et al., 2006). The apparent increase has continued in more recent years, being documented in both the UK (Eastham et al. 2004, Spencer et al. 2006, Fletcher et al. 2006) and Taiwan (Lin et al., 2006) with reports of between three and fivefold rises in rates.

2.2 Aetiology

Pathogens traditionally associated with empyema include *S. aureus, S. pneumoniae, S. pyogenes, Mycobacterium tuberculosis,* and *Haemophilus influenzae* type B. Causative organisms reflect, to an extent, circulating pathogens within a community which may, in turn, be influenced by immunization programmes, for example, universal use of conjugate pneumococcal vaccine. Apparent differences are also seen between populations. Although these may reflect, in part, differences between studies, genuine variation in epidemiology is certainly present too. For instance, in developing countries, tuberculosis may make up a significant proportion of the empyemas seen. A recent review of cases in Pakistan found 35 of 50 (66%) of children with large pleural effusion had tuberculosis (Memon, Shaikh, 2007). In India, when mycobacterial infections were excluded, 77% of those in whom a pathogen was isolated had *S. aureus* and only 8% had pneumococcus (Baranwal et al., 2003). Conversely, in Taiwan, where 42% of 59 children with empyema had a pathogen culture confirmed, 50% had pneumococcus, and 47% had mycoplasma on serology (Shen et al., 2006). *S. aureus* has also been documented as the predominant pathogen in Portugal (Martins et al., 2007)

and although *S. pyogenes* is also found marginally more frequently than *S. aureus* in France, USA, and the UK, pneumococcus predominates in these countries (Monnier et al., 2006; Byington et al., 2002; Eastham et al., 2004; Fletcher et al., 2006).

2.3 Risk Factors for Progression to Empyema

The likelihood of progression of pneumonia to empyema depends on interactions between host and organism. How many children do so is unquantified, although it may be inferred from a cohort of children seen in hospital with pneumonia, where 9% had an effusion and 3% an empyema (Clark et al., ADC 2007).

The innate immune response is likely to be important in the control of invasive pneumococcal disease and the transcription factor NF-kappaB plays a central role. NFKBIA encodes the IkappaB family of inhibitors mediating NF-kappaB. Recent work has indicated that minor polymorphisms in the NFKBIA promoter region were associated with protection from invasive pneumococcal disease (IPD). NFKBIA polymorphisms associate with susceptibility to IPD and genetic variation in an inhibitor of NF-kappaB may therefore influence the development of common infectious diseases (Chapman et al., 2007).

Pathogen is clearly relevant, with pneumococcus firmly associated with empyema. A clear seasonal variation in empyema rates, with increases usually within the winter, mimics trends for invasive pneumococcal disease. However, some organisms appear to be more likely to progress to empyema from pneumonia than others. Both *S. pyogenes* and *S. aureus* are more frequently found in empyema than in pneumonia (Byington et al., 2002) and in one study 83% of confirmed *S. pyogenes* pneumonia an effusion was present, compared to 32% for *S. pneumoniae* (Al-Kaabi et al., 2006).

Univariate analysis of children with pneumonia, with and without empyema, identified some interesting possible associations (Byington et al., 2002). Children with empyema were more likely to have had no antibiotics prior to presentation, though if an antibiotic was given this was more likely to have been ceftriaxone. There was also a significant association with preadmission ibuprofen use. Both these latter cases may have been due to differences in initial presentation of these children, such as higher fever and being more unwell. Other studies suggest that children with empyema, when compared with those with pneumonia, are more likely to be over 2–years-old, white and have an associated fever for longer before admission but less likely to have an underlying disease (Hsieh et al., 2004; Tan et al., 2002).

Despite a possible socioeconomic association with pneumonia (Clark et al., 2007) no such evidence is yet obvious for empyema.

3 Potential Reasons for Increasing Incidence of Empyema

There are many hypotheses suggested for the recent increase in the incidence of empyema. These include viral infections, climate, environmental factors, change in initial treatment regimes, or changes in pathogens.

The most obvious potential cause, and that in which there has been most interest, is the latter. There is little evidence to suggest that bacterial species that are more likely to progress to empyema from pneumonia, such as *S. pyogenes*, are becoming more prevalent. As the predominant pathogen is usually *S. pneumoniae,* is there evidence for change in its resistance or virulence? There is no support for a role for increasing antibiotic resistance, as no such rise has been seen in the UK (Eastham et al., 2004) and no differences were found in the rates of pneumococcal antibiotic resistance between empyema and pneumonia (Byington et al., 2002; Tan et al., 2002; Hsieh et al., 2004).

3.1 Serotype 1

Streptococcus pneumoniae has over 90 different serotypes, but only a small proportion is predominately responsible for human disease. In the UK, the most prevalent serotypes responsible for invasive pneumococcal disease in children have been 14 followed by 19 then 6 (George et al., 2001). However, in empyema, serotype 1 predominates, being responsible for 24% to 50% (Tan et al., 2002; Byington et al., 2002) in the USA and between 53% and 62% in the UK (Eastham et al., 2004; Fletcher et al., 2006). This serotype is unusual, in that nasopharyngeal carriage is rarely found and it has been implicated in invasive disease outbreaks with high attack rates, as well as within enclosed communities (Kirkham et al., 2006). Serotype 1 predominance in empyema is reflected not only in North America and the UK, but also Europe (Bekri et al., 2007), although is not consistent throughout the world. In Taiwan, where increasing empyema rates have also been described, serotype 14 was most frequently identified, with serotype 1 almost completely absent (Hsieh et al., 2004). This suggests that, if the increases in incidence noted in different areas are due to a common pathogen-related factor, serotype may be a marker of this rather than the cause.

3.2 Sequence Types

Further genetic characterization of *S. pneumoniae* has been possible by multi-locus sequence typing (MLST), which specifically sequences fragments of seven housekeeping genes to give an allelic profile, which then generates a sequence type (ST) or clone. Interestingly, a particular ST (ST306) was associated with an increase in invasive disease also associated with serotype 1 in Sweden in 1997

(Henriques et al., 2001). ST306 also predominated among increased serotype 1-invasive pneumococcal disease isolates in Scotland since 2001 (Kirkham et al., 2006). Within the UK, Europe, USA, and Australia, ST227 and ST306 have been identified as the major sequence types found among invasive serotype 1 isolates (Brueggeman, 2003). Little is yet known about the distribution of ST in empyema, though an important start has been made, where a handful of paediatric serotype 1 isolates from two different UK centres (Bristol and Newcastle) have been typed. ST306 was implicated in 5 of 7 (71%) empyemas from Bristol but in only 1 of 5 (20%) from Newcastle (Bibby et al., 2007), with ST 227 accounting for the remainder.

4 Management

4.1 Prevention

As ever in infectious diseases, prevention is always better than cure, and as much of empyema is caused by pneumococcus, the advent of the conjugate pneumococcal vaccine could be expected to have significant impact. The seven valent pneumococcal vaccines contain serotypes 4, 6B, 9 V, 14, 18C, 19F, and 23F. Serotype 1 is notably absent. This conjugate vaccine was introduced in the USA in 2000 and several studies since then have revealed a decrease in total incidence (Buckingham et al., 2003; Schultz et al., 2004 and Alfaro et al., 2005). However, alongside this, the decrease in pneumococcal isolates has been accompanied by a significant increase in MRSA-associated empyema. More worryingly, one study found an increase in rates when comparing pre- with post-conjugate pneumococcal vaccine empyema rates. Non-vaccine pneumococcal strains increased from 63% to 86% whilst vaccine serotypes decreased from 37% to 14%. Interestingly, the most significant increases were serotypes 3 and 19A with serotype 1 remaining static (Byington et al., 2006)

4.2 Drainage of Fluid

Objectives of treatment of empyema include eradication of infection, relief of pain and respiratory distress, evacuation of pleural cavity contents and re-expansion of lung parenchyma, a return to normal function and prevention of chronic disease, early hospital discharge, and a good cosmetic result. Antibiotics are essential and require good pleural penetration. These are achieved well with penicillin, with good levels also for carbenicillin, clindamycin, amikacin, ciprofloxacin, and cefuroxime (Teixeira et al., 2000; Balfour-Lynn et al., 2005). There are no good data to guide duration of antibiotics treatment which is often guided by clinical response.

In significant pleural effusion with empyema, there is general agreement that evacuation of pleural contents is usually necessary for resolution. Medical treatment may include insertion of a chest drain with or without instillation of fibrinolytic agents. Surgical treatment may include video-assisted thoracoscopic surgery (VATS) or open drainage including thoracotomy with or without decortication. There are strong advocates for both medical and surgical approaches and the recent published literature includes several case series, often from the US, describing successful outcomes using VATS. A recent systematic review found 25 studies (363 children) of operative intervention, three studies (64 children) of fibrinolysis and 54 studies (3418 children) of chest drain and antibiotics only (non-operative) (Avansino et al., 2005). Of children undergoing only the latter intervention, 76% recovered, although they endured a chest tube for much longer, stayed in hospital for double the length of time, and had a higher overall mortality.

The evidence-based recommendations of British Thoracic Society (BTS) on the management for pleural empyema suggest intrapleural fibrinolytics (grade B evidence) initially. Failure of chest tube, antibiotics, and fibrinolytics should prompt early discussion with a thoracic surgeon (evidence grade D). However, if a general anaesthetic is used to place a chest drain, then VATS or mini thoracotomy could be considered as a first-line procedure.

These guidelines reflect the ongoing discussion about optimal treatment. A randomized controlled trial showed good outcome in children with fibrinolysis and a small percutaneous drain (Thomson et al., 2002). A follow-up case series from the same group presented a very low failure rate with only 2% requiring surgery (Barnes et al., 2005). Case series in centres where an initial surgical approach is practiced with mini thoracotomy also indicate very good outcomes and short hospital stays (Carey et al., 1998). Since the BTS guidelines were published, a further randomized control trial comparing fibrinolytic therapy with VATS indicated no difference in hospital stay, although VATS was a more expensive option (Sonnappa et al., 2006). Locally, practice is usually dictated by local expertise and cardiothoracic experience and enthusiasm.

5 Summary

Empyema is apparently becoming more common, with pneumococcus being the most common pathogen detected in Europe and the USA. However, group A streptococcus and *S. aureus* pneumonia are individually more likely to progress to empyema. Serotype 1 pneumococcus is frequently implicated and the reasons for an apparent increase in incidence remain unclear. Management requires antibiotics and removal of pus either by fibrinolysis or primary drainage.

References

Alfaro C, Fergie J, Purcell K. Emergence of community-acquired methicillin-resistant *staphylococcus aureus* in complicated parapneumonic effusions. Pediatr Infect Dis J 2005, **24(3)**: 274–275.

Al-Kaabi N, Solh Z, Pacheco S, et al. A comparison of Group A Streptococcus versus Streptococcus pneumoniae Pneumonia. Pediatr Infect Dis J 2006, **25**: 1008–1012.

Avansino J R, Goldman B, Sawin R S, et al. Primary operative versus nonoperative therapy for pediatric empyema, a meta-analysis. Pediatrics 2005, **115(6)**: 1652–1659.

Balfour-Lynn I M, Abrahamson E, Cohen G, et al. BTS guidelines for the management of pleural infection in children. Thorax 2005, **60**: i1–i21.

Baranwal A K, Singh M, Marwaha R K, et al Empyema thoracis: a 10-year comparative review of hospitalised children from south Asia. Arch Dis Child 2003, **88**: 1009–14.

Barnes N P, Hull J, Thomson A H. Medical management of parapneumonic pleural disease. Pediatr Pulmonol 2005, **39(2)**: 127–43.

Behrman R E, Vaughan V C. 1987. Nelson textbook of pediatrics. 13th ed. Philadelphia. WB Saunders Company.

Bekri H, Cohen R, Varon E, et al. Streptococcus pneumoniae serotypes involved in children with pleural empyemas in France. Archives de Pediatrie 2007, **14(3)**: 239–243

Bibby A C, Leeming J P, Fletcher M et al. 25th Annual meeting of ESPID. 2007, abstract 468: 244.

Brueggeman A B, Spratt B G. Geographic Distribution and clonal diversity of Streptococcus pneumoniae Septypr 1 isolates. 2003, **41**: 4966–4970.

Buckingham S C, King M D, Miller ML Incidence and etiologies of complicated parapneumonic effusions in children, 1996–2001. Pediatr Infect Dis J 2003, **22(6)**: 499–504.

Byington C L, Korgenski K, Daly J, et al. Impact of the pneumococcal conjugate vaccine on pneumococcal parapneumonic empyema. Pediatr Infect Dis J 2006, **25(3)**: 250–254.

Byington C L, Spencer L Y, Johnson T A, et al. An epidemiological investigation of a sustained high rate or pediatric parapneumonic empyema: risk factors and microbiological associations. CID 2002, **34**: 434–440.

Carey J A, Hamilton J R L, Spencer D A, et al. Empyema thoracis: a role for open thoracotomy and decortication. Arch Dis Child 1998, **79**: 510–513.

Chapman S J, Khor C C, Vannberg F O et al. IkappaB genetic polymorphisms and invasive pneumococcal disease. Am J Respir Crit Care Med 2007 Jul 15, **176(2)**: 181–7.

Clark J E, Hammal D, Hampton F, et al. Epidemiology of community-acquired pneumonia in children seen in hospital. Epidemiol Infect 2007, **135**: 262–269.

Clark J E, Hammal D, Spencer D A, Hampton F. Children with pneumonia – how do they present and how are they managed? Arch Dis Child 2007 May, **92(5)**: 394–8.

Eastham K M, Freeman R, Kearns A M et al. Clinical features, aetiology and outcome of empyema in children in the North East of England. Thorax 2004, **59**: 522–525.

Fletcher M, Leeming J, Cartwright K, et al. Childhood empyema: Limited potential impact of 7-valent pneumococcal conjugate vaccine. Pediatrc Infect Dis J 2006, **25**: 559–560.

George A C, Melegaro A. Invasive pneumococcal infection in England and Wales 1999. CDR Weekly 2001:11–21.

Hardie W, Bokulie R, Garcia V F, et al. Pneumococcal pleural empymas in children. Clin Infect Dis 1996, **22(6)**: 1057–63.

Henriques N, Kalin M, Ortqvist A et al. Dynamics of penicillin-susceptible clones in invasive pneumococcal disease. J Infect Dis 2001, **184**: 861–869.

Hsieh Y C, Hsueh P R, Lu C Y, et al. Clinical manifestations and molecular epidemiology of necrotizing pneumonia and empyema caused by *streptococcus pneumoniae* in children in Taiwan. CID 2004, **38**: 830–835.

Kirkham L A S, Jefferies J M C, Kerr A R, et al. Identification of invasive serotype 1 pneumococcal isolates that express nonhemolytic pneumolysin. J Clin Microbiol 2006, **44**: 151–159.

Light R W. Parapneumonic effusions and empyema. Proc Am Thorac Soc 2006, **3(1)**: 75–80.

Lin C J, Chen P Y, Huang F L, et al. Radiographic, clinical, and prognostic features of complicated and uncomplicated community-acquired lobar pneumonia in children. J Microbiol Immunol Infect 2006, **39**: 489–495.

Martins S, Valente S, David T N, et al. Complicated pleural effusion in children – therapeutical approach. Revista Portugesa de Pneumologia 2007, **13(1)**: 53–70.

Memon S A B, Shaikh S J. The etiology of pleural effusion in children: Hyderabad experience. Pak J Med Sci 2007, **23(1)**: 86–87.

Monnier A L, Carbonnelle E, Zahar J R, et al. Microbiological diagnosis of empyema in children: Comparative evaluations by culture, polymerase chain reaction, and pneumococcal antigen detection in pleural fluids. CID 2006, **42**: 1135–40.

Rees J., Spencer D., Parikh D., Weller P. Increase in incidence of childhood empyema in West Midlands, UK. Lancet 1997 Feb 8, **349**(9049): 402.

Schultz K D, Fan L L, Pinsky J, et al. The changing face of pleural empyemas in children: Epidemiology and management. Pediatrics 2004, **113(6)**: 1735–1740.

Shen Y H, Hwang K P, Niu C K. Complicated parapneumonic effusion and empyema in children. J Micro Imm Infect 2006, **39**: 483–488.

Singh M, Singh S K, Chowdhary S K. Management of empyema thoracic in children. Indian Pediatr 2002 Feb, **39(2)**: 145–57.

Sonnappa S, Cohen G, Owens C M, et al. Comparison of urokinase and video-assisted thoracoscopic surgery for treatment of childhood empyema. Am J Respir Crit Care Med 2006, **174**: 221–227.

Spencer D A, Iqbal S M, Hassan A, et al. Empyema thoraces is still increasing in UK children. BMJ 2006, **332**: 1333–1333.

Tan T, Mason E, Wald E R, et al. Clinical characteristics of children with complicated pneumonia caused by *Streptococcus pneumoniae*. Pediatrics 2002, **110**: 1–6.

Teixeira L R, Sasse S A, Villarino M A, et al. Antibiotic levels in pleural fluid. Chest 2000, **117**: 1734–1739.

Thomson A H, Hull J, Kumar M R, et al. Randomised trial of intrapleural urokinas in the treatment of childhood empyema. Thorax 2002, **57**: 343–347.

Thompson A, Reid A, Shields M, Steen H, Taylor R. Increased incidence in childhood empyema thoracis in Northern Ireland. Ir Med J 1999, **92(7)**: 438.

Pneumococcal Conjugate Vaccines Probe Studies: The Solution Points to the Problem

Ron Dagan

1 Observed Versus Expected Effect of a Vaccine – The Vaccine Probe Concept

Understanding the effect of vaccines must include information derived from earlier studies (Phase I and Phase II), efficacy studies (Phase III), and data derived after introduction of the vaccines. When information is accumulated, one needs to assess to compare the 'observed effects' with what was expected from the vaccine ('expected effects'). Observed effects can be divided schematically into five components (1) Observed outcomes that were expected to occur; (2) observed outcomes where no prevaccination estimates could be calculated, but some effect was still expected; (3) effect in contacts of vaccinees through herd immunity; (4) unexpected beneficial outcomes; and (5) unexpected deleterious effects. The term 'vaccine probe' is used to describe an attempt to understand pathogenesis, epidemiology, and disease burden through observing unexpected or unpredictable responses to vaccines.

When this approach is applied to pneumococcal diseases, we need first to list the main vaccine outcomes that may be measured after vaccination with pneumococcal conjugate vaccines (PCVs) which are incidence of (1) invasive pneumococcal disease (IPD) – that is, bacteremia and meningitis; (2) pneumonia; (3) acute otitis media (AOM); (4) reduction in antibiotic resistance; and (5) all other outcomes including less common and unexpected outcomes. In addition, effects on contacts (herd immunity) must be considered. Of these, substantial falls in IPD caused by the serotypes included in the vaccine and perhaps some other serotypes with common capsular epitopes (termed vaccine-related serotype) were expected. Some reduction in AOM incidence and fall in observed antibiotic-resistance rates were also expected. The effect on pneumonia could not be predicted accurately due to lack of knowledge about the size of the role of *Streptococcus pneumoniae* in pneumonia. The extent of any reduction in disease

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rates among contacts (herd immunity) could also not be predicted. Thus, potentially a lot can be learned from the observed effects of PCVs on pneumococcal disease in vaccinees and their contacts. In this chapter, several examples of such studies will be discussed.

2 Vaccine Probe-Derived Insights into PCVs – Selected Examples

2.1 Probe Studies in Pneumonia – the 'pneumonia detective'

A major obstacle to determining pathogen-specific causes of pneumonia is the lack of sensitive tests for diagnosing bacterial pneumonia (Madhi and Klugman, 2007). It is estimated that 146 million cases of pneumonia occur in children <5 years of age globally every year and that a considerable proportion of these are caused by *S. pneumoniae* (Rudan et al., 2004). Furthermore, it is estimated that 1.8 million deaths caused by acute respiratory infections occur globally every year, and that the main pathogen causing death is *S. pneumoniae* (Bryce et al., 2005). However, the size of the role of *S. pneumoniae* and thus the proportion of cases that can be prevented by PCVs is not known. Furthermore, it is impossible to describe with accuracy a characteristic radiological picture of pneumococcal pneumonia (Cherian et al., 2005). Therefore, predicting the impact of PCVs on pneumonia was not possible prior to the initiation of PCV studies. The maximal effect of PCVs was expected in alveolar pneumonia (lobar consolidation). In contrast, clinical pneumonia cases without consolidation were expected to be reduced only minimally, if at all (Cherian et al., 2005).

One major surprise following PCV studies, and later after PCV introduction, was the very prominent effect of the vaccines on rates of lower respiratory infection without consolidation. The first suggestion that protection against lower respiratory infections could be achieved in cases without even a clear picture of pneumonia was the observation in a daycare study in Beer-Sheva, Israel, where the effect of a nine-valent PCV (containing serotypes 1, 4, 5, 6B, 9 V, 14, 18C, 19F, and 23F; PCV9) on lower respiratory infections was compared with a control vaccine (Dagan et al., 2001). A 23% reduction in occurrence of lower respiratory infections (mainly bronchitis/bronchiolitis) was observed in vaccine recipients compared to controls (Fig. 1). Subsequently, a comprehensive double-blind, placebo-controlled study conducted in South Africa demonstrated that PCV9 provided 31–50% protection against admissions with 'viral pneumonia' in children younger than 2 years (Madhi et al., 2004; Madhi et al., 2006). Viral pneumonia was defined in this study as any admission with pneumonia in which a virus was detected. The efficacy was 34%, 32%, 41%, 31%, and 50% in cases of pneumonia associated with influenza virus, RSV, parainfluenza virus, adenovirus, and metapneumovirus, respectively (Madhi et al., 2004; Madhi et al., 2006).

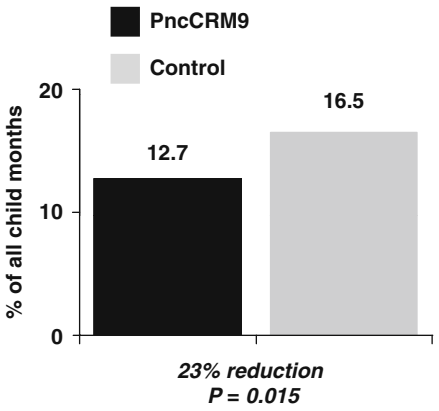


Fig. 1 Reduction in lower respiratory morbidity in daycare center attendees (aged under 15–35 months) receiving PCV9 versus those receiving control vaccine (Dagan et al., 2001)

Further analysis of the South African set of data revealed that if alveolar consolidation alone had been considered in efficacy studies, much of the effect of PCV on reduction of pneumonia disease burden would have been missed (Madhi et al., 2005). Vaccine efficacy against a first episode of chest X-ray confirmed that alveolar pneumonia was 20% (95% CI 3-35) in HIV-negative children aged <2.5 years. This could be translated to 100 cases of alveolar pneumonia that were prevented per 100,000 vaccinated children. However, if 'all' cases of clinical pneumonia (regardless of chest X-ray findings) are considered, the efficacy was not much lower (17%; 95% CI 7–26), but since now a much larger group of children are considered, an additional 167 cases per 100,000 vaccinated children could be prevented. This study taught us that pneumococcal pneumonia can take many additional forms to alveolar (or lobar) pneumonia, and that PCVs may be preventing more cases of pneumonia than expected.

Confirmation of this new insight came after introduction of PCV7 in the US. PCV7 reduced unexpectedly high number of cases of pneumonia in all ages annually (Grijalva et al., 2007). For example, for children <2 years, hospital admissions for all-cause pneumonia were reduced by 506 cases per 100,000 children, extrapolated to a total of 41,000 cases annually in the US. As a comparison, the annual reduction of admission for invasive pneumococcal disease (IPD) in this age group was estimated to be 3,000 – over tenfold fewer than the reduction in pneumonia admissions (Dagan et al., 2001).

Thus, using vaccine probes, several lessons could be learned from studying PCVs in regard to pneumonia: (1) *S. pneumoniae* can be involved in lower respiratory infections even in X-ray-negative cases; (2) pneumococcal pneumonia does not present uniquely as alveolar pneumonia; (3) *S. pneumoniae* plays an important role as a copathogen with viruses; and (4) PCVs prevent more than tenfold more admissions for pneumonia than admissions for IPD.

3 Potential Role of PCVs in the Prevention of Mortality in Developing Populations

In The Gambia, PCV9 prevented 50% (95% CI 27–45) of all IPD, 37% (95% CI 27–45) of first episodes of radiologically proven pneumonia, and 15% (95% CI 7–21) of all-cause hospital admissions in children <30-months old.

Most importantly, this was the only study of PCVs in which the outcome of 'mortality' was studied. Surprisingly, a 16% reduction in all-cause mortality (95% CI 3–28) was demonstrated in children <30-months-old, a much higher efficacy than expected (Cutts et al., 2005). This vaccine-probe study could demonstrate both the major role of *S. pneumoniae* in infant mortality in Africa and a major potential role for PCVs in reducing mortality in this continent.

4 Indirect Effect of PCV – the Herd Immunity Effect

As stated before, the extent of the reduction in disease caused by serotypes included in PCV7 in contacts due to reduction of spread of these serotypes from vaccinees could not be predicted before introduction of the vaccine into universal immunization programs. The extensive reduction of IPD in age groups that were not vaccinated surprised a lot of skeptical people (Whitney et al., 2003). One of the most impressive set of results came from observing the incidence of IPD in the elderly (aged ≥65 years) among whom a dramatic 73% reduction in IPD incidence from an average of 33.6 cases/100,000 in the years 1998–1999 to 9.0/100,000 in 2004 was seen (Lexau et al., 2005) (Fig. 2).

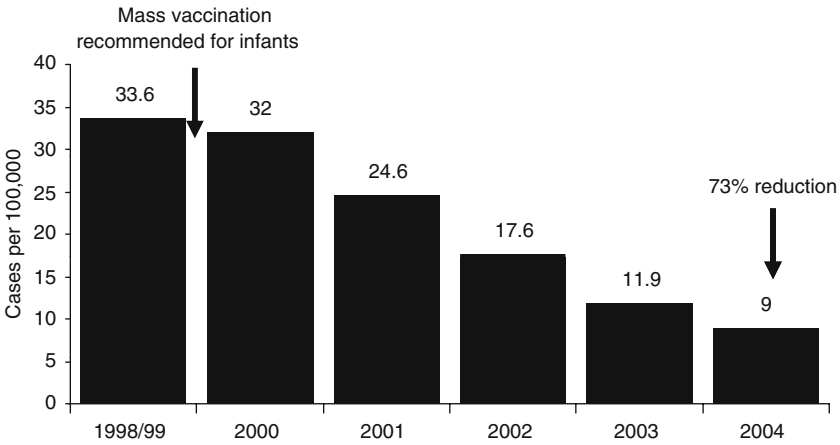


Fig. 2 An example of indirect effect (herd immunity) in adults following childhood universal vaccination in the US: rates of IPD in adults ≥ 65 years old 1998–2004 (data derived from Lexau et al., 2005, presented by Cynthia Whitney, MD, IDSA 2005)

This reduction was not seen for serotypes not included in PCV7, confirming the causative role of childhood PCV7 administration. The overall indirect effect in protection against IPD was measured in an active surveillance program conducted by the Centers for Diseases Control and Prevention (CDC) – the 'ABC' surveillance. When the number of cases of IPD caused by serotypes included in PCV7 in 2003 was compared to those in the prevaccine era, for each case prevented in a vaccinated individual ('direct PCV7 effect'), 2.2 cases were prevented in nonvaccinated contacts ('indirect effect' or 'herd immunity') (CDC, 2005). Recently, a similar effect was demonstrated in regard to admissions for all-cause pneumonia, when a reduction of 27.4 cases/100,000 (95% CI, −4.6 to −45.1) admissions for pneumonia in the age group 18–39 years could be demonstrated in 2004 compared to the prevaccine era (Grijalva et al., 2007).

By using the vaccine-probe approach important lessons were learnt: (1) young children are responsible for the transmission of a considerable amount of pneumococcal disease to adults; and (2) vaccination of young children protects adults from pneumococcal diseases, through reduction of transmission.

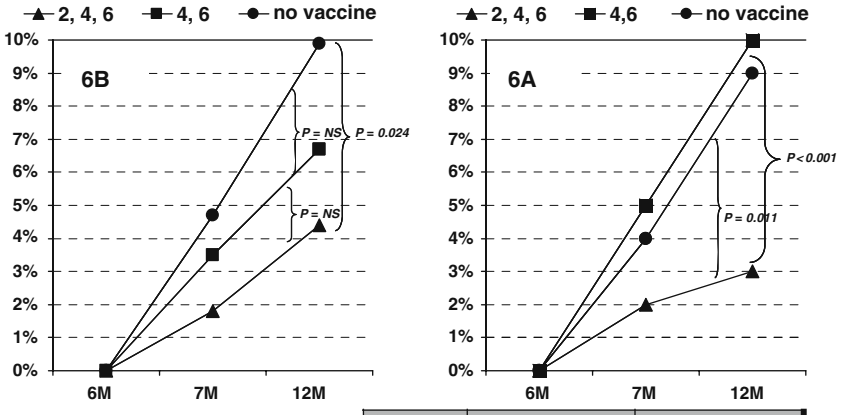
5 How Many Doses of PCV Are Appropriate for Infant Immunization Programs?

In a recent noncomparative study published in 2006, Goldblatt et al. suggested that immunogenicity of a three-dose regimen of PCV9 (primary series of two doses at 2 and 4 months and a booster dose at 12 months) resulted in antibody concentrations that were similar to a four-dose regimen (consisting of a primary series of 2, 3, and 4 months and a booster dose at 12 months). These data were the main basis for the decision of the government Dept of Health in the UK to introduce PCV7 in September 2006 to all infants as a three-dose regimen at ages 2, 4, and 13 months. However, the noncomparative nature of this study and data from other studies, suggesting that a two-dose primary series may be less immunogenic than a three-dose primary series (Lockhart et al., 2006) led us to conduct a randomized study in which the immunogenicity of a regimen with three doses for primary series (at 2, 4, and 6 months) was compared to that of two doses for the primary series (at 4 and 6 months). Anticapsular-specific IgG was tested at 7 months (1 month after primary series of two or three doses).

The proportion of children with antibody concentration ≥ 0.35 μg/ml was significantly higher for three serotypes in children receiving three primary doses compared with those receiving two doses: 6B (87% vs. 61%), 18C (96% vs. 90%), and 23F (83% vs. 70%). Furthermore, the geometric mean concentration (in μg/ml) was significantly higher in four serotypes in those receiving three doses: 6B (2.1 vs. 0.6), 14 (5.2 vs. 3.5), 18C (1.7 vs. 1.2), and 23F (1.1 vs. 0.6). Thus, our comparative study could establish that the immunogenicity of the reduced-dose regimen group at 4 and 6 months was not equivalent to that of the licensed regimen of 2, 4, and 6 months.

To test whether the observed, reduced immunogenicity in the reduced-dose regimen may be associated with any biological effect, we also studied nasopharyngeal acquisition of *S. pneumoniae* at age 7 and 12 months (1 month and 6 months after last dose) and attempted to correlate this to antibody concentrations. Only two serotypes were carried frequently enough to be tested: serotype 6B and the related serotype 6A. As can be clearly seen in Fig. 3, children receiving three doses acquired serotype 6B significantly less frequently than those not receiving any vaccine. In contrast, those receiving only two doses did not differ significantly from those not receiving vaccine, although some effect was suggested (without reaching statistical significance). When the related serotype 6A was tested, new acquisition events in infants receiving three doses were dramatically reduced compared to those not receiving any vaccine. Those receiving two doses acquired serotype 6A at an identical frequency to those not receiving any vaccine. As discussed previously, the differences in immunogenicity of serotype 6B antigen were the most dramatic among all the serotypes where the two-dose and three-dose primary series were compared. Thus, at least for the 6B component of PCV7, the two-dose regimen not only resulted in decreased anti-6B IgG but also showed inferior protection against acquisition of both serotype 6B and serotype 6A.

Thus, the vaccine probe approach taught us two important lessons: (1) The immunogenicity of the reduced two-dose regiment is not equal to that of the licensed three-dose regimen; and (2) at least for serotypes 6B and 6A, the difference between the two regimens is of enough magnitude to be associated



Group	N tested at 7m	N tested at 12m
2, 4, 6	329	307
4, 6	171	156
No PCV	172	153

Fig. 3 New acquisitions of nasopharyngeal carriage of serotype 6B and 6A per 100 children after three PCV doses (at 2, 4, and 6 months) or after two PCV doses (at 4 and 6 months) or in nonvaccinated children (Dagan et al., 2007). The numbers in the table represent the number of children tested in each group for each visit

with reduced protection against new nasopharyngeal acquisition. These findings are of importance as more countries worldwide adopt the reduced-dose regimens. However, all countries also add a booster dose during the second year of life. Evaluation of immunogenicity of these two regimens following booster and evaluation of carriage following booster is currently being conducted in our study. At this time it is not clear whether the reduced dose is indeed inferior when introduced as a universal vaccine program with a booster during the second year of life.

6 Hypothesis Generating Results: A Study on Social Mixing During Infancy and Antibody Response to PCV7 at 1 Year of Age

A study conducted by the Oxford Group (Salt et al., 2007) tested immunogenicity of one PCV7 dose given at 12 months of age. Serotype-specific, anticapsular IgG was tested 1 month later. The vaccine was immunogenic for all seven serotypes. The surprising finding was that for four serotypes, not including serotype 6B, antibodies were higher if the children had attended a daycare facility or had older siblings during the first year of life. These two factors were significantly associated with higher antibody concentrations to serotypes 4, 9 V, 14, and 23F.

This came as a surprise since natural exposure to *S. pneumoniae* is not expected to prime for a high anticapsular response. In this case, the vaccine-probe approach confronts us with an observation that needs an explanation, and any speculation about the reasons why previous social mixing improved immunogenicity needs to be further tested. The study results generate several potential explanations. The authors provide two potential hypotheses: (1) Exposure to pneumococci in the first year of life induce immunological priming by a yet unknown mechanism; or (2) differences in immunological experience (i.e., increased exposure to respiratory viral infections in early childhood) alter the response to vaccines possibly by affecting the balance between Th1 and Th2 cytokines. This case exemplifies how unexpected results may provide clues to new immunological mechanisms if thoughtful analysis is performed, as was done by the Oxford Group.

7 Conclusions

From the examples given in this chapter we learn that careful study design can provide us with new insight into pathogenesis and epidemiology of disease. Furthermore, we also learn that careful analysis may reveal unexpected results, which in turn generate additional studies. Much of our understanding of pneumococcal disease to date derives from unexpected results. Additional examples not discussed in this chapter are the replacement of carriage of the

serotypes included in PCV7 by nonvaccine serotypes (and to some extent also replacement in disease) (Hicks et al., 2007), the inability of a combined PCV7–PPV23 immunization schedule to reduce AOM cases in children with established recurrent AOM (Veenhoven et al., 2003), the reduction in antibiotic use by vaccinated children (Dagan et al., 2001; Fireman et al., 2003), and altered immunogenicity of meningococcal conjugate vaccines when coadministered with PCV9 (Buttery et al., 2005).

It confirms that we need to adopt the philosophy of Albert Einstein who said: "The most important thing is not to stop questioning"

References

Bryce, J., Boschi-Pinto, C., Shibuya, K. and Black, R.E. (2005). WHO estimates of the causes of death in children. *The Lancet*, **365**, 1147–1152.

Buttery, J.P., Riddell, A., McVernon, J., Chantler, T., Lane, L., Bowen-Morris, J., Diggle, L., Morris, R., Harnden, A., Lockhart, S., Pollard, A.J., Cartwright, K. and Moxon, E.R. (2005). Immunogenicity and safety of a combination pneumococcal–meningococcal vaccine in infants: a randomized controlled trial. *JAMA* **293**, 1751–1758.

CDC. (2005). Direct and indirect effects of routine vaccination of children with 7-valent pneumococcal conjugate vaccine on incidence of invasive pneumococcal disease - United States, 1998–2003. *MMWR*, **54**, 893–897.

Cherian, T., Mulholland, E.K., Carlin, J.B., Ostensen, H., Amin, R., de Campo, M., Greenberg, D., Lagos, R., Lucero, M., Madhi, S.A., O'Brien, K.L., Obaro, S. and Steinhoff, M.C. (2005). Standardized interpretation of paediatric chest radiographs for the diagnosis of pneumonia in epidemiological studies. *Bull World Health Organ*, **83**, 353–359.

Cutts, F.T., Zaman, S.M., Enwere, G., Jaffar, S., Levine, O.S., Okoko, J.B., Oluwalana, C., Vaughan, A., Obaro, S.K., Leach, A., McAdam, K.P., Biney, E., Saaka, M., Onwuchekwa, U., Yallop, F., Pierce, N.F., Greenwood, B.M. and Adegbola, R.A. (2005). Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia: randomised, double-blind, placebo-controlled trial. The *Lancet*, **365**, 365–1146.

Dagan, R., Givon-Lavi, N., Abu-Abed, J., Rosenblum, H. and Greenberg, D. (2007). Nasopharyngeal pneumococcal carriage in first year of life following administration of two or three doses of 7-valent CRM-conjugate vaccine [G-1241]. 47th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), September 17–20, 2007, Chicago, IL.

Dagan, R., Sikuler-Cohen, M., Zamir, O., Janco, J., Givon-Lavi, N. and Fraser, D. (2001). Effect of a conjugate pneumococcal vaccine on the occurrence of respiratory infections and antibiotic use in day-care center attendees. *Pediatr. Infect. Dis. J.*, **20**, 20–958.

Fireman, B., Black, S.B., Shinefield, H.R., Lee, J., Lewis, E. and Ray, P. (2003). Impact of the pneumococcal conjugate vaccine on otitis media. *Pediatr. Infect. Dis. J.* **22**, 22–16.

Goldblatt, D., Southern, J., Ashton, L., Richmond, P., Burbidge, P., Tasevska, J., Crowley-Luke, A., Andrews, N., Morris, R., Borrow, R., Cartwright, K. and Miller, E. (2006). Immunogenicity and boosting after a reduced number of doses of a pneumococcal conjugate vaccine in infants and toddlers. *Pediatr. Infect. Dis. J.* **25**, 25–319.

Grijalva, C.G., Nuorti, J.P., Arbogast, P.G., Martin, S.W., Edwards, K.M. and Griffin, M.R. (2007). Decline in pneumonia admissions after routine childhood immunisation with pneumococcal conjugate vaccine in the USA: a time-series analysis. *Lancet* **369**, 369–1186.

Hicks, L.A., Harrison, L.H., Flannery, B., Hadler, J.L., Schaffner, W., Craig, A.S., Jackson, D., Thomas, A., Beall, B., Lynfield, R., Reingold, A., Farley, M.M. and Whitney, C.G. (2007). Incidence of Pneumococcal Disease Due to Non-Pneumococcal Conjugate Vaccine (PCV7) Serotypes in the United States during the Era of Widespread PCV7 Vaccination, 1998–2004. *J. Infect. Dis.* **196**, 196–1354.

Lexau, C.A., Lynfield, R., Danila, R., Pilishvili, T., Facklam, R., Farley, M.M., Harrison, L.H., Schaffner, W., Reingold, A., Bennett, N.M., Hadler, J., Cieslak, P.R. and Whitney, C.G. (2005). Changing epidemiology of invasive pneumococcal disease among older adults in the era of pediatric pneumococcal conjugate vaccine. *JAMA* **294**, 294–2051.

Lockhart, S.P., Hackell, J.G. and Fritzell, B. (2006). Pneumococcal conjugate vaccines: emerging clinical information and its implications. *Expert Rev. Vaccines*, **5**, 5–564.

Madhi, S.A. and Klugman, K.P. (2007). World Health Organisation definition of "radiologically-confirmed pneumonia" may under-estimate the true public health value of conjugate pneumococcal vaccines. *Vaccine* **25**, 25–2419.

Madhi, S.A., Klugman, K.P. and The Vaccine Trialist, G. (2004). A role for *Streptococcus pnemoniae* in virus-associated pneumonia. *Nat. Med.* **10**, 10–813.

Madhi, S.A., Kuwanda, L., Cutland, C. and Klugman, K.P. (2005). The impact of a 9-valent pneumococcal conjugate vaccine on the public health burden of pneumonia in HIV-infected and -uninfected children. *Clin. Infect. Dis.* **40**, 40–1518.

Madhi, S.A., Ludewick, H., Kuwanda, L., Niekerk, N., Cutland, C., Little, T. and Klugman, K.P. (2006). Pneumococcal coinfection with human metapneumovirus. *J. Infect. Dis.* **193**, 193–1243.

Rudan, I., Tomaskovic, L., Boschi-Pinto, C. and Campbell, H. (2004). Global estimate of the incidence of clinical pneumonia among children under five years of age. *Bull. World Health Organ.* **82**, 82–903.

Salt, P., Banner, C., Oh, S., Yu, L.M., Lewis, S., Pan, D., Griffiths, D., Ferry, B. and Pollard, A. (2007). Social mixing with other children during infancy enhances antibody response to a pneumococcal conjugate vaccine in early childhood. *Clin. Vaccine Immunol.* **14**, 14–599.

Veenhoven, R., Bogaert, D., Uiterwaal, C., Brouwer, C., Kiezebrink, H., Bruin, J., lJzerman, E., Hermans, P., de Groot, R., Zegers, B., Kuis, W., Rijkers, G., Schilder, A. and Sanders, E. (2003). Effect of conjugate pneumococcal vaccine followed by polysaccharide pneumococcal vaccine on recurrent acute otitis media: a randomized study. *The Lancet*, **361**, 361–2195.

Whitney, C.G., Farley, M.M., Hadler, J., Harrison, L.H., Bennett, N.M., Lynfield, R., Reingold, A., Cieslak, P.R., Pilishvili, T., Jackson, D., Facklam, R.R., Jorgensen, J.H. and Schuchat, A. (2003). Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. *N. Engl. J. Med.* **348**, 348–1746.

New Findings in Primary Immunodeficiency

Andrew R. Gennery and Andrew J. Cant

1 Introduction

Primary immunodeficiencies (PIDs) are a group of genetically diverse diseases which affect distinct components of innate and acquired immunity, including the development and function of complement proteins, dendritic cells, granulocytes, natural killer cells, and T and B lymphocytes. The genetic basis of many of these diseases has now been established (Geha et al., 2007). Recently, a number of new single-gene defects have been discovered which confer vulnerability to multiple infections. Additionally, a whole range of disorders is being elucidated where gene defects cause pathogen-specific immunodeficiency, adding a new group of primary immunodeficiency disorders and broadening our concept of immune system failure (Fig. 1), bringing us closer to the notion that severe invasive infection with, for example, the ubiquitous organisms causing pneumococcal meningitis or herpes simplex encephalitis may not be caused by 'bad luck', but rather an inbuilt susceptibility to these agents. This brief review highlights these recent discoveries.

2 Severe Combined Immunodeficiency

Genetic defects causing absence or disordered function of T lymphocytes (and sometimes, B lymphocytes and natural killer cells) are collectively known as severe combined immunodeficiency (SCID). Without treatment, death follows within the first 12–18 months of life. The molecular causes of many forms of SCID have previously been described (Table 1). They can be broadly categorized as defects in cytokine-dependent survival signalling, defects of purine salvage, defects of T and B lymphocyte receptor recombination, and defects of pre-T receptor cell TCR and TCR signalling (Cavazzana-Calvo and Fischer,

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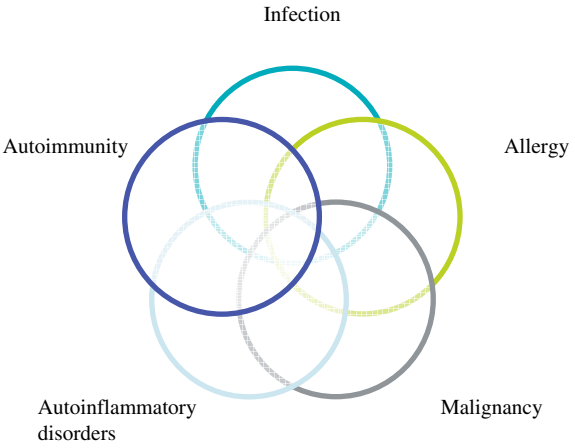

Fig. 1 Immune system failure (Reproduced with permission courtesy of the Paediatric Immunology Unit, Newcastle General Hospital)

2007). Four new causes of severe combined immunodeficiency have recently been discovered, two with defects linked to the T lymphocyte receptor signalling pathway, and two with defects in the lymphocyte receptor recombination pathway.

Table 1 Molecular defects in severe combined immunodeficiency

Category of defect	Type of SCID	Lymphocyte phenotype
Cytokine-dependent survival signalling	CγC deficiency	T-B + NK- (XL)
	JAK3 deficiency	T-B + NK- (AR)
	IL7Rα deficiency	T-B + NK +
Defects of purine salvage	ADA deficiency	T-B-NK-
	PNP deficiency	$T^{low}B^{low}NK^{low}$
Defects of T and B lymphocyte receptor recombination	RAG 1/2 deficiency	T-B-NK +
	Artemis deficiency	T-B-NK + (RS)
	DNA ligase IV	T^{low}B-NK + (RS)
	Cernunnos/XLF deficiency	T^{low}B-NK + (RS)
Defects of pre-TCR and TCR signalling	CD45 deficiency	T^{low}B + NK +
	CD3δ/CD3ε/CD3ζ deficiency	T-B + NK +
	Orai1	T + B + NK +
Others	Reticular dysgenesis	T-B-NK- (often absent neutrophils, platelets)
	Complete DiGeorge syndrome	T-B + NK +
	CHARGE syndrome	T-B + NK +

TCR, T cell receptor; CγC, common gamma chain; JAK3, janus-associated kinase 3; IL7Rα, interleukin 7 receptor alpha; ADA, adenosine deaminase; PNP, purine nucleoside phosphorylase RAG, recombinase-activating gene; RS, radiosensitive

2.1 New T Lymphocyte Receptor Signalling Defects

2.1.1 CD3 ζ Subunit Deficiency

A form of T-B + NK + severe combined immunodeficiency due to a defect of the CD3 ζ subunit of the TCR complex has been described (Roberts et al., 2007). The patient presented with a chronic cough, recurrent otitis media, and cytomegalovirus infection. Lymphocyte phenotype showed extremely low levels of normal mature, oligoclonal T lymphocytes with increased numbers of B lymphocytes and normal numbers of natural killer cells. There was no evidence of thymic production of T lymphocytes and a profound depression of T lymphocyte proliferative responses to mitogens. Serum IgA and IgM levels were elevated. The patient had a homozygous mutation in the CD3 ζ chain gene, causing a complete deficiency of the subunit of CD3, which is critical for survival and efficient transport of the T lymphocyte receptor complex from the endoplasmic reticulum to the cell plasma membrane. Surface expression of the T lymphocyte receptor complex is critical to antigen recognition and signal transduction upon ligand binding of the antigen-capture region with antigen. Lack of CD3 ζ expression leads to severe, incomplete, thymocyte development block. Previously, CD3 ζ chain deficiency, partially corrected by somatic mutations, had been described in a patient, whose phenotype was not so severe, with recurrent infection and a decreased number of peripheral T lymphocytes (Rieux-Laucat et al., 2006).

2.1.2 ORAI Deficiency

The second new form of SCID due to a defect in the TCR signalling pathway involves a defect in calcium signalling (Feske et al., 2006). The gene (*ORAI1*), identified by linkage mapping using a genome-wide SNP array screen and a genome-wide RNAi screen for NFAT regulators in *Drosophila*, encodes a structural component of membrane-calcium channels that enable calcium influx across the plasma membrane from endoplasmic reticulum calcium stores. Patients with this form of SCID have peripheral T cells which fail to activate in response to stimuli because of defective nuclear translocation of NFAT due to deranged calcium signalling.

2.2 New Defects of T and B Lymphocyte Receptor Recombination

2.2.1 DNA Ligase IV Deficiency

Two forms of severe combined immunodeficiency due to defects in the DNA repair pathway have been described recently. The first was in a girl of consanguineous Turkish parents who reached normal developmental milestones during the first year of life with no mental retardation and then developed recurrent

severe respiratory tract infections and candidiasis in the second year of life, with chronic diarrhoea, fever, and failure to thrive. She had normal NK cell numbers with very low numbers of T lymphocytes and B lymphocytes and reduced proliferative responses to phytohaemagylutin (PHA). She had low levels of IgG and IgA but normal levels of IgM. A homozygous deletion in DNA ligase 4 was found (van der Burg et al., 2006). DNA ligase 4 is required to repair DNA double-strand breaks initiated by the Recombinase Activity gene (RAG) proteins during VDJ recombination. Interestingly, a number of other patients have been described with defects in DNA ligase 4 who have presented at an older age with combined immunodeficiency and associated microcephaly. Two further reports of patients with SCID due to mutations in DNA ligase 4 have subsequently been published (Buck et al., 2006a, Enders et al., 2006). Previous reports have described immunologically normal individuals who develop leukaemia, or those with a combined immunodeficiency phenotype (Riballo et al., 1999, O'Driscoll et al., 2001).

2.2.2 Cernunnos-XLF Deficiency

A further cause of T-B-NK + SCID has also been described recently (Dai et al., 2003). A patient had T-B-NK + SCID with radio sensitivity. No defect was found in Artemis or DNA ligase 4, factors known to be associated with immunodeficiency in radio sensitivity. Subsequently, a new factor Cernunnos-XLF was discovered to be the defect in this patient as well as others (Buck et al., 2006b, Ahnesorg et al., 2006). Cernunnos-XLF interacts with DNA ligase 4 and defects disable effective VDJ recombination and T and B lymohocyte receptor formation.

3 X-Linked Lymphoproliferative Syndromes

A group of genetically inherited primary immunodeficiencies can lead to defective regulation of the immune response resulting in susceptibility to particular infections (particularly with herpes viruses), as well as haemophagocytic lymphohistiocytosis (HLH) and lymphoma. The first of these syndromes, X-linked lymphoproliferative syndrome (XLP), is characterized by susceptibility to infection by Epstein Barr virus (EBV), with HLH or lymphoma. Other manifestations include hypogammaglobulinaemia, and less commonly, vasculitis or aplastic anaemia. The genetic defect is in *SH2D1A* (Coffey et al., 1998), and patients have low numbers of natural killer T (NKT) lymphocytes. However, 20–40% of patients with XLP disease have no demonstrable gene mutation. Recently, a new form of XLP has been reported caused by mutations in *BIRC4* which encodes the X-linked inhibitor of apoptosis protein (XIAP) (Rigaud et al., 2006). Like *SH2D1A,* XIAP is required for the survival or differentiation of NKT lymphocytes, but unlike classical XLP patients, there is no impairment of 2B4-mediated cytotoxicity. XIAP patients have a similar clinical presentation

to those with XLP-1 deficiency. Patients are susceptible to EBV infection, and complications including fulminant disease and HLH. Splenomegaly and haemorrhagic colitis are features that are not described in XLP-1. Lymphoma has not yet been described in XIAP patients. Haematopoietic stem cell transplantation is curative for both XLP-1 and XLP-2.

4 Common Variable Immunodeficiency

Common variable immunodeficiency (CVID) is characterized by recurrent bacterial sinopulmonary infections, often due to encapsulated bacteria, and with bacterial gastrointestinal infection. Granulomatous disease, autoimmunity, lymphoproliferation, and malignancy are also features emphasizing the breadth of presentation of immune system failure (Fig. 1). Five distinct genetic disorders have now been described in CVID, although the underlying molecular problem has yet to be defined for the majority of patients.

4.1 Inducible Costimulator Deficiency

Inducible costimulator (ICOS) deficiency, first described in adults with CVID (Grimbacher et al., 2003), has subsequently been described in children (Salzer et al., 2004). ICOS, exclusively expressed on activated T lymphocytes, is essential for T-lymphocyte-dependent B lymphocyte responses including the switch of immunoglobulin class from IgM to IgA and IgG (class switch recombination, CSR). Lack of ICOS results in impaired germinal centre development, terminal B lymphocyte differentiation, and hypogammaglobulinaemia (Warnatz et al., 2006).

4.2 CD19 Deficiency

CD19 is only expressed on B lymphocytes, and deficiency has been described in only a few patients (van Zelm et al., 2006, Kanegane et al., 2007). CD19 deficiency leads to impaired antigen-induced B-lymphocyte activation with hypogammaglobulinaemia.

4.3 Transmembrane Activator and Calcium-Modulating Cyclophilin Ligand Interactor Deficiency

Transmembrane activator and calcium-modulating cyclophilin ligand interactor (TACI) is a tumour necrosis factor-like receptor expressed on B lymphocytes and is involved in the control of B lymphocyte apoptosis, survival, and

differentiation. Other molecules involved in this pathway include the B-cell-activating factor receptor (BAFF-R). Defects in both of these molecules have been found in CVID patients and those with IgA deficiency (Salzer et al., 2005, Castigli et al., 2005, Warnatz et al., 2005). Heterozygous mutations in TACI are found in normal individuals, as well as those with disease, and so constitute a risk factor for developing CVID. As more individuals with mutations are described, the complex interactions of molecules in this pathway, as well as the effects of specific mutations and modifier genes, are likely to become clearer.

4.4 MutS Homologue 5 Defects

The MutS homologue 5 (MSH5) is critical in DNA mismatch repair and has a role in CSR. Defects have been found in patients with CVID and associated IgA deficiency (Sekine et al., 2007), but there appears to be incomplete penetrance, as healthy controls have been found with the same mutations.

Whilst our knowledge of the pathways involved in B-lymphocyte maturation and differentiation has grown, there is still much to be learnt in relation to disease-causing genotypes and those conferring risk, as well as the interplay between the many different molecules and pathways.

5 Defects in Innate Immunity

Our understanding of defects in innate immunity has increased exponentially over the last few years, with some exciting developments. Our understanding of congenital neutropenia has improved, but new areas of biology are also revealing their roles in human immunodeficiency.

5.1 Severe Congenital Neutropenia

Severe congenital neutropenia (SCN), first described by Kostmann in a Swedish family in 1956 (Kostmann, 1956) and defined by absolute neutrophil counts $<500/\mu l$, leaves affected individuals susceptible to severe invasive bacterial and fungal infection, and development of myelodysplasia and leukaemia. Defects in neutrophil elastase (*ELA2*) causing both autosomal dominant SCN and cyclical neutropenia have been described previously (Dale et al., 2000) as have activating missense mutations in the Wiskott–Aldrich protein gene, which cause X-linked SCN (Devriendt et al. 2001). More recently, the gene, *HAX1,* mutated in the original family described by Kostmann was shown to be a cause of autosomal recessive SCN (Klein et al., 2007). HAX1, a mitochondrial protein involved in stabilizing the inner mitochondrial membrane potential, is required

to prevent mitochondrial-mediated apoptosis. Hence, HAX1-deficient individuals have increased neutrophil apoptosis. Although other cell types can also be shown to have reduced membrane potential stability, the biological effect is seen only in myeloid-derived cells.

Congenital neutropenia or neutrophil dysfunction with associated hypopigmentation is recognized in patients with Chediak–Higashi syndrome (Kaplan et al., 2008), Griscelli syndrome (Menasche et al., 2003), and Hermansky–Pudlak syndrome (Wei, 2006). The common feature of these diseases is defects in proteins which control lysosomal secretion in melanocytes and immune cells. A new defect causing skin hypopigmentation, with SCN and B lymphocyte dysfunction with short stature, has now been described in a Mennonite family. Patients suffered recurrent pneumococcal bronchopulmonary infection (Bohn et al., 2007). The affected protein, p14, is an adaptor molecule that is involved in control of late endosome trafficking. The method by which this leads to neutropenia remains unclear.

5.2 Hyperimmunoglobulin E Syndrome

Hyperimmunoglobulin E syndrome (HIES) is a congenital immunodeficiency characterized by severe dermatitis and recurrent staphylococcal skin infections. Patients lack features of typical inflammation (so-called cold abscesses) and characteristically have pneumonia due to *Staphylococcus aureus*, often with superadded aspergilloma, as well as an elevated serum IgE and eosinophilia.

5.2.1 Autosomal Dominant HIES

Two forms of disease are recognized – the first is multi-system disease where patients have concurrent skeletal, dental, and connective tissue manifestations with scoliosis, pathological fractures, and hyperextensibility of the joints. Distinctive facial appearances include coarse texture of facial skin, asymmetric facial appearance with a prominent forehead, deep set eyes, and a broad nasal bridge. Additionally, there is a delay in dental exfoliation. The molecular pathophysiology of HIES has remained elusive for more than 30 years but a genetic cause for both forms has now been described. Recently, patients with autosomal-dominant HIES were found to be defective in signal transducer and activator of transcription 3 (STAT3), downstream of Tyk2 and important in the transcription of IL-6 and –10, but not IL-12 or IFNα 9 (Minegishi et al., 2007, Holland et al., 2007). All mutations were in the SRC homology 2 or DNA-binding domains, which do not affect protein levels. Many of the systemic features found in HIES can now be explained; in mice, *stat3* deficiency in the lung leads to excessive lung inflammation and airway enlargement, consistent with pneumatocoele formation in patients. Downregulation of T_H2 cytokines including IL-6 and -10 impairs inflammatory responses, causing 'cold abscesses'.

5.2.2 Autosomal Recessive HIES

A further autosomal-recessive-inherited disease is recognized without skeletal and dental abnormalities, or pneumatocoele formation. Additionally, patients suffer from severe viral infection including chronic refractory *molluscum contagiosum* and herpes simplex virus infections. The gene defect causing this form of disease is tyrosine kinase 2 (Tyk2) deficiency (Minegishi et al., 2006). Tyk2 is a *janus kinase* family kinase involved in signalling through many cytokine pathways. Cells from affected patients show severe defects in response to type 1 cytokines including interferon (IFN) γ, IL-6,-10, -12, and -23. The predisposition to viral infections may be explained by the defects in type I (IFN) signalling.

6 Toll-Like Receptor Pathway Deficiencies

Conventionally, primary immunodeficiencies have been thought to consist of Mendelian traits conferring predisposition to multiple infectious diseases, the nature and range of which vary according to the disorder. Newly identified immunodeficiencies predisposing otherwise healthy individuals to a single or very narrow range of infections are now being recognized.

6.1 Defects in the NFκB Essential Modulator Signalling Pathway

The transcription factors of the NF-κB family are activated by signals received when innate immune responses are triggered in cells such as neutrophils and macrophages. They play an important role in regulating immune and stress responses, inflammatory reactions, and immunity to infection in humans. A number of diseases are associated with defects in this pathway including incontinentia pigmentii and ectodermal dysplasia. Patients with susceptibility to a narrow range of infections, particularly *Streptococcus pneumoniae, Staphylococcus aureus*, and weakly pathogenic mycobacteria, often with an absent inflammatory response, have now been described with mutations in a number of genes coding for proteins in this signaling pathway. The clinical entity of ectodermal dysplasia with polysaccharide-antibody deficiency in male patients was described over 10 years ago, emphasizing the importance of meticulous clinical description in the recognition of new genetic diseases (Abinun et al., 1996). Hypomorphic mutations in *NEMO*, which codes for NFκB Essential Modulator, were subsequently discovered in a number of patients (Döffinger et al., 2001), whilst stop codon mutations in the same gene are responsible for osteopetrosis and/or lymphoedema with ectodermal dysplasia and immunodeficiency (Dupuis-Girod et al., 2002). Subsequently, immunodeficiency without ectodermal dysplasia has also been described in patients with mutations in *NEMO* (Orange et al., 2004, Niehues et al., 2004). Hypermorphic mutations

in IκBα, a protein that acts downstream of NFκB, have been described in a patient with ectodermal dysplasia and immunodeficiency (Courtois et al., 2003). The mutation impairs NFκB activation, resulting in a similar clinical phenotype to *NEMO* defects. Further, a patient deficient in IκBα, who presented with ectodermal dysplasia and immunodeficiency, has also been described (Janssen et al., 2004). Invasive *S. pneumoniae* infections are also seen in patients with IRAK-4 deficiency (Ku et al., 2007). IRAK-4, a kinase downstream of toll-like receptors, is important for the onset and propagation of inflammation, and patients have a poor or delayed inflammatory response. Whilst potentially life threatening in childhood, infections are rare as patients reach adulthood.

6.2 Toll-Like Receptor Deficiency

There are10 known toll-like receptors (TLR) in humans, each of which recognizes a distinct, but limited repertoire of conserved microbial products which, when bound by the relevant TLR on neutrophils or macrophages, triggers a number of signalling pathways that involve NFκB, which activate cells, stimulate proliferation, and cause cytokine release. Defects have been discovered in one TLR and the signalling pathway. The first to be discovered was in individuals who had experienced herpes simplex encephalitis, but no other unusual infectious disease, and had controlled other viral infections. Defects were found in *UNC-93B*, which encodes for a protein expressed in the endoplasmic reticulum involved in signalling through the TLR-3, -7, -8, and -9 (Casrouge et al., 2006). More recently, defects in the gene-encoding TLR-3 have been identified in children who appear susceptible only to herpes simplex encephalitis (Zhang et al., 2007). No doubt, other pathogen-specific immunodeficiencies associated with different TLRs or the associated signalling pathways exist.

7 Regulatory T Lymphocyte-Mediated Autoimmunity

It has been recognized for some time that absence of regulatory T lymphocytes leads to the autoimmune disease, immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome in humans. This disease is characterized by severe autoimmune enteropathy and dermatitis, early onset endocrinopathies and, often autoimmune cytopenias (Bennett et al., 2001). There is an absence of $CD4+CD25^{BRIGHT}+T$ lymphocytes (Fig. 2) and no FOXP3-containing $CD4+CD25^{BRIGHT}+T$ lymphocytes are seen on flow cytometry. The IgE level is often elevated. Not all patients with IPEX syndrome have mutations in *FOXP3* and female patients with IPEX-like features are described. A further genetic defect has recently been described.

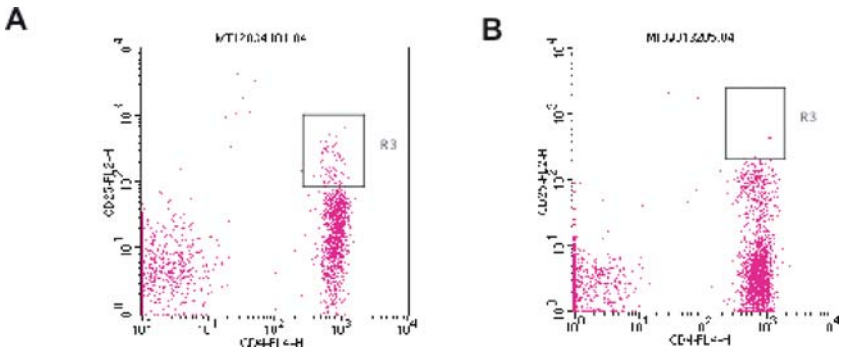

Fig. 2 Flow cytometry plots showing **A**. a normal control with CD4 + CD25^{BRIGHT} + T lymphocytes in the box R3 and **B**. absence of CD4 + CD25^{BRIGHT} + T lymphocytes in a patient with IPEX syndrome (Reproduced with permission courtesy of the Paediatric Immunology Unit, Newcastle General Hospital)

7.1 CD25 Deficiency

A defect in *CD25* was described in two different patients, neither of whom had CD25-expressing T lymphocytes (Sharfe et al., 1997, Caudy et al., 2007). Clinical features were similar to those with FOXP3 deficiency, including severe autoimmune enteropathy, other endocrinopathies, and hepatosplenomegaly with lymphadenopathy. IgE levels were only mildly elevated.

7.2 Other Immunodeficiencies Associated with Reduced Regulatory T Lymphocytes

Autoimmune polyendocrinopathy, candidiasis, and ectodermal dystrophy (APECED) are characterized by systemic autoimmunity, particularly of endocrine glands, but also, in many patients, mucocutaneous candidiasis (Perheentupa, 2006). Mutations in *AIRE*, a transcription factor required for ectopic expression of tissue-specific antigen in the thymus, are present in patients with APECED (Finnish–German APECED consortium, 1997). Regulatory T lymphocytes are derived from the thymus, and AIRE may play a role in their development (Aschenbrenner et al., 2007). Two studies in patients have found decreased numbers of regulatory T lymphocytes with decreased function (Kekäläinen et al., 2007, Ryan et al., 2005). The role of these cells in the disease is unknown. Finally, Omenn syndrome is a disease due to hypomorphic mutations in the recombinase-activating genes required for T and B lymphocyte receptor formation (Sobacchi et al., 2006). Patients present with erythroderma, hepatosplenomegaly, lymphadenopathy, and alopecia. Laboratory findings include normal or elevated peripheral blood T lymphocyte counts exhibiting a restricted

oligoclonal T lymphocyte receptor repertoire. The T lymphocytes are activated and often skewed toward a TH2 phenotype. An associated eosinophilia and high immunoglobulin E level is found, most likely secondary to the TH2-type cytokine secretion. The role of regulatory T lymphocytes has not been investigated in these patients, but in a murine model, these cells were markedly reduced (Marrella et al., 2007).

8 Conclusion

The field of immunodeficiency continues to grow, as our understanding of the interplay between immunodeficiency, immunoregulation, control of infection and malignancy increases. Further genetic discoveries are likely, but we expect further insights into gene regulation and the interplay of molecular pathways to bring further insights into human immune system failure.

References

Abinun, M., Spickett, G., Appleton, A.L., Flood, T., and Cant, A.J. (1996). Anhidrotic ectodermal dysplasia associated with specific antibody deficiency. *Eur J Pediatr*. 155:146–147.

Ahnesorg, P., Smith, P., and Jackson, S.P. (2006). XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. *Cell*. 124:301–313.

Aschenbrenner, K., D'Cruz, L.M., Vollmann, E.H., Hinterberger, M., Emmerich, J., Swee, L.K., Rolink, A., and Klein, L. (2007). Selection of Foxp3+ regulatory T cells specific for self antigen expressed and presented by Aire+ medullary thymic epithelial cells. *Nat Immunol*. 8:351–358.

Bennett, C.L., Christie, J., Ramsdell, F., Brunkow, M.E., Ferguson, P.J., Whitesell, L., Kelly, T.E., Saulsbury, F.T., Chance, P.F., and Ochs, H.D. (2001). The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet*. 27:20–21.

Bohn, G., Allroth, A., Brandes, .G., Thiel, J., Glocker, E., Schäffe, A.A., Rathinam, C., Taub, N., Tei, D., Zeidler, C., Dewey, R.A., Geffers, R., Buer, J., Huber, L.A., Welte, K., Grimbacher, B., and Klein, C. (2007). A novel human primary immunodeficiency syndrome caused by deficiency of the endosomal adaptor protein p14. *Nat Med*. 13:38–45.

Buck, D., Moshous, D., de Chasseval, R., Ma, Y., le Deist, F., Cavazzana-Calvo, M., Fischer, A., Casanova, J.L., Lieber, M.R., and de Villartay, J.P. (2006a). Severe combined immunodeficiency and microcephaly in siblings with hypomorphic mutations in DNA ligase IV. *Eur J Immunol*. 36:224–235.

Buck, D., Maliver, L., de Chasseval, R., Barraud, A., Fondanèche, M.C., Sanal, O., Plebani, A., Stéphan, J.L., Hufnagel, M., le Deist, F., Fischer, A., Durandy, A., de Villartay, J.P., and Revy, P. (2006b). Cernunnos, a novel nonhomologous end-joining factor, is mutated in human immunodeficiency with microcephaly. *Cell*. 124:287–299.

Casrouge, A., Zhang, S.Y., Eidenschenk, C., Jouanguy, E., Puel, A., Yang, K., Alcais, A., Picard, C., Mahfoufi, N., Nicolas, N., Lorenzo, L., Plancoulaine, S., Sénéchal, B., Geissmann, F., Tabeta, K., Hoebe, K., Du, X., Miller, R.L., Héron, B., Mignot, C., de Villemeur, T.B., Lebon, P., Dulac, O., Rozenberg, F., Beutler, B., Tardieu, M., Abel, L.,

and Casanova, J.L. (2006). Herpes simplex virus encephalitis in human UNC-93B deficiency. *Science*. 314:308–312.

Castigli, E., Wilson, S.A., Garibyan, L., Rachid, R., Bonilla, F., Schneider, L., and Geha, R.S. (2005). TACI is mutant in common variable immunodeficiency and IgA deficiency. *Nat Genet*. 37:829–834.

Caudy, A.A., Reddy, S.T., Chatila, T., Atkinson, J.P., and Verbsky, J.W. (2007). CD25 deficiency causes an immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like syndrome, and defective IL-10 expression from CD4 lymphocytes. *J Allergy Clin Immunol*. 119:482–487.

Cavazzana-Calvo M., and Fischer, A. (2007). Gene therapy for severe combined immunodeficiency: are we there yet? *J Clin Invest*. 117:1456–1465

Coffey, A.J., Heath, P., Wray, P., Pavitt, R., Wilkinson, J., Leversha, M., Huckle, E., Shaw-Smith, C.J., Dunham, A., Rhodes, S., Schuster, V., Porta, G., Yin, L., Serafini, P., Sylla, B., Zollo, M., Franco, B., Bolino, A., Seri, M., Lanyi, A., Davis, J.R., Webster, D., Harris, A., Lenoir, G., de St Basile, G., Jones, A., Behloradsky, B.H., Achatz, H., Murken, J., Fassle,r R., Sumegi, J., Romeo, G., Vaudin, M., Ross, M.T., Meindl, A., and Bentley, D.R. (1998). Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet*. 20:129–135.

Courtois, G., Smahi, A., Reichenbach, J., Döffinger, R., Cancrini, C., Bonnet, M., Puel, A., Chable-Bessia, C., Yamaoka, S., Feinberg, J., Dupuis-Girod, S., Bodemer, C., Livadiotti, S., Novelli, F., Rossi, P., Fischer, A., Israël, A., Munnich, A., Le Deist, F., and Casanova, J.L. (2003). A hypermorphic IkappaBalpha mutation is associated with autosomal dominant anhidrotic ectodermal dysplasia and T cell immunodeficiency. *J Clin Invest*. 112:1108–1115.

Dai, Y., Kysela, B., Hanakahi, L.A., Manolis, K., Riballo, E., Stumm, M., Harville, T.O., West, S.C., Oettinger, M.A., and Jeggo, P.A. (2003). Nonhomologous end joining and V(D)J recombination require an additional factor. *Proc Natl Acad Sci U S A*. 100:2462–2467.

Dale, D.C., Person, R.E., Bolyard, A.A., Aprikyan, A.G., Bos, C., Bonilla, M.A., Boxer, L.A., Kannourakis, G., Zeidler, C., Welte, K., Benson, K.F., and Horwitz, M. (2000). Mutations in the gene encoding neutrophil elastase in congenital and cyclic neutropenia. *Blood*. ;96:2317–2322.

Devriendt, K., Kim, A.S., Mathijs, G., Frints, S.G., Schwartz, M., Van Den Oord, J.J., Verhoef, G.E., Boogaerts, M.A., Fryns, J.P., You, D., Rosen, M.K., and Vandenberghe, P. (2001). Constitutively activating mutation in WASP causes X-linked severe congenital neutropenia. *Nat Genet*. 27:313–317.

Döffinger, R., Smahi, A., Bessia, C., Geissmann, F. Feinberg, J., Durandy, A., Bodemer, C., Kenwrick, S., Dupuis-Girod, S., Blanche, S., Wood, P., Rabia, S.H., Headon, D.J., Overbeek, P.A., Le Deist, F., Holland, S.M., Belani, K., Kumararatne, D.S., Fischer, A., Shapiro, R., Conley, M.E., Reimund, E., Kalhoff, H., Abinun, M., Munnich, A., Israël, A., Courtois, G., and Casanova, J.L. (2001). X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-kappaB signaling. *Nat Genet*. 27:277–285.

Dupuis-Girod, S., Corradini, N., Hadj-Rabia, S., Fournet, J.C., Faivre, L., Le Deist, F., Durand, P., Döffinger, R., Smahi, A., Israel, A., Courtois, G., Brousse, N., Blanche, S., Munnich, A., Fischer, A., Casanova, J.L., Bodemer, C. (2002). Ostespetrosis, lymphedema, anhidrotic ectodermal dysplasia, and immunodeficiency in a boy and incartinentia pigmenti in his mother. Pediatrics. Jun; 109(6):e97.

Enders, A., Fisch, P., Schwarz, K., Duffner, U., Pannicke, U., Nikolopoulos, E., Peters, A., Orlowska-Volk, M., Schindler, D., Friedrich, W., Selle, B., Niemeyer, C., and Eh,l S. (2006). A severe form of human combined immunodeficiency due to mutations in DNA ligase IV. *J Immunol*. 176:5060–5068.

Feske, S., Gwack, Y., Prakriya, M., Srikanth, S., Puppel, S.H., Tanasa, B., Hogan, P.G., Lewis, R.S., Daly, M., and Rao, A. (2006). A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature*. 441:179–185.

Geha, R.S., Notarangelo, L.D., Casanova, J.L., Chapel, H., Conley, M.E., Fischer, A., Hammarström, L., Nonoyama, S., Ochs, H.D., Puck, J.M., Roifman, C., Seger, R., and Wedgwood, J.; International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee. (2007). Primary immunodeficiency diseases: an update from the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee. *J Allergy Clin Immunol*. 120:776–794.

Grimbacher, B., Hutloff, A., Schlesier, M., Glocker, E., Warnatz, K., Dräger, R., Eibel, H., Fischer, B., Schäffe, A.A., Mages, H.W., Kroczek, R.A., and Peter, H.H. (2003). Homozygous loss of ICOS is associated with adult-onset common variable immunodeficiency. *Nat Immunol*. 4:261–268.

Holland, S.M., DeLeo, F.R., Elloumi, H.Z., Hsu, A.P., Uzel, G., Brodsky, N., Freeman, A.F., Demidowich, A., Davis, J., Turner, M.L., Anderson, V.L., Darnell, D.N., Welch, P.A., Kuhns, D.B., Frucht, D.M., Malech, H.L., Gallin, J.I., Kobayashi, S.D., Whitney, A.R., Voyich, J.M., Musser, J.M., Woellner, C., Schäffer, A.A., Puck, J.M., and Grimbacher, B. (2007). STAT3 mutations in the hyper-IgE syndrome. *N Engl J Med*. 357:1608–1619.

Janssen, R., van Wengen, A., Hoeve, M.A., ten Dam, M., van der Burg, M., van Dongen, J., van de Vosse, E., van Tol, M., Bredius, R., Ottenhoff, T.H., Weemaes, C., van Dissel, J.T., and Lankester, A. (2004). The same IkappaBalpha mutation in two related individuals leads to completely different clinical syndromes. *J Exp Med*. 200:559–568.

Kanegane, H., Agematsu, K., Futatani, T., Sira, M.M., Suga, K., Sekiguchi, T., van Zelm, M.C., Miyawaki, T. (2007). Novel mutations in a Japanese patient with CD19 deficiency. Genes Immun. Dec; 8(8):663–670.

Kaplan, J., De Domenico, I., and Ward, D.M. (2008). Chediak-Higashi syndrome. *Curr Opin Hematol*. 15:22–29.

Kekäläinen, E., Tuovinen, H., Joensuu, J., Gylling, M., Franssila, R., Pöntynen, N., Talvensaari, K., Perheentupa, J., Miettinen, A., and Arstila, T.P. (2007). A defect of regulatory T cells in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J Immunol*. 178:1208–1215.

Klein,C., Grudzie, M., Appaswamy, G., Germeshausen, M., Sandrock, I., Schäffer, A.A., Rathinam, C., Boztug, K., Schwinzer, B., Rezaei, N., Bohn, G., Melin, M., Carlsson, G., Fadeel, B., Dahl, N., Palmblad, J., Henter, J.I., Zeidler, C., Grimbacher, B., and Welte, K. (2007). HAX1 deficiency causes autosomal recessive severe congenital neutropenia (Kostmann disease). *Nat Genet*. 39:86–92.

Kostmann, R. (1956). Infantile genetic agranulocytosis; agranulocytosis infantilis hereditaria. *Acta Paediatr*. 45(Suppl 105):1–78.

Ku, C.L., von Bernuth, H., Picard, C., Zhang, S.Y., Chang, H.H., Yang, K., Chrabieh, M., Issekutz, A.C., Cunningham, C.K., Gallin, J., Holland, S.M., Roifman, C., Ehl, S., Smart, J., Tang, M., Barrat, F.J., Levy, O., McDonald, D., Day-Good, N.K., Miller, R., Takada, H., Hara, T., Al-Hajjar, S., Al-Ghonaium, A., Speert, D, Sanlaville, D., Li, X., Geissmann, F., Vivier, E., Maródi, L., Garty, B.Z., Chapel, H., Rodriguez-Gallego, C., Bossuyt, X., Abel, L., Puel, A., and Casanova, J.L. (2007). Selective predisposition to bacterial infections in IRAK-4-deficient children: IRAK-4-dependent TLRs are otherwise redundant in protective immunity. *J Exp Med*. 204: 2407–2422.

Marrella, V., Poliani, P.L., Casati, A., Rucci, F., Frascoli, L., Gougeon, M.L., Lemercier, B., Bosticardo, M., Ravanini, M., Battaglia, M., Roncarolo, M.G., Cavazzana-Calvo, M., Facchetti, F., Notarangelo, L.D., Vezzoni, P., Grassi, F., and Villa, A. (2007). A hypomorphic R229Q Rag2 mouse mutant recapitulates human Omenn syndrome. *J Clin Invest*. 117:1260–1269.

Menasche, G., Feldmann, J., Houdusse, A., Desaymard, C., Fischer, A., Goud, B.,and de Saint Basile, G. (2003). Biochemical and functional characterization of Rab27a mutations occurring in Griscelli syndrome patients. *Blood*. 2003 101:2736–2742.

Minegishi,Y., Saito, M., Morio, T., Watanabe, K., Agematsu, K., Tsuchiya, S., Takada, H., Hara, T., Kawamura, N., Ariga, T., Kaneko, H., Kondo, N., Tsuge, I., Yachie, A., Sakiyama, Y., Iwata, T., Bessho, F., Ohishi, T., Joh, K., Imai, K., Kogawa, K., Shinohara, M., Fujieda, M., Wakiguchi, H., Pasic, S., Abinun, M., Ochs, H.D., Renner, E.D., Jansson, A., Belohradsky, B.H., Metin, A., Shimizu, N., Mizutani, S., Miyawaki, T., Nonoyama, S., and Karasuyama, H. (2006). Human tyrosine kinase 2 deficiency reveals its requisite roles in multiple cytokine signals involved in innate and acquired immunity. *Immunity*. 25:745–755.

Minegishi, Y., Saito, M., Tsuchiya, S., Tsuge, I., Takada, H., Hara, T., Kawamura, N., Ariga, T., Pasic, S., Stojkovic, O., Metin, A., and Karasuyama, H. (2007). Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. *Nature*. 448:1058–1062.

Niehues, T., Reichenbach, J., Neubert, J., Gudowius, S., Puel, A., Horneff, G., Lainka, E., Dirksen, U., Schroten, H., Döffinger, R., Casanova, J.L., and Wahn, V. (2004). Nuclear factor kappaB essential modulator-deficient child with immunodeficiency yet without anhidrotic ectodermal dysplasia. *J Allergy Clin Immunol*. 114:1456–1462.

O'Driscoll, M., Cerosaletti, K.M., Girard, P.M., Dai, Y., Stumm, M., Kysela, B., Hirsch, B., Gennery, A., Palmer, S.E., Seidel, J., Gatti, R.A., Varon, R., Oettinger, M.A., Neitzel, H., Jeggo, P.A., and Concannon, P. (2001). DNA ligase IV mutations identified in patients exhibiting developmental delay and immunodeficiency. *Mol Cell*. 8:1175–1185.

Orange, J.S., Levy, O., Brodeur, S.R., Krzewski, K., Roy, R.M., Niemela, J.E., Fleisher, T.A., Bonilla, F.A., and Geha, R.S. (2004). Human nuclear factor kappa B essential modulator mutation can result in immunodeficiency without ectodermal dysplasia. *J Allergy Clin Immunol*. 114:650–656.

Perheentupa, J. (2006). Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J Clin Endocrinol Metab*. 91:2843–2850.

Riballo, E., Critchlow, S.E., Teo, S.H., Doherty, A.J., Priestley, A., Broughton, B., Kysela, B., Beamish, H., Plowman, N., Arlett, C.F., Lehmann, A.R., Jackson, S.P., and Jeggo, P.A. (1999). Identification of a defect in DNA ligase IV in a radiosensitive leukaemia patient. *Curr Biol*. 9:699–702.

Rieux-Laucat, F., Hivroz, C., Lim, A., Mateo, V., Pellier, I., Selz, F., Fischer, A., and Le Deist, F. (2006). Inherited and somatic CD3zeta mutations in a patient with T-cell deficiency. *N Engl J Med*. 354:1913–1921.

Rigaud, S., Fondanèche, M.C., Lamber, N., Pasquier, B., Mateo, V., Soulas, P., Galicier, L., Le Deist, F., Rieux-Laucat, F., Revy, P., Fischer, A., de Saint Basile, G., and Latour, S. (2006). XIAP deficiency in humans causes an X-linked lymphoproliferative syndrome. *Nature*. 444:110–114.

Roberts. J.L., Lauritsen, J.P., Cooney, M., Parrott, R.E., Sajaroff, E.O., Win, C.M., Keller, M.D., Carpenter, J.H., Carabana, J., Krangel, M.S., Sarzotti, M., Zhong, X.P., Wiest, D.L., and Buckley, R.H0. (2007). T-B + NK + severe combined immunodeficiency caused by complete deficiency of the CD3zeta subunit of the T-cell antigen receptor complex. *Blood*. 109:3198–3206.

Ryan, K.R., Lawson, C.A., Lorenzi, A.R., Arkwright, P.D., Isaacs, J.D., and Lilic, D. (2005). CD4 + CD25 + T-regulatory cells are decreased in patients with autoimmune polyendocrinopathy candidiasis ectodermal dystrophy. *J Allergy Clin Immunol*. 116:1158–1159.

Salzer, U., Maul-Pavicic, A., Cunningham-Rundles, C., Urschel, S., Belohradsky, B.H., Litzman, J., Holm, A., Franco, J.L., Pleban, A., Hammarstrom, L., Skrab, A., Schwinger, W., and Grimbacher, B. (2004). ICOS deficiency in patients with common variable immunodeficiency. *Clin Immunol*. 113:234–240.

Salzer, U., Chapel, H.M. Webster, A.D., Pan-Hammarström, Q., Schmitt-Graeff, A., Schlesier, M., Peter, H.H., Rockstroh, J.K., Schneider, P., Schäffer, A.A., Hammarström, L., and Grimbacher, B. (2005). Mutations in TNFRSF13B encoding TACI are associated with common variable immunodeficiency in humans. *Nat Genet*. 37:820–828.

Sekine, H., Ferreira, R.C., Pan-Hammarström, Q., Graham, R.R., Ziemba, B., de Vries, S.S., Liu, J., Hippen, K., Koeuth, T., Ortmann, W., Iwahori, A., Elliott, M.K., Offer, S., Skon, C., Du, L., Novitzke, J., Lee, A.T., Zhao, N., Tompkins, J.D., Altshuler, D., Gregersen, P.K., Cunningham-Rundles, C., Harris, R.S., Her, C., Nelson, D.L., Hammarström, L., Gilkeson, G.S., and Behrens, T.W. (2007). Role for Msh5 in the regulation of Ig class switch recombination. *Proc Natl Acad Sci U S A*. 104:7193–7198.

Sharfe, N., Dadi, H.K., Shahar, M., and Roifman, C.M. (1997). Human immune disorder arising from mutation of the alpha chain of the interleukin-2 receptor. *Proc Natl Acad Sci U S A*. 94:3168–3171.

Sobacchi, C., Marrella, V., Rucci, F., Vezzoni, P., and Villa, A. (2006). RAG-dependent primary immunodeficiencies. *Hum Mutat*. 27:1174–1184.

van der Burg, M., van Veelen, L.R., Verkaik, N.S., Wiegant, W.W., Hartwig, N.G., Barendregt, B.H., Brugmans, L., Raams, A., Jaspers, N.G., Zdzienicka, M.Z., van Dongen, J.J., and van Gent, D.C. (2006). A new type of radiosensitive T-B-NK + severe combined immunodeficiency caused by a LIG4 mutation. *J Clin Invest*. 116:137–145.

van Zelm, M.C., Reisli, I., van der Burg, M., Castaño, D., van Noesel, C.J., van Tol, M.J., Woellner, C., Grimbacher, B., Patiño, P.J., van Dongen, J.J., and Franco, J.L. (2006). An antibody-deficiency syndrome due to mutations in the CD19 gene. *N Engl J Med*. 354:1901–1912.

Warnatz, K., Salzer,U., Gutenberger, S., Schlesier, M., Grimbacher,B., Peter, H.H., and Eibel, H. (2005). Finally Found: Human BAFF-R Deficiency Causes Hypogammaglobulinemia. *Clin Immunol*. 115 (Suppl 1): S20

Warnatz, K., Bossaller, L., Salze,r U., Skrabl-Baumgartner, A., Schwinger, W., van der Burg, M., van Donge, J.J., Orlowska-Volk, M., Knoth, R., Durandy, A., Draeger, R, Schlesier, M., Peter, H.H., and Grimbacher, B. (2006). Human ICOS deficiency abroga.tes the germinal center reaction and provides a monogenic model for common variable immunodeficiency. *Blood*. 107:3045–3052.

Wei, M.L. (2006). Hermansky-Pudlak syndrome: a disease of protein trafficking and organelle function. *Pigment Cell Res*. 19:19–42.

Zhang, S.Y., Jouanguy, E., Ugolini, S., Smahi, A., Elain, G., Romero, P., Segal, D., Sancho-Shimizu, V., Lorenzo, L., Puel, A., Picard, C., Chapgier, A., Plancoulaine, S., Titeux, M., Cognet, C., von Bernuth, H., Ku, C.L., Casrouge, A., Zhang, X.X., Barreiro, L., Leonard, J., Hamilton, C., Lebon, P., Héron, B., Vallée, L., Quintana-Murci, L., Hovnanian, A., Rozenberg, F., Vivier, E., Geissmann, F., Tardieu, M., Abel, L., and Casanova, J.L. (2007). TLR3 deficiency in patients with herpes simplex encephalitis. *Science*.317:1522–1527.

Routine Use of Influenza Vaccine

David Isaacs

1 Background

Influenza is a contagious illness of huge global importance. About 20% of children and 5% of adults worldwide develop symptomatic influenza A or B each year (Nicholson et al., 2003). Influenza can be asymptomatic or can cause a broad range of illness. In children, influenza causes various respiratory syndromes, including otitis media, croup, bronchiolitis, bronchitis, asthma, and pneumonia that can vary from mild to fulminant primary viral or secondary bacterial pneumonia. The most common clinical picture in hospitalized children in Finland was high fever, cough, and rhinorrhoea (Peltola et al., 2003). Children may also be hospitalized for febrile convulsions and encephalitis (Peltola et al., 2003; Centers for Disease Control and Prevention (CDC), 2003; Bueving et al., 2005; Poehling et al., 2006; Nguyen-Van-Tam, 2006), and children with underlying neurological problems have an increased mortality from influenza (CDC, 2003). Influenza is underdiagnosed in young children, for whom outpatient visits associated with influenza are 10–250 times as common as hospitalizations in the US, but few influenza infections are recognized clinically (Poehling et al., 2006).

2 Virology

Influenza viruses are RNA viruses that exhibit great diversity. There are three types of influenza viruses: A, B, and C, but only types A and B cause widespread outbreaks. Influenza A viruses are classified into subtypes based on antigenic differences between their two surface glycoproteins, haemagglutinin and neuraminidase. There are 15 described haemagglutinin subtypes (H1–H15) and nine neuraminidase subtypes (N1–N9). Viruses of all haemagglutinin and

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neuraminidase subtypes have been recovered from aquatic birds, but only three haemagglutinin subtypes (H1, H2, and H3) and two neuraminidase subtypes (N1 and N2) have established stable lineages in the human population since 1918. Only one subtype of haemagglutinin and one of neuraminidase are recognized for influenza B viruses (Nicholson et al., 2003).

3 Epidemiology

The epidemiological behaviour of influenza in humans is a result of antigenic drift and antigenic shift. These occur due to antigenic variation of the envelope glycoproteins, haemagglutinin, and neuraminidase.

During antigenic drift, new strains of virus evolve by accumulation of point mutations in the surface glycoproteins. The new strains are antigenic variants but are related to those circulating during preceding epidemics. This feature enables the virus to evade immune recognition, leading to repeated outbreaks during interpandemic years.

Antigenic shift occurs with the emergence of a 'new', potentially pandemic, influenza A virus that possesses a novel haemagglutinin alone or with a novel neuraminidase. The new virus is antigenically distinct from earlier human viruses and could not have arisen from them by mutation. Antigenic shift can arise when genes for one or both surface glycoproteins, usually the haemagglutinin, are introduced into people by direct transmission of an avian virus from birds 'and' there are mutations which make the avian virus able to replicate in man and transmissible between humans. In the case of the current H5N1 virus, direct infection of humans has occurred but there have not yet been the necessary mutations to allow efficient human–to-human transmission and spread has been limited. An alternative means of antigenic shift could occur when avian and human virus genetically reassort in a species (e.g. pig), which can support replication of both viruses.

4 Transmission

Most influenza infections are spread through virus-laden droplets expelled by coughing or sneezing. Although the initial site of replication is probably tracheobronchial-ciliated epithelium, the whole respiratory tract can be involved. Virus can be detected in secretions shortly before the onset of illness, usually within 24 h. The viral load rises to a peak after a day, remains high for 24–72 h, and falls to low values by the fifth day. In young children, high titre virus is shed for longer, and virus can be recovered up to several weeks after symptom onset.

5 Severity of Influenza in Children

Young children are more likely to catch influenza than older children and adults (Nicholson et al., 2003). Our early understanding of the burden of influenza in children comes from a series of large, prospective, community studies of children and their families, carried out in the 1960s and 1970s in the USA (Nguyen-Van-Tam, 2006). The studies in Tecumseh (Monto and Kioumehr, 1975), Seattle (Hall, Cooney and Fox, 1973), Houston (Frank et al., 1983), and elsewhere combined clinical surveillance with varying degrees of virus isolation and serology. They consistently showed that the highest serological attack rates for influenza each season occur in children (typically 15–40%) compared with adults (12–20%), although there is far less consensus on whether the peak rate is in teenagers, school age children (5–11 years), or children (<5 years).

Children have very high rates of hospitalization for influenza. Initial attempts to quantify hospital admissions in children due to influenza used retrospective data and compared the excess hospitalization rate in epidemic compared with non-epidemic years. However, other viruses, particularly RSV, can cause winter respiratory epidemics, and early studies did not consider the potential confounding effect of concurrent RSV infections. However, recent studies have incorporated data on the timing of influenza and respiratory syncytial virus (RSV) infections when calculating the probable incidence of influenza from excess winter hospitalizations. These studies have shown very high rates in children. The US studies from the 1990s (Neuzil et al., 2000; Izurieta et al., 2000; O'Brien et al., 2004) are summarized in Table 1. The rates in children <5 years are comparable to those in the elderly. The highest rate in children was in infants <12-months old and the rate in children <6 months was over 1% and only exceeded by the rate in adults >85-years old (Neuzil et al., 2000; Izurieta et al., 2000; O'Brien et al., 2004; Iwane et al., 2004; Grijalva et al., 2006; Thompson et al., 2004). The highest reported rates of hospitalization for children with influenza were from Hong Kong: 2800 per

Table 1 Estimated rates of hospitalization per 100,000 due to influenza by age in the 1990s in the USA (Neuzil et al., 2000; Izurieta et al., 2000; O'Brien et al., 2004; Iwane et al., 2004; Grijalva et al., 2006; Thompson et al., 2004)

Age	Hospitalizations per 100,000
<6 months	1000
6–11 months	500
1 to <3 years	190
3 to <5 years	90
5 to <15 years	40
15–49 years	20
50–64 years	80
65–69 years	200

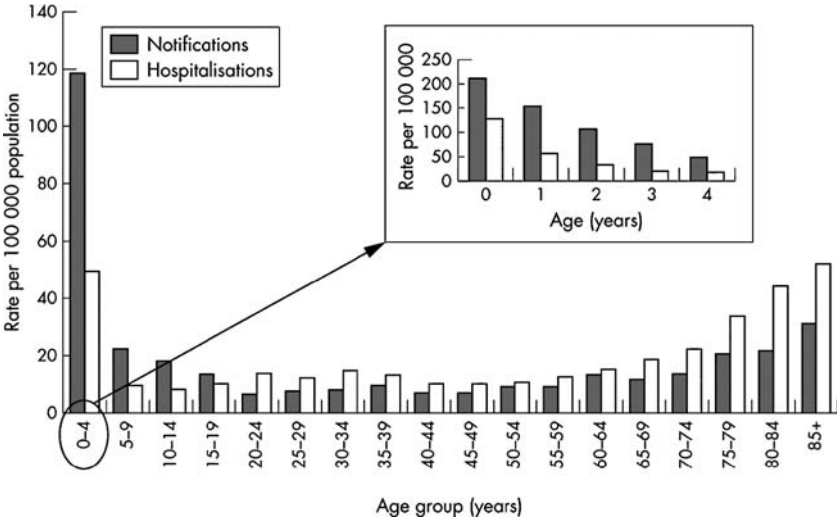

Fig. 1 Influenza notification rates 2002 and hospitalization rates 2000–02, Australia, by age group (Chiu et al., 2002)

100,000 (2.8%) in children <1 year of age, 2100 in children 1 to <2 years of age; and 1000 per 10,000 children 2 to <5 years of age (Chiu et al., 2002). The rates in Sydney, calculated using the same methods, were intermediate between Hong Kong and the USA (see Fig. 1) (Beard et al., 2006). It is unclear why the rates of excess hospitalization in Hong Kong are so much higher than in Sydney and in the USA. Possible explanations include other undiagnosed virus infections causing admission, true differences in the incidence of influenza in children in different countries and different thresholds for hospital admission.

Whatever the reason for the differences, an important message is that children and particularly infants have very high rates of hospitalization for influenza.

In contrast, the mortality from influenza is many times higher in the elderly than in children (Bhat et al., 2003; Advisory Committee on Immunization Practices, Smith et al., 2006). Although children do occasionally die from influenza, the rate in non-pandemic years in the USA is lower in children than at any other age (see Table 2). It is estimated that an average of 92 children

Table 2 Estimated death rate per 100,000 from influenza by age in the USA in inter-pandemic years (Bhat et al., 2003; Advisory Committee on Immunization Practices, Smith et al., 2006)

Age in years	Deaths per 100,000	Average number of deaths per year
<5	0.4	93
0–49	0.4–0.6	
50–64	7.5	
≥65	98.3	32,651

<5 years died annually from influenza in the 1990s in the USA compared with an average of 32,651 deaths a year in adults >65 years (Bhat et al., 2003; Advisory Committee on Immunization Practices; Smith et al., 2006).

6 Children as a Source of Infection

The North American community studies provide convincing data that children introduce and spread influenza infection in households and communities (Nguyen-Van-Tam, 2006; Monto and Kioumehr, 1975; Hall et al., 1973; Frank et al., 1983; Neuzil et al., 2000.

The elderly may be particularly vulnerable to infection introduced by children. An epidemiological study (Reichert et al., 2001) retrospectively reviewed deaths in Japan and the United States from 1949 to 1998. In the USA, where the elderly are routinely immunized against influenza, death rates were nearly constant over time. In Japan, where the elderly are not routinely immunized against influenza, excess winter deaths attributed to influenza were 3–4 times higher than those in the USA. After introducing a vaccination program for schoolchildren in Japan, excess mortality rates in the elderly dropped to values similar to those in the USA. The authors estimated that vaccination of Japanese children prevented about 37,000–49,000 deaths of elderly persons per year, or about 1 death for every 420 children vaccinated. When the vaccination of schoolchildren was discontinued, the excess mortality rates in Japan increased (Reichert et al., 2001).

Mathematical modelling using a stochastic simulation model of influenza transmission, clinical illness, and economic costs was used to estimate the population-wide benefits of routinely vaccinating US children (ages 6 months–18 years) against influenza (Weycker et al., 2005). Disease burden was projected to decline as a result of both reduced susceptibility to infection among vaccinated children and reductions in disease transmission to others in the community. It was estimated that vaccination of 20% of children would reduce the total number of influenza cases in the US by 46%; and 80% coverage would reduce the total by 91%. It was estimated that there would be similar reductions in influenza-related mortality and economic costs (Weycker et al., 2005).

Immunizing all children ought to be an attractive option for reducing influenza in the population. What is the evidence that it works?

[Question: For the population, does immunizing children against influenza, compared with not immunizing them, reduce the incidence of influenza in adults? Literature review: A search revealed eight randomized controlled trials and a non-Cochrane systematic review (Jordan et al., 2006). The results suggested possible benefit but were inconclusive.]

A systematic review examined the evidence of the effectiveness of vaccinating healthy children to protect others (Jordan et al., 2006). The authors of the

review found eight randomized controlled trials (RCTs), three community studies, and three economic evaluations that met their inclusion criteria. The quality of the studies was variable and often weak. The results were presented in a range of different ways and used different methods for diagnosis of illness, and were not suitable for combined analysis. The authors concluded that the evidence suggests vaccinating healthy children against influenza has the potential for reducing the impact of influenza epidemics, but further evidence is needed because limitations of study design or execution mean that the community benefits are difficult to quantify (Jordan et al., 2006).

7 Influenza and Otitis Media

Influenza infection is associated with acute otitis media, but other respiratory viruses such as rhinoviruses and RSV are far more common causes (Heikkinen et al., 1999; Nokso-Koivisto et al., 2004; Bulut et al., 2006). Viruses can be detected by culture of middle-ear fluid or nasopharyngeal aspirate on the same specimens by rapid tests such as immunofluorescence, ELISA, and polymerase chain reaction (PCR) (Heikkinen et al., 1999; Nokso-Koivisto et al., 2004; Bulut et al., 2006; Neuzil et al., 2002) or by serology (Raty et al., 2004). A review of the literature found 32 longitudinal studies of acute otitis media and virus detection. Influenza virus could be detected by one or more of the methods in association with acute otitis media in 2.5–6.7% of children per year, with a median value of 3–4%. Thus, the incidence of influenza-associated otitis media is about 3–4% in children <5 years. The tests are all relatively insensitive, however, and the true role of influenza virus in acute otitis media might be much higher.

One way of testing whether otitis media due to influenza is underdiagnosed is to use the so-called vaccine probe method. In this method, the incidence of the condition (otitis media) in a group of children who have been vaccinated against the pathogen (influenza virus) is compared with the incidence in children who have not been vaccinated against influenza. The vaccine probe method has been used to look at the proportion of episodes of pneumonia caused by *Haemophilus influenzae* type b (Hib) after the introduction of Hib immunization and by pneumococci after the introduction of conjugate pneumococcal vaccine.

[Question: For children, does influenza vaccine, compared with no vaccine or placebo, reduce the incidence of acute otitis media or otitis media with effusion? Literature review: A search found nine RCTs of influenza vaccine and otitis media. Six studies looked at efficacy of inactivated (3), live attenuated nasal (2), and virosomal inactivated vaccines (1). Three studies looked at safety of the live, attenuated vaccine.**]**

An RCT of 186 children in day care showed that giving inactivated influenza vaccine was associated with a 31% reduction in acute otitis media (OR = 0.69, 95% CI, 0.49–0.98) (Clements et al., 1995). A recent RCT of 119 children aged

6–60 months showed inactivated vaccine was associated with significant reductions in the incidence of acute otitis media from 5.2% to 2.3% and of otitis media with effusion from 31.1% to 22.8% (Ozgur et al., 2006). The largest RCT of 786 children aged 6–24 months found inactivated vaccine was not associated with any reduction in acute otitis media, otitis media with effusion, or healthcare use (Hoberman et al., 2003).

A placebo-controlled trial of intranasal, live, attenuated vaccine studied 1602 children aged 15–71 months (Belshe and Gruber, 2000). The annual incidence of influenza-associated acute otitis media was 3.8% in the placebo group, and the vaccine was 94–98% effective against such influenza-associated acute otitis media. The vaccine was associated with a 33% reduction in all episodes of febrile otitis media in the first year compared with placebo (from an average of 0.20–0.14 episodes per person) and a 16% reduction in the second year (from 0.13 to 0.11) (Belshe and Gruber, 2000). These figures suggest that the intranasal, live, attenuated vaccine is highly effective against otitis media due to influenza. The absolute reduction in all episodes of febrile otitis media was 0.06 episodes per person (from 0.20 to 0.14) or 6%, compared with 3.8% for influenza-associated otitis media. This suggests that a small number of episodes of otitis media are due to undiagnosed influenza and are potentially preventable by influenza immunization.

In a study to evaluate the efficacy of an intranasal, inactivated, virosomal subunit influenza vaccine for prevention of new episodes of acute otitis media in children with recurrent acute otitis media (Marchisio et al., 2002), 133 children aged 1–5 years were randomized to receive the vaccine ($n = 67$) or no vaccination ($n = 66$). During a 6-month period, 24 (35.8%) vaccine recipients had 32 episodes of acute otitis media; 42 (63.6%) control subjects had 64 episodes. The overall efficacy of vaccination in preventing acute otitis media in these children was 43.7% (95% confidence interval, 18.6–61.1; $P = 0.002$).

8 Influenza Immunization

There are two possible schools of thought about influenza immunization for children. The first is expressed by the US Advisory Committee on Immunization Practices (ACIP) who encouraged influenza immunization of healthy 6–23-month-old children during 2002–2003 and 2003–2004 influenza seasons (Advisory Committee on Immunization Practices, Smith et al., 2006). For the 2004–2005 season, the ACIP strengthened this policy by making it a full recommendation to immunize all 6–23-month-old children annually against influenza, a strategy that was endorsed by the American Academy of Pediatrics and the American Academy of Family Physicians (Advisory Committee on Immunization Practices, Smith et al., 2006). The major reason was the recent data documenting high rates of influenza-associated hospitalization among children <2-years old (Bueving et al., 2005; Nguyen-Van-Tam, 2006; Neuzil

et al., 2000; Izurieta et al., 2000; O'Brien et al., 2004; Iwane et al., 2004; Grijalva et al., 2006; Thompson et al., 2004). From 2006, the ACIP recommendation was extended to children aged 6–59 months (Advisory Committee on Immunization Practices, Smith et al., 2006), because of the high number of emergency department visits in 24–59-month-old children Poehling et al., 2006). Other reasons for recommending universal influenza immunization of young children are to reduce the incidence of influenza-associated acute otitis media and to protect others, particularly the elderly and infants <6 months who are too young to be immunized (Advisory Committee on Immunization Practices, Smith et al., 2006).

An alternate view points out the gap between policy and evidence regarding influenza vaccination (Jefferson, 2006). The evidence is weak that influenza immunization protects young children <2 years old, although it is due to lack of evidence of benefit of influenza vaccine rather than evidence that the vaccine is ineffective, that is, more studies need to be done. This view describes the ACIP's broadening recommendations as 'availability creep: policy makers like to recommend what is available even without evidence (Jefferson, 2006). The problems with introducing universal influenza immunization of children without good evidence are:

a) the vaccine may be ineffective, and thus a waste of resources
b) the vaccine may be harmful
c) introducing vaccines before getting the evidence means RCTs will not be done, yet may be necessary to convince providers and parents of the efficacy.

9 Immunogenicity of Influenza Vaccines in Children

Inactivated influenza vaccines are significantly less immunogenic in children (Groothuis et al., 1991; Piedra et al., 1993; Englund et al., 2005; Neuzil et al., 2006). It is recommended to give two doses of vaccine to children <9 years who have not received a previous dose, and the need for a second dose to induce an adequate response has been confirmed (Neuzil et al., 2006). Although most studies show adequate antibody responses after two doses in children <24-months old (Groothuis et al., 1991; Piedra et al., 1993; Englund et al., 2005; Neuzil et al., 2006), there is concern that the vaccine response is poorest in the youngest children who are at highest risk for hospitalization (Negri et al., 2005). Many studies on 6–24-months-old children have failed to do a subgroup analysis to give the vaccine immunogenicity in 6–12-month-olds (Negri et al., 2005). A small study from Japan found that children aged 6–12 months had poor antibody responses to inactivated vaccine (Kumagai et al. (2004).

Live, attenuated, intranasal vaccines (Zangwill et al., 2001) and virosome-adjuvanted vaccines (Kanra et al., 2004) may be more immunogenic in infants than inactivated vaccines.

10 Safety of Influenza Vaccines

Influenza vaccines are generally described as being safe and as causing only minor adverse events. There have been some concerns that inactivated vaccine might cause exacerbations of asthma in adults and children. A Cochrane systematic review found two trials of influenza vaccine involving 2306 people with asthma (Cates et al., 2003). The pooled results did not demonstrate a significant increase in asthma exacerbations in the two weeks following influenza vaccination (Risk Difference 0.00; 95% CI −0.02 to +0.02).

Another Cochrane review reported that most adverse events associated with inactivated vaccines were minor and self-limiting. The most common were fever and local reactions (Smith et al., 2006). A meta-analysis of the safety outcome data was not feasible (Smith et al., 2006).

Live, attenuated, intranasal vaccines are associated with runny nose or nasal congestion, vomiting, muscle aches, and fever (Piedra et al., 2005; Bergen et al., 2004; Fleming et al., 2006; Vesikari et al., 2006). There have been some concerns about asthma exacerbations. Most studies suggest the occasional reports of increased asthma events are due to chance as a result of multiple subgroup analyses (Piedra et al., 2005; Bergen et al., 2004; Fleming et al., 2006; Vesikari et al., 2006). However, the largest study, in which 8352 children received either live, attenuated or inactivated influenza vaccine, found a non-significant increase in wheezing, but a significantly higher rate of any-cause hospitalization (6.1% vs. 2.6%) in children aged 6–11 months who received live, attenuated vaccine compared to inactivated vaccine (Belshe et al., 2007).

11 Vaccine Efficacy for High-Risk Children

Some groups of children are at risk of more severe illness with influenza. These include children with immunodeficiency, asthma, cystic fibrosis, and congenital heart disease (Nicholson et al., 2003; Peltola et al., 2003; Centers for Disease Control and Prevention (CDC), 2003; Bueving et al., 2005; Poehling et al., 2006; Nguyen-Van-Tams, 2006; Advisory Committee on Immunization Practices, Smith et al., 2006). Routine influenza immunization was recommended for these high-risk children in the US in the past, before universal immunization was recommended, and immunization of high-risk children continues to be recommended in most other countries.

Influenza vaccine is safe and effective in adult HIV-infected patients (Tasker et al., 1999). The live, attenuated vaccine is safe and immunogenic in HIV-infected children (King et al., 2001). We could find no RCTs of influenza-vaccine efficacy in immunodeficient children, including those with HIV infection.

A Cochrane review of influenza vaccine in asthma found nine RCTs, but could find no evidence that influenza vaccination reduces the frequency of

exacerbations of asthma or even of influenza-related exacerbations (Smith et al., 2006). We found one subsequent RCT of 696 children that found no reduction in the incidence or severity of influenza-related asthma exacerbations with vaccine, but a trend towards shorter duration (Bueving et al., 2004). The exacerbations lasted 3.1 days shorter on average in the vaccine group (95% CI −6.2 to 0.002 days, $p = 0.06$) (Bueving et al., 2004).

A Cochrane review of influenza vaccine and cystic fibrosis found four studies, but each study compared one vaccine with another and there was no placebo group in any of the studies (Bhalla et al., 2000).

We could find no RCTs of influenza vaccine and congenital heart disease.

In summary, the recommendation to immunize high-risk children routinely against influenza is based on their being at increased risk for severe disease, but not on evidence that the vaccine is effective. There is no evidence of harm from the vaccine and the recommendation to immunize high-risk children is not unreasonable, but it lacks good supportive evidence of benefit from trials.

12 Vaccine Efficacy for Universal Childhood Vaccination

[Question: For healthy children, does influenza immunization compared with no-influenza vaccine or placebo reduce the incidence of influenza? Literature review: A search found 14 RCTs, a Cochrane review (Smith et al., 2006), and a non-Cochrane meta-analysis of influenza vaccines in healthy children (Negri et al., 2005).]

A Cochrane review (Smith et al., 2006) and a non-Cochrane meta-analysis (Negri et al., 2005) of influenza vaccine efficacy in children reached very similar conclusions. Live vaccines were more efficacious against influenza than inactivated vaccines. The efficacy of live vaccines in the Cochrane review (Smith et al., 2006) was 79% (95% CI 48–92%) in children >2 years compared with placebo or no intervention. There were no studies of live vaccine in children under 2 years old.

Inactivated vaccines had an overall efficacy of 59% in children (95% CI 41–71%). The efficacy of inactivated vaccines in children >6 years old was 69% (95% CI 55–78%). In contrast, the efficacy for children <6 years was 39% (95% CI –8% to 63%). For children <2 years the efficacy of inactivated vaccine was described as 'similar to placebo', but this was because there were insufficient data to say whether or not the vaccines were effective. The Forest plots that summarize the results of the Cochrane review are shown in Figs. 2 and 3.

A retrospective cohort study of children aged 6–23 months found that two doses of vaccine were 49% protective against pneumonia and influenza and 25% against influenza-like illness, but one dose was ineffective (Ritzwoller et al., 2005). Only 7.5% of children were fully immunized and this sort of study is very subject to bias.

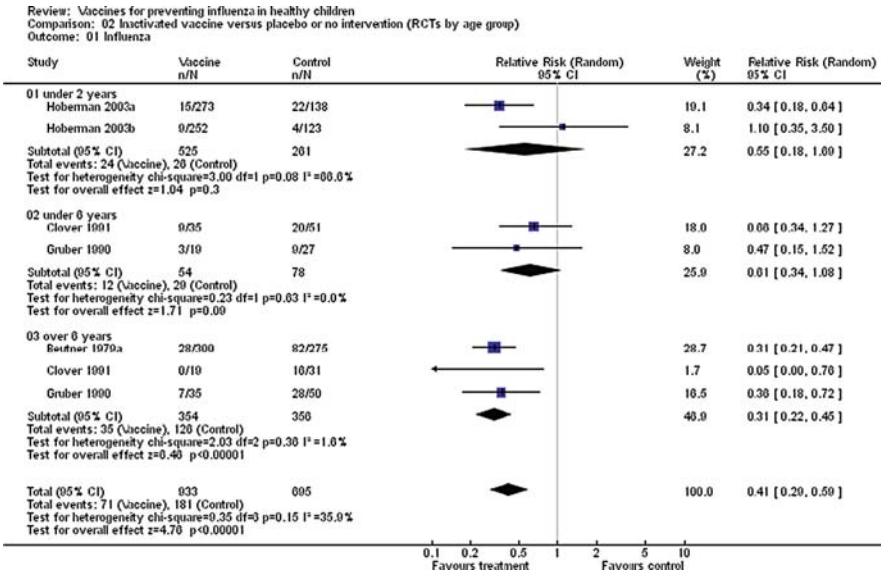

Fig. 2 Efficacy of inactivated influenza vaccine versus placebo or no intervention for preventing influenza. From *Cochrane review* (Smith et al., 2006)

In a recent large RCT comparing live, attenuated vaccine with inactivated vaccine, but with no placebo group, the live, attenuated vaccine was significantly better than inactivated vaccine for children over 3 years old, but there was no significant difference between the vaccines for children aged 6–35 months (Belshe et al., 2007).

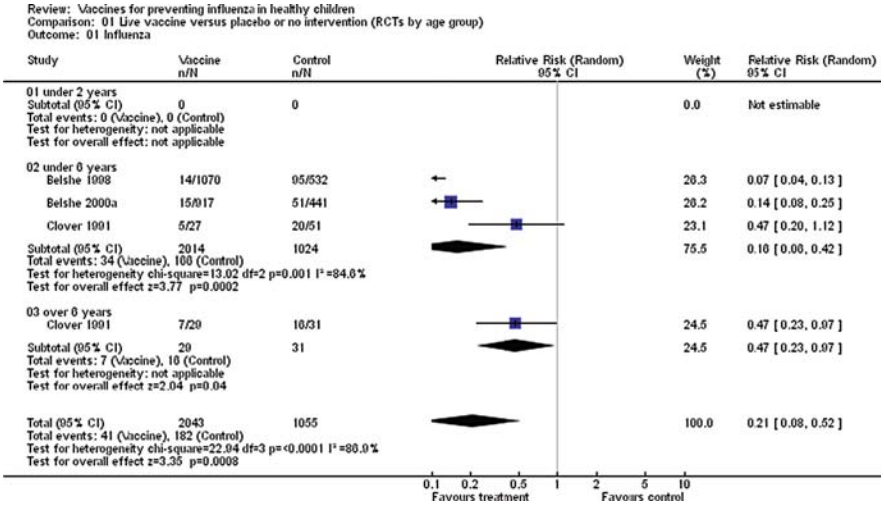

Fig. 3 Efficacy of live, attenuated, intranasal, influenza vaccine versus placebo or no intervention for preventing influenza. From Cochrane review (Smith et al., 2006)

These results cast some doubt over whether immunization of children <2 years old, the highest-risk group for hospital admission, is likely to be successful in preventing influenza, even using live, attenuated vaccines.

13 Cost-Effectiveness of Universal Childhood Influenza Immunization

[Question: For normal children, is universal influenza immunization compared with immunization only of children at high risk of severe influenza cost effective? Literature review: A search found seven cost-effectiveness analyses in children (Office of Technology Assessment, 1981; White et al., 1999; Pisu et al., 2005; Luce et al., 2001; Hall and Katz, 2005; Salo et al, 2006; Skowronski et al, 2006.)

We found five studies from the USA. In 1981, a cost-effectiveness analysis of influenza vaccine at any age suggested the cost effectiveness of vaccination increased with age (Office of Technology Assessment, 1981). An analysis of schoolchildren found that influenza vaccination might just be cost saving but depended on indirect costs such as parental time off work (White et al, 1999). A day care study found that from both the household and societal perspectives, there were no economic benefits to households from vaccinating day care children against influenza virus (Pisu et al, 2005). A study found that live, attenuated vaccine might be cost effective for healthy children aged 15–71 months, but only if delivered on a group vaccination basis and only if the price was <$28 per dose (Luce et al., 2001). A hospital study found that vaccination would be cost effective if it prevented hospitalization of low-risk children, which depends on efficacy (Hall and Katz, 2005).

A Finnish study found that vaccination of children <13 years was cost effective at all ages, assuming vaccine efficacy of 60% and including societal costs (Salo et al., 2006). In contrast, even including societal costs, a Canadian study found that influenza vaccination of infants and toddlers was not likely to be cost effective unless the attack rate was over 55%, compared with their model of a 25% attack rate, or unless the vaccine costed less than $6.81 compared with $15 in the model (Skowronski et al., 2006).

Of course, if the vaccine has very low or no efficacy in children aged 6–23 months, vaccination at this age will not be cost effective.

14 Delivery of Vaccine

Delivery of influenza vaccine is not necessarily straightforward, even if vaccination is universally recommended. Vaccine has to be delivered annually before the winter, so it does not fit into routine childhood immunization schedules. Despite a firm recommendation for universal immunization of children aged

Table 3 Vaccine coverage for 6–23-months-old children, USA, 2003–2005 (Hall and Katz, 2005)

Year	Two doses	One dose
2003–2004	8.4%	17.5%
2004–2005	17.8%	33.4%

6–23 months in the US, initial vaccine uptake was low (Centers for Disease Control and Prevention (CDC), 2006). Vaccine uptake doubled in 2004–2005 compared with the preceding year, following media coverage of the dangers of a severe outbreak and reassuring messages about vaccine safety (Ma et al., 2006; Daley et al., 2006), but still only 17.8% of children received two doses (see Table 3).

15 Conclusions

Routine childhood immunization against influenza is currently recommended in most countries for high-risk children. In the USA, annual influenza vaccine is recommended for all children aged 6–59 months.

There are no good studies of vaccine efficacy in high-risk children, but vaccines are safe and immunogenic for children over 2 years old and the benefits are likely to outweigh any harms.

Studies show that live, attenuated vaccines are more effective than inactivated vaccines for healthy children but both are efficacious for children >6-years old. The data for children <2-years old are weak.

Uptake of vaccine in children <2 years old cannot be assumed and is currently suboptimal in the USA.

There is a danger that recommendations about universal immunization have preceded essential studies and that it will be impossible now to do those studies in the USA. Nevertheless, such studies would be extremely valuable in helping decide whether routine immunization of children is indicated and helping to educate parents and providers.

References

Advisory Committee on Immunization Practices; Smith NM, Bresee JS, Shay DK, Uyeki TM, Cox NJ, Strikas RA. Prevention and Control of Influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2006; 55(RR-10): 1–42.

Beard F, McIntyre P, Gidding H, et al. Influenza-related hospitalisations in Sydney, New South Wales, Australia. Arch Dis Child 2006; 91: 20–5.

Belshe RB, Edwards KM, Vesikari T, et al. Live attenuated versus inactivated influenza vaccine in infants and young children. N Engl J Med 2007; 356: 685–96.

Belshe RB, Gruber WC. Prevention of otitis media in children with live attenuated influenza vaccine given intranasally. Pediatr Infect Dis J 2000; 19(5 Suppl): S66–71.

Belshe RB, Gruber WC. Safety, efficacy and effectiveness of cold-adapted, live, attenuated, trivalent, intranasal influenza vaccine in adults and children. Philos Trans R Soc Lond B Biol Sci. 2001; 356: 1947–51.

Bergen R, Black S, Shinefield H, et al. Safety of cold-adapted live attenuated influenza vaccine in a large cohort of children and adolescents. Pediatr Infect Dis J 2004; 23: 138–44.

Bhalla P, Tan A, Smyth R. Vaccines for preventing influenza in people with cystic fibrosis. *Cochrane Database of Systematic Reviews* 2000, Issue 1. Art. No.: CD001753. DOI: 10.1002/14651858.CD001753.

Bhat N, Wright JG, Broder KR, et al. Influenza-associated deaths among children in the United States, 2003–2004. N Engl J Med 2003; 353: 2559–67.

Bueving HJ, Bernsen RM, de Jongste JC, et al. Influenza vaccination in children with asthma: randomized double-blind placebo-controlled trial. Am J Respir Crit Care Med 2004; 169: 488–93.

Bueving HJ, van der Wouden JC, Berger MY, Thomas S. Incidence of influenza and associated illness in children aged 0–19 years: a systematic review. Rev Med Virol 2005; 15: 383–91.

Bulut Y, Guven M, Otlu B, et al. Acute otitis media and respiratory viruses. Eur J Pediatr 2006 Sep 12.

Cates CJ, Jefferson TO, Bara AI, Rowe BH. Vaccines for preventing influenza in people with asthma. *Cochrane Database of Systematic Reviews* 2003, Issue 4. Art. No.: CD000364. DOI: 10.1002/14651858.CD000364.pub2.

Centers for Disease Control and Prevention (CDC). Childhood influenza vaccination coverage–United States, 2004–05 influenza season. MMWR Morb Mortal Wkly Rep 2006; 55: 1062–5.

Centers for Disease Control and Prevention (CDC). Severe morbidity and mortality associated with influenza in children and young adults-Michigan, 2003. MMWR Morb Mortal Wkly Rep 2003; 52: 837–40.

Chiu SS, Lau YL, Chan KH, et al. Influenza-related hospitalizations among children in Hong Kong. N Engl J Med 2002; 347: 2097–103.

Clements DA, Langdon L, Bland C, Walter E. Influenza A vaccine decreases the incidence of otitis media in 6- to 30-month-old children in day care. Arch Pediatr Adolesc Med 1995; 149: 1113–7.

Daley MF, Crane LA, Chandramouli V, et al. Influenza among healthy young children: changes in parental attitudes and predictors of immunization during the 2003 to 2004 influenza season. Pediatrics 2006; 117: e268–77.

Englund JA, Walter EB, Fairchok MP, Monto AS, Neuzil KM. A comparison of 2 influenza vaccine schedules in 6- to 23-month-old children. Pediatrics 2005; 115: 1039–47.

Fleming DM, Crovari P, Wahn U, et al. Comparison of the efficacy and safety of live attenuated cold-adapted influenza vaccine, trivalent, with trivalent inactivated influenza virus vaccine in children and adolescents with asthma. Pediatr Infect Dis J 2006; 25: 860–9.

Frank AL, Taber LH, Glezen WP, et al. Influenza B virus infections in the community and the family: the epidemics of 1976–77 and 1979–80 in Houston, Texas. Am J Epidemiol 1983; 118: 313–25.

Grijalva CG, Craig AS, Dupont WD, et al. Estimating influenza hospitalizations among children. EID 2006; 12: 103–9.

Groothuis JR, Levin MJ, Rabalais GP, Meiklejohn G, Lauer BA. Immunization of high-risk infants younger than 18 months with split product influenza vaccine. Pediatrics 1991; 87: 823–8.

Hall CE, Cooney MK, Fox JP. The Seattle Virus Watch: IV. Comparative epidemiologic observations of infections with influenza A and B viruses, 1965–69, in families with young children. Am J Epidemiol 1973; 98: 365–80.

Hall JL, Katz BZ. Cost of influenza hospitalization at a tertiary care children's hospital and its impact on the cost-benefit analysis of the recommendation for universal influenza immunization in children age 6 to 23 months. J Pediatr 2005; 147: 807–11.

Heikkinen T, Thint M, Chonmaitree T. Prevalence of various respiratory viruses in the middle ear during acute otitis media. N Engl J Med 1999; 340: 260–4.

Hoberman A, Greenberg DP, Paradise JL, et al. Effectiveness of inactivated influenza vaccine in preventing acute otitis media in young children: a randomized controlled trial. JAMA 2003; 290: 1608–16.

Iwane MK, Edwards KM, Szilagyi PG, et al. Population-based surveillance for hospitalizations associated with respiratory syncytial virus, influenza virus, and parainfluenza viruses among young children. Pediatrics 2004; 113: 1758–64.

Izurieta HS, Thompson WW, Kramarz P, et al. Influenza and the rates of hospitalization for respiratory disease among infants and young children. N Engl J Med 2000; 342: 232–9.

Jefferson T. Influenza vaccination: policy versus evidence. BMJ 2006; 333: 912–5.

Jordan R, Connock M, Albon E, Fry-Smith A, Olowokure B, Hawker J, Burls A. Universal vaccination of children against influenza: are there indirect benefits to the community? A systematic review of the evidence. Vaccine 2006; 24: 1047–62.

Kanra G, Marchisio P, Feiterna-Sperling C, et al. Comparison of immunogenicity and tolerability of a virosome-adjuvanted and a split influenza vaccine in children. Pediatr Infect Dis J 2004; 23: 300–6.

King JC Jr, Fast PE, Zangwill KM, et al. Safety, vaccine virus shedding and immunogenicity of trivalent, cold-adapted, live attenuated influenza vaccine administered to human immunodeficiency virus-infected and noninfected children. Pediatr Infect Dis J 2001; 20: 1124–31.

Kumagai T, Nagai K, Okui T, et al. Poor immune responses to influenza vaccination in infants. Vaccine 2004; 22: 3404–10.

Luce BR, Zangwill KM, Palmer CS, et al. Cost-effectiveness analysis of an intranasal influenza vaccine for the prevention of influenza in healthy children. Pediatrics 2001; 108: E24.

Ma KK, Schaffner W, Colmenares C, Howser J, Jones J, Poehling KA. Influenza vaccinations of young children increased with media coverage in 2003. Pediatrics 2006; 117: e157–63.

Marchisio P, Cavagna R, Maspes B, et al. Efficacy of intranasal virosomal influenza vaccine in the preventio of recurrent acute otitis media in children. Clin Infect Dis 2002; 35: 168–74.

Monto AS, Kioumehr F. The Tecumseh study of respiratory illness IX. Occurrence of influenza in the community, 1966–71. Am J Epidemiol 1975; 102: 553–63.

Negri E, Colombo C, Giordano L, Groth N, Apolone G, La Vecchia C. Influenza vaccine in healthy children: a meta-analysis. Vaccine 2005; 23: 2851–61.

Neuzil KM, Jackson LA, Nelson J, et al. Immunogenicity and reactogenicity of 1 versus 2 doses of trivalent inactivated influenza vaccine in vaccine-naive 5-8-year-old children. J Infect Dis 2006; 194: 1032–9.

Neuzil KM, Mellen BG, Wright PF, et al. The effect of influenza on hospitalizations, outpatient visits, and courses of antibiotics in children. N Engl J Med 2000; 342: 225–31.

Neuzil KM, Zhu Y, Griffin MR, et al. Burden of interpandemic influenza in children younger than 5 years: a 25-year prospective study. J Infect Dis 2002; 185: 147–52.

Nguyen-Van-Tam JS. Influenza related hospital admissions in children: evidence about the burden keeps growing but the route to policy change remains uncertain. Arch Dis Child 2006; 91: 5–7.

Nicholson KG, Wood JM, Zambon M. Influenza. Lancet 2003; 362: 1733–45.

Nokso-Koivisto J, Raty R, Blomqvist S, et al. Presence of specific viruses in the middle ear fluids and respiratory secretions of young children with acute otitis media. J Med Virol 2004; 72: 241–8.

O'Brien MA, Uyeki TM, Shay DK, et al. Incidence of outpatient visits and hospitalizations related to influenza in infants and young children. Pediatrics 2004; 113: 585–93.
Office of Technology Assessment. Cost effectiveness of influenza vaccination. In: U.S. Congress. Washington, DC: Office of Technology Assessment; 1981.
Ozgur SK, Beyazova U, Kemaloglu YK, et al. Effectiveness of inactivated influenza vaccine for prevention of otitis media in children. Pediatr Infect Dis J 2006; 25: 401–4.
Peltola V, Ziegler T, Ruuskanen O. Influenza A and B virus infections in children. Clin Infect Dis 2003; 36: 299–305.
Piedra PA, Gaglani MJ, Riggs M, et al. Live attenuated influenza vaccine, trivalent, is safe in healthy children 18 months to 4 years, 5 to 9 years, and 10 to 18 years of age in a community-based, nonrandomized, open-label trial. Pediatrics. 2005; 116: e397–407.
Piedra PA, Glezen WP, Mbawuike I, et al. Studies on reactogenicity and immunogenicity of attenuated bivalent cold recombinant influenza type A (CRA) and inactivated trivalent influenza virus (TI) vaccines in infants and young children. Vaccine 1993; 11: 718–24.
Pisu M, Meltzer MI, Hurwitz ES, Haber M. Household-based costs and benefits of vaccinating healthy children in day care against influenza virus: results from a pilot study. Pharmacoeconomics 2005; 23: 55–67.
Poehling KA, Edwards KM, Weinberg GA, et al. The underrecognized burden of influenza in young children. N Engl J Med. 2006; 355: 31–40.
Raty R, Ziegler T, Kleemola M. The value of virus serology in epidemiological studies of acute otitis media in children. J Clin Virol 2004; 29: 315–9.
Reichert TA, Sugaya N, Fedson DS, Glezen WP, Simonsen L, Tashiro M. The Japanese experience with vaccinating schoolchildren against influenza. N Engl J Med 2001; 344: 889–96.
Ritzwoller DP, Bridges CB, Shetterly S, Yamasaki K, Kolczak M, France EK. Effectiveness of the 2003–2004 influenza vaccine among children 6 months to 8 years of age, with 1 vs 2 doses. Pediatrics 2005; 116: 153–9.
Salo H, Kilpi T, Sintonen H, Linna M, Peltola V, Heikkinen T. Cost-effectiveness of influenza vaccination of healthy children. Vaccine 2006; 24: 4934–41.
Skowronski DM, Woolcott JC, Tweed SA, Brunham RC, Marra F. Potential cost-effectiveness of annual influenza immunization for infants and toddlers: experience from Canada. Vaccine 2006; 24: 4222–32.
Smith S, Demicheli V, Di Pietrantonj C, Harnden AR, Jefferson T, Matheson NJ, Rivetti A. Vaccines for preventing influenza in healthy children. *Cochrane Database of Systematic Reviews* 2006, Issue 1. Art. No.: CD004879. DOI: 10.1002/14651858.CD004879.pub2.
Tasker SA, Treanor JJ, Paxton WB, Wallace MR. Efficacy of influenza vaccination in HIV-infected persons. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 1999; 131: 430–3.
Thompson WW, Shay DK, Weintraub E, et al. Influenza-associated hospitalizations in the United States. JAMA 2004; 292: 1333–40.
Vesikari T, Fleming DM, Aristegui JF, et al. Safety, efficacy, and effectiveness of cold-adapted influenza vaccine-trivalent against community-acquired, culture-confirmed influenza in young children attending day care. Pediatrics. 2006; 118: 2298–312.
Weycker D, Edelsberg J, M.E. Halloran ME, et al. Population-wide benefits of routine vaccination of children against influenza. Vaccine 2005; 23: 1284–93.
White T, Lavoie S, Nettleman MD. Potential cost savings attributable to influenza vaccination of school-aged children. Pediatrics 1999; 103: e73.
Zangwill KM, Droge J, Mendelman P, et al. Prospective, randomized, placebo-controlled evaluation of the safety and immunogenicity of three lots of intranasal trivalent influenza vaccine among young children. Pediatr Infect Dis J 2001; 20: 740–6.

Challenges in the Evaluation and Management of Bone and Joint Infections and the Role of New Antibiotics for Gram Positive Infections

Sheldon L. Kaplan

1 Introduction

Osteomyelitis and septic arthritis are two of the more common invasive bacterial infections in children (Krogstad, 2004). Changes in the epidemiology of these infections, mostly related to the emergence of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) as well advances in molecular microbiology and imaging techniques have led to modifications in the approach to the diagnosis and management of children with osteomyelitis and/or septic arthritis, which will be reviewed in this chapter.

2 Site of Acute Hematogenous Osteomyelitis in Children

The majority of osteomyelitis cases in children arise hematogenously and typically occur in the metaphysis of long bones such as the femur, tibia, and humerus, which together account for about two-thirds of cases (Krogstad, 2004). Vertebral and pelvic locations are noted in about 2 % and 8% of cases, respectively.

3 Etiology

Staphylococcus aureus is by far the most common bacterial pathogen causing osteomyelitis in children in all age groups (Krogstad, 2004). Group A Streptococcus (especially complicating varicella) (Ibia, Imoisili et al., 2003), *Streptococcus pneumoniae* (Bradley, Kaplan et al., 1998), and *Kingella kingae* (Yagupsky, 2004) are the next most common organisms in infants and children. Group B

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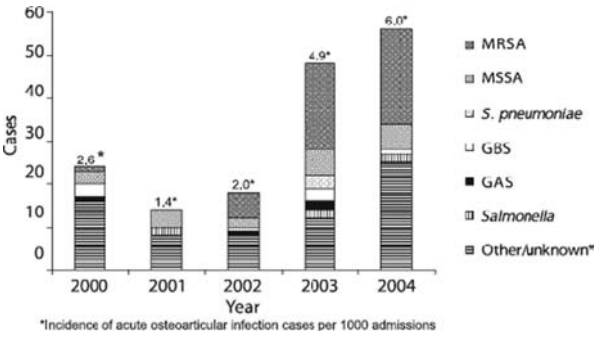

Fig. 1 Etiology of acute osteoarticular infections 2000–2004 at Le Bonheur Children's Medical Center, Memphis, TN

Streptococcus and Gram-negative enterics are important agents in the neonatal period. In children with hemoglobinopathies, *Salmonella* species are the most common cause of osteomyelitis (Burnett, Bass et al., 1998). *Pseudomonas* spp. are particularly associated with puncture wounds of the calcaneous, metatarsal, and tarsal bones (Jacobs, McCarthy et al., 1989). An etiology is never established in almost half of children with acute osteomyelitis (Floyed and Steele, 2003). However, by polymerase chain reaction (PCR), *Kingella kingae* was determined to be the most common etiology of osteoarticular infections among children seen at a pediatric hospital in Lyon, France (Chometon, Benito et al., 2007). Chronic osteomyelitis is most commonly caused by *S. aureus* and Gram-negative enterics. Polymicrobial etiologies are found in a high proportion of children with osteomyelitis, secondary to trauma or infected contiguous soft tissue (Dubey, Krasinski et al., 1988).

CA-MRSA has become a major cause of acute hematogenous osteomyelitis in children in many areas of the world (Sdougkos, Chini et al., 2007). In some studies, CA-MRSA strains are now the most common isolates recovered from children with acute osteomyelitis. (Fig. 1) (Arnold, Elias et al., 2006; Bocchini, Hulten et al., 2006).

4 Clinical Manifestations

Previously unusual manifestations of acute osteomyelitis have been associated with some clones of CA-MRSA, especially those that carry the genes encoding Panton–Valentine leukocidin (*pvl*). Generally, in children with acute hematogenous osteomyelitis caused by *S. aureus,* only one site of infection is noted. Bocchini, Hulten et al. (2006) found that 15% of children with acute osteomyelitis caused by *pvl*+ *S. aureus* isolates have multiple sites of infection. Furthermore, myositis or pyomyositis and intraosseous/subperiosteal abscesses are more commonly seen in association with *pvl*+ isolates than for *pvl*– isolates. Chronic osteomyelitis also is more likely to be present at the time of diagnosis or

at follow-up with *pvl*+ isolates than with *pvl*– isolates (Martinez-Aguilar, Avalos-Mishaan et al., 2004).

Severe life-threatening infections in adolescents in association with CA-MRSA osteomyelitis are increasingly encountered (Gonzalez, Martinez-Aguilar et al., 2005). These patients typically present with hypotension or in septic shock with prolonged bacteremia and pulmonary distress usually requiring intubation and mechanical ventilation. Deep venous thrombosis was not a common complication of the course of *S. aureus* osteomyelitis in the past but has now been reported from several centers, and in some cases with septic pulmonary emboli, as an important problem associated with CA-MRSA osteomyelitis as well as CA-methicillin susceptible *pvl*+ *S. aureus* isolates (Crary, Buchanan et al., 2006; Gonzalez, Teruya et al., 2006; Dohin, Gillet et al., 2007; Nourse, Starr et al., 2007).

5 Diagnosis

If acute osteomyelitis is a consideration following a complete history and physical examination, laboratory and diagnostic imaging evaluation are required to confirm the diagnosis. Standard indicators of acute inflammation such as the total white blood cell count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) are all generally elevated. The CRP is perhaps the most reliably increased marker of acute inflammation and was elevated in all (98%) but one of 44 children compared to an elevated ESR in 92% (35/38) in one series (Unkila-Kallio, Kallio et al., 1994). In one study, the ESR and the CRP at the time of initial evaluation as well as the maximum value achieved during hospitalization were significantly higher in children with *pvl*+ *S. aureus* isolates than in children with *pvl*– *S. aureus* isolates (Bocchini, Hulten et al., 2006). Blood cultures are positive in up to 50–60% of patients and are also more commonly positive in children with osteomyelitis caused by *pvl*+ *S. aureus* isolates (Krogstad, 2004; Bocchini, Hulten et al., 2006). Bone aspiration especially under computed tomography (CT) or ultrasound guidance may reveal an etiologic agent when the blood cultures are negative (Karwowska, Davies et al., 1998).

PCR may determine the etiology of osteomyelitis in cases in which blood or bone cultures are negative and is already useful for cases caused by *Bartonella henselae* or *K. kingae* (Moumile, Merckx et al., 2003) Placing bone aspirates or synovial fluid into blood culture bottles enhances the yield of isolating *K. kingae* and may also serve as a medium for detecting16S rRNA of *K. kingae* by PCR.

Plain radiographs early in the clinical course usually show nonspecific changes such as soft-tissue swelling and obliteration of tissue planes but bone abnormalities such as periosteal elevation or lytic lesions are typically not identified until 10–14 days into the course. Plain films are useful for demonstrating fractures or bone malignancies, which are included in the differential diagnosis of osteomyelitis. Technetium-labeled methylene diphosphate bone scan is about 90% sensitive

in detecting osteomyelitis and is especially useful if multifocal osteomyelitis is a concern or the site of infection is in an unusual location such as in the pelvic region (Rifai and Nyman 1997; Connolly, Connolly et al., 2002; Darville and Jacobs, 2004). In children with sickle cell disease, ultrasound can readily detect periosteal abscesses, which is useful for helping to distinguish infection from infarction in bone (Rifai and Nyman, 1997). Distinguishing diskitis from vertebral osteomyelitis can be problematic but clinical differences between the two have been highlighted (Fernandez, Carrol et al., 2000).

Magnetic resonance imaging (MRI) is now the most sensitive modality for detecting changes in bone consistent with acute osteomyelitis (Chung, 2002; Connolly, Connolly et al., 2002) (Fig. 2). However, these changes are not specific so the findings have to be interpreted within the clinical context (Chung, 2002). MRI also readily detects myositis or pyomyositis contiguous to the site of osteomyelitis, findings that are more common in patients with *pvl*+ *S. aureus* isolates compared with *pvl*– isolates ($p = 0.05$) (Bocchini, Hulten et al., 2006). In addition, subperiosteal or intraosseal abscesses are more common is association with pvl+ *S. aureus* isolates than *pvl*– strains. MRI is especially useful for evaluating children with acute nontraumatic hip pain without evidence of hip joint infection but in whom there is concern about pelvic or vertebral osteomyelitis (Karmazyn, Loder et al., 2007). Finally, because of greater anatomic detail, MRI allows the orthopedic surgeon to plan the optimal surgical management for the patient. The disadvantages of MRI include the increased time required for scanning, need for sedation in younger children, and cost when compared with CT.

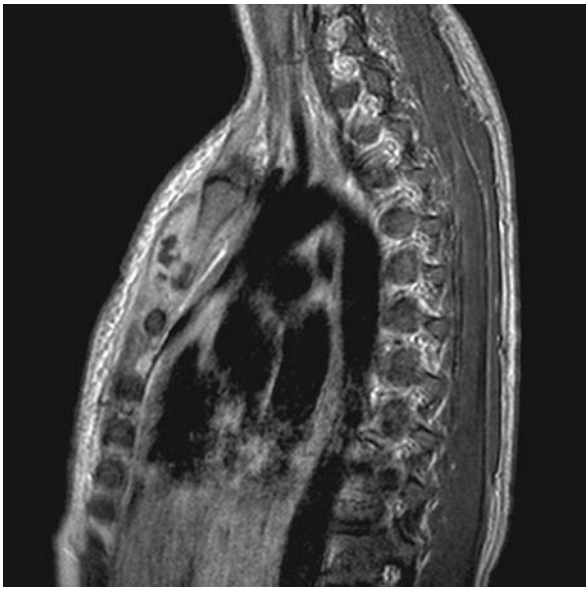

Fig. 2 Magnetic resonance imaging of the sternum of a 14-year-old male with primary hematogenous osteomyelitis of the sternum caused by community-acquired, methicillin-resistant *Staphylococcus aureus*. There is evidence of a cortical breach with a presternal soft-tissue abnormality and moderate-sized fluid collection

6 Treatment

The initial empiric treatment of acute osteomyelitis in children always includes an agent directed against *S. aureus*, including CA-MRSA isolates (Kaplan, 2005). Some experts recommend that once the rate of methicillin resistance among community *S. aureus* isolates is $\geq$10% (others would argue for lower rates), antibiotics effective against CA-MRSA should be administered from the onset of treatment. Vancomycin is the gold standard for treating MRSA infections. The addition of gentamicin and/or rifampin for some synergistic activity has not been proven beneficial. In most regions, over 90% of CA-MRSA isolates are susceptible to clindamycin, which is effective for the treatment of acute musculoskeletal infections caused by CA-MRSA when the *S. aureus* isolate is fully susceptible to clindamycin (Martinez-Aguilar, Hammerman et al., 2003; Arnold, Elias et al., 2006). Musculoskeletal infections caused by *S. aureus* isolates with inducible-clindamycin resistance should not be treated with clindamycin since the risk of inducing resistance in vivo is substantial with resultant treatment failure (Frank, Marcinak et al., 2002). Thus, the 'D-test' should be used routinely by the microbiology laboratory to screen for inducible macrolide–lincosamide–streptogramin resistance (Lewis and Jorgensen, 2005). Clindamycin is not recommended for initial empiric treatment in areas where clindamycin-resistance rates among community *S. aureus* isolates exceed 10–15%.

Vancomycin and clindamycin are also active against almost all isolates of *S. pyogenes* and *S. pneumoniae*, the other two main causes of acute hematogenous osteomyelitis in otherwise normal children, but not against *K. kingae*. In the normal child with presumed hematogenous osteomyelitis, initiatiation of one antibiotic is preferred. In areas with a rate of CA-MRSA $\geq$ 10% among community-acquired *S. aureus* isolates, clindamycin or vancomycin is the agent of choice. Nafcillin or oxacillin (or flucloxacillin in the UK) is the agent of choice if CA-MRSA is not a concern (Pickering et al., 2006). If an organism is not isolated in a child who is clearly improving, the etiologic pathogen is likely susceptible to clindamycin, vancomycin, or nafcillin. For patients with venous thromboses associated with acute osteomyelitis, early collaboration with hematology colleagues with expertise in the management of thromboses, which usually includes anticoagulation therapy, is recommended (Gonzalez, Teruya et al., 2006)

Once an organism is isolated and antibiotic susceptibilities are known, antibiotic treatment may be modified. Nafcillin or oxacillin is the agent of choice for treating methicillin-susceptible *S. aureus*. An oral agent such as dicloxacillin or cephalexin can be used to complete therapy after the patient has responded for some period of time to intravenous treatment (Bryson, Connor et al., 1979; Peltola, Unkila-Kallio et al., 1997). Clindamycin is effective for treating musculoskeletal infections caused by methicillin-susceptible *S. aureus* in patients allergic to or intolerant of nafcillin or as a primary option in the treatment of

CA-MRSA osteomyelitis when the organism is fully susceptible to clindamycin (Kaplan, Mason et al., 1982; Martinez-Aguilar, Hammerman et al., 2003). Clindamycin can be administered orally when appropriate following intravenous treatment. Adverse events seen most commonly with clindamycin are loose stools and rashes.

Other options for treating CA-MRSA musculoskeletal infection are not well studied. Very little information is available on the use of trimethoprim–sulfamethoxazole (TMP/SMX) in the treatment of MRSA osteomyelitis (Ardati, Thirumoorthi et al., 1979).

Long-acting tetracyclines such as minocycline or doxycycline have good in vitro activity against most CA-MRSA isolates but data are insufficient to recommend their use in osteomyelitis or septic arthritis (Ruhe, Monson et al., 2005). Minocycline or doxycycline is only a consideration in children over 8 years of age.

Linezolid is an oxazolidinone antibiotic with excellent in vitro activity against MRSA isolates, is approved for use in children, and was equivalent to vancomycin for the treatment of infections caused by resistant Gram-positive bacteria and can be administered intravenously or orally (Kaplan, Deville et al., 2003). Although linezolid has not been evaluated in a formal manner for treatment of osteomyelitis, it has been used successfully in a compassionate-use program as well as in several case series in adult patients (Rayner, Baddour et al., 2004; Falagas, Siempos et al., 2007). In the only published experience for children, 10 of 12 patients with MRSA osteomyelitis had successful treatment with linezolid (Chen, Chiu et al., 2007). In the future, linezolid may be an important oral agent for initial treatment as well as for completing therapy for osteomyelitis caused by MRSA isolates resistant to clindamycin. Treatment with linezolid for more that 2 weeks has been associated with a decrease in hemoglobin or platelets (Gerson, Kaplan et al., 2002). Linezolid is associated with minimal myelosuppression in the first 2 weeks of therapy in children (Meissner, Townsend et al., 2003). Long-term linezolid use in adults has been associated rarely with a peripheral neuropathy, optic neuritis, or lactic acidosis. Prospective trials are needed to determine the safety and efficacy of linezolid for treating CA-MRSA musculoskeletal infections in children.

Daptomycin is an intravenous, cyclic-lipopeptide antibiotic with rapid bactericidal activity against MRSA in vitro and has been found to be efficacious in the treatment of MRSA bacteremia and right-sided endocarditis in adults (Carpenter and Chambers, 2004; Fowler, Boucher et al., 2006). Daptomycin is not optimal for treating pneumonia, in part, because concentrations of daptomycin are low in the bronchial–alveolar lining fluid and lung parenchyma as well as inhibition of daptomycin by surfactant (Carpenter and Chambers, 2004). Preliminary data in adults suggest that daptomycin is efficacious in the treatment of MRSA osteomyelitis (Lamp, Friedrich et al., 2007). Pharmacokinetic data in children following a single 4 mg/kg dose of daptomycin in children have been presented, and daptomycin has been given to very ill children in preliminary studies (Ardura et al., 2007; Kearns et al., 2007). However,

pulmonary involvement occurs in many of the children with *S. aureus* osteomyelitis in which case daptomycin would be added to another agent more active in the lung (Gonzalez, Hulten et al., 2005).

Antibiotic treatment is provided for a minimum of 21 days (Dich, Nelson et al., 1975). Transitioning to an oral agent to complete therapy is an option that allows more convenient administration of drug; it avoids complications of central lines and has been found to be equally efficacious as intravenous therapy for musculoskeletal infections (Le Saux, Howard et al., 2002; Ruebner, Keren et al., 2006). The author generally recommends continuing treatment until the C-RP and ESR are both within a normal range, which typically requires 4–6 weeks of total therapy.

7 Surgical Therapy

The author believes that drainage of subperiosteal or intraosseous abscesses is indicated in patients with acute hematogenous osteomyelitis; multiple drainage procedures are not unusual (Dohin, Gillet et al., 2007). In many instances, this can be accomplished through interventional radiology. Surgical debridement is more critical for optimal treatment of osteomyelitis associated with contiguous infections, direct inoculation, or chronic osteomyelitis.

8 Summary

CA-MRSA isolates are increasingly common pathogens in children with acute, hematogenously acquired osteomyelitis and clinicians have to consider this pathogen in the selection of initial empiric treatment. With the emergence of *pvl*+ CA-MRSA isolates, unusual manifestations of acute osteomyelitis have become common including venous thromboses and multiple sites of infection. MRI is currently the imaging modality of choice for osteomyelitis. Drainage of purulent collections is also more common in association with the *pvl*+ strains. The role of newer antibiotics such as linezolid and daptomycin for the treatment of CA-MRSA musculoskeletal infections in children needs further study.

References

Ardati, K. O., M. C. Thirumoorthi, et al. (1979). "Intravenous trimethoprim-sulfamethoxazole in the treatment of serious infections in children." *J Pediatr* **95**(5 Pt 1): 801–806.

Ardura M, I., Mejias A et al. (2007). "Daptomycin therapy for invasive Gram-positive bacterial infections in children." *Pediatr Infect Dis* J **26**(12): 1128–1132.

Arnold, S. R., D. Elias, et al. (2006). "Changing patterns of acute hematogenous osteomyelitis and septic arthritis: emergence of community-associated methicillin-resistant *Staphylococcus aureus*." *J Pediatr Orthop* **26**(6): 703–708.

Bocchini, C. E., K. G. Hulten, et al. (2006). "Panton-Valentine leukocidin genes are associated with enhanced inflammatory response and local disease in acute hematogenous *Staphylococcus aureus* osteomyelitis in children." *Pediatrics* **117**(2): 433–440.

Bradley, J. S., S. L. Kaplan, et al. (1998). "Pediatric pneumococcal bone and joint infections. The Pediatric Multicenter Pneumococcal Surveillance Study Group (PMPSSG)." *Pediatrics* **102**(6): 1376–1382.

Bryson, Y. J., J. D. Connor, et al. (1979). "High-dose oral dicloxacillin treatment of acute staphylococcal osteomyelitis in children." *J Pediatr* **94**(4): 673–675.

Burnett, M. W., J. W. Bass, et al. (1998). "Etiology of osteomyelitis complicating sickle cell disease." *Pediatrics* **101**(2): 296–297.

Carpenter, C. F. and H. F. Chambers (2004). "Daptomycin: another novel agent for treating infections due to drug-resistant gram-positive pathogens." *Clin Infect Dis* **38**(7): 994–1000.

Chen, C. J., C. H. Chiu, et al. (2007). "Experience With Linezolid Therapy in Children With Osteoarticular Infections." *Pediatr Infect Dis J* **26**(11): 985–988.

Chometon, S., Y. Benito, et al. (2007). "Specific real-time polymerase chain reaction places *Kingella kingae* as the most common cause of osteoarticular infections in young children." *Pediatr Infect Dis J* **26**(5): 377–381.

Chung, T. (2002). "Magnetic resonance imaging in acute osteomyelitis in children." *Pediatr Infect Dis J* **21**(9): 869–870.

Connolly, L. P., S. A. Connolly, et al. (2002). "Acute hematogenous osteomyelitis of children: assessment of skeletal scintigraphy-based diagnosis in the era of MRI." *J Nucl Med* **43**(10): 1310–1316.

Crary, S. E., G. R. Buchanan, et al. (2006). "Venous thrombosis and thromboembolism in children with osteomyelitis." *J Pediatr* **149**(4): 537–541.

Darville, T. and R. F. Jacobs (2004). "Management of acute hematogenous osteomyelitis in children." *Pediatr Infect Dis J* **23**(3): 255–257.

Dich, V. Q., J. D. Nelson, et al. (1975). "Osteomyelitis in infants and children. A review of 163 cases." *Am J Dis Child* **129**(11): 1273–1278.

Dohin, B., Y. Gillet, et al. (2007). "Pediatric Bone and Joint Infections Caused by Panton-Valentine Leukocidin-Positive *Staphylococcus aureus*." *Pediatr Infect Dis J* **26**(11): 1042–1048.

Dubey, L., K. Krasinski, et al. (1988). "Osteomyelitis secondary to trauma or infected contiguous soft tissue." *Pediatr Infect Dis J* **7**(1): 26–34.

Falagas, M. E., Siempos, II, et al. (2007). "Linezolid for the treatment of adults with bone and joint infections." *Int J Antimicrob Agents* **29**(3): 233–239.

Fernandez, M., C. L. Carrol, et al. (2000). "Discitis and vertebral osteomyelitis in children: an 18-year review." *Pediatrics* **105**(6): 1299–304.

Floyed, R. L. and R. W. Steele (2003). "Culture-negative osteomyelitis." *Pediatr Infect Dis J* **22**(8): 731–736.

Fowler, V. G., Jr., H. W. Boucher, et al. (2006). "Daptomycin versus standard therapy for bacteremia and endocarditis caused by *Staphylococcus aureus*." *N Engl J Med* **355**(7): 653–665.

Frank, A. L., J. F. Marcinak, et al. (2002). "Clindamycin treatment of methicillin-resistant *Staphylococcus aureus* infections in children." *Pediatr Infect Dis J* **21**(6): 530–534.

Gerson, S. L., S. L. Kaplan, et al. (2002). "Hematologic effects of linezolid: summary of clinical experience." *Antimicrob Agents Chemother* **46**(8): 2723–2726.

Gonzalez, B. E., K. G. Hulten, et al. (2005). "Pulmonary manifestations in children with invasive community-acquired *Staphylococcus aureus* infection." *Clin Infect Dis* **41**(5): 583–590.

Gonzalez, B. E., G. Martinez-Aguilar, et al. (2005). "Severe Staphylococcal sepsis in adolescents in the era of community-acquired methicillin-resistant *Staphylococcus aureus*." *Pediatrics* **115**(3): 642–648.

Gonzalez, B. E., J. Teruya, et al. (2006). "Venous thrombosis associated with staphylococcal osteomyelitis in children." *Pediatrics* **117**(5): 1673–1679.

Ibia, E. O., M. Imoisili, et al. (2003). "Group A beta-hemolytic streptococcal osteomyelitis in children." *Pediatrics* **112**(1 Pt 1): e22–26.

Jacobs, R. F., R. E. McCarthy, et al. (1989). "Pseudomonas osteochondritis complicating puncture wounds of the foot in children: a 10-year evaluation." *J Infect Dis* **160**(4): 657–661.

Kaplan, S. L. (2005). "Osteomyelitis in children." *Infect Dis Clin North Am* **19**(4): 787–797, vii.

Kaplan, S. L., J. G. Deville, et al. (2003). "Linezolid versus vancomycin for treatment of resistant Gram-positive infections in children." *Pediatr Infect Dis J* **22**(8): 677–686.

Kaplan, S. L., E. O. Mason, Jr., et al. (1982). "Clindamycin versus nafcillin or methicillin in the treatment of *Staphylococcus aureus* osteomyelitis in children." *South Med J* **75**(2): 138–142.

Karmazyn, B., R. T. Loder, et al. (2007). "The role of pelvic magnetic resonance in evaluating nonhip sources of infection in children with acute nontraumatic hip pain." *J Pediatr Orthop* **27**(2): 158–164.

Karwowska, A., H. D. Davies, et al. (1998). "Epidemiology and outcome of osteomyelitis in the era of sequential intravenous-oral therapy." *Pediatr Infect Dis J* **17**(11): 1021–1026.

Kearns GL, J. R., Jafri HS, Hong EF and Benziger BD (2007). Pharmacokinetics of daptomycin in pediatric patients. *Pediatric Academic Societies' Annual Meeting*. Toronto.

Krogstad (2004). Osteomyelitis and septic arthritis. *Textbook of Pediatric Infectious Diseases*. J. RD Feigin, GJ Demmler, SL Kaplan Philadelphia, Saunders: 713–736.

Lamp, K. C., L. V. Friedrich, et al. (2007). "Clinical experience with daptomycin for the treatment of patients with osteomyelitis." *Am J Med* **120**(10 Suppl 1): S13–S20.

Le Saux, N., A. Howard, et al. (2002). "Shorter courses of parenteral antibiotic therapy do not appear to influence response rates for children with acute hematogenous osteomyelitis: a systematic review." *BMC Infect Dis* **2**: 16.

Lewis, J. S., 2nd and J. H. Jorgensen (2005). "Inducible clindamycin resistance in Staphylococci: should clinicians and microbiologists be concerned?" *Clin Infect Dis* **40**(2): 280–285.

Martinez-Aguilar, G., A. Avalos-Mishaan, et al. (2004). "Community-acquired, methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* musculoskeletal infections in children." *Pediatr Infect Dis J* **23**(8): 701–706.

Martinez-Aguilar, G., W. A. Hammerman, et al. (2003). "Clindamycin treatment of invasive infections caused by community-acquired, methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in children." *Pediatr Infect Dis J* **22**(7): 593–598.

Meissner, H. C., T. Townsend, et al. (2003). "Hematologic effects of linezolid in young children." *Pediatr Infect Dis J* **22**(9 Suppl): S186–S192.

Moumile, K., J. Merckx, et al. (2003). "Osteoarticular infections caused by *Kingella kingae* in children: contribution of polymerase chain reaction to the microbiologic diagnosis." *Pediatr Infect Dis J* **22**(9): 837–839.

Nourse, C., M. Starr, et al. (2007). "Community-acquired methicillin-resistant *Staphylococcus aureus* causes severe disseminated infection and deep venous thrombosis in children: literature review and recommendations for management." *J Paediatr Child Health* **43**(10): 656–661.

Peltola, H., L. Unkila-Kallio, et al. (1997). "Simplified treatment of acute staphylococcal osteomyelitis of childhood. The Finnish Study Group." *Pediatrics* **99**(6): 846–850.

Pickering L. K., B. C., S. S. Long, J. A. McMillan (2006). Staphylococcal Infections. *Red Book: 2006 Report of the Committee on Infectious Diseases*. B. C. Pickering LK, Long SS, McMillan JA. Elk Grove, American academy of Pediatrics: 598–610.

Rayner, C. R., L. M. Baddour, et al. (2004). "Linezolid in the treatment of osteomyelitis: results of compassionate use experience." *Infection* **32**(1): 8–14.

Rifai, A. and R. Nyman (1997). "Scintigraphy and ultrasonography in differentiating osteomyelitis from bone infarction in sickle cell disease." *Acta Radiol* **38**(1): 139–143.

Ruebner, R., R. Keren, et al. (2006). "Complications of central venous catheters used for the treatment of acute hematogenous osteomyelitis." *Pediatrics* **117**(4): 1210–1215.

Ruhe, J. J., T. Monson, et al. (2005). "Use of long-acting tetracyclines for methicillin-resistant *Staphylococcus aureus* infections: case series and review of the literature." *Clin Infect Dis* **40**(10): 1429–1434.

Sdougkos, G., V. Chini, et al. (2007). "Methicillin-resistant *Staphylococcus aureus* producing Panton-Valentine leukocidin as a cause of acute osteomyelitis in children." *Clin Microbiol Infect* **13**(6): 651–654.

Unkila-Kallio, L., M. J. Kallio, et al. (1994). "Serum C-reactive protein, erythrocyte sedimentation rate, and white blood cell count in acute hematogenous osteomyelitis of children." *Pediatrics* **93**(1): 59–62.

Yagupsky, P. (2004). "*Kingella kingae* infections of the skeletal system in children: diagnosis and therapy." *Expert Rev Anti Infect Ther* **2**(5): 787–794.

The Significance of Serotype Replacement for Pneumococcal Disease and Antibiotic Resistance

Keith P. Klugman

1 Introduction

Acute respiratory infections are the leading cause of death in children and the leading infectious cause of death in adults (WHO, 2005). There are in excess of 4 million deaths a year due to pneumonia and this estimate is conservative as it excludes HIV-associated deaths (WHO, 2005). Of these deaths, nearly two million are in children and the largest fraction of these deaths is vaccine preventable and caused by *Streptococcus pneumoniae*. There has been remarkable progress in the roll-out of pneumococcal conjugate vaccine in developed countries, but the cost of the vaccine has proven to be a considerable barrier to getting it to children at highest risk of death in developing countries. Two randomized trials in Africa have demonstrated the efficacy of a nine – valent conjugate to prevent pneumonia in HIV-uninfected (Klugman, Madhi et al., 2003; Cutts, Zaman et al., 2005) and HIV-infected children (Madhi, Kuwanda et al., 2005), as well as a 16% reduction in all-cause pneumonia (Cutts, Zaman et al., 2005). Effectiveness studies in the US have demonstrated not only a reduction in invasive disease among children immunized with seven-valent conjugate vaccine, but also through herd immunity in adults (Whitney, Farley et al., 2003) and young infants too young to have been immunized (Poehling, Talbot et al., 2006). Conjugate vaccine has also reduced the burden of pneumonia in the USA through direct protection of infants and herd immunity (Grijalva, Nuorti et al., 2007), and antibiotic resistance has similarly declined in both children and adults (Stephens, Zughaier et al., 2005; Kyaw, Lynfield et al., 2006). Thus, the impact of the vaccine in developed countries is clear, but there are some caveats to unbridled optimism that pneumococcal disease has been defeated. The current vaccine only protects

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against 7 of 91 serotypes, with partial protection against serotype 6A, a type closely related to 6B which is in the vaccine.

2 Replacement in Carriage

The first study to demonstrate that children exposed to pneumococcal conjugate vaccine appeared to be carrying an excess of non-vaccine types was a small study in the Gambia in which children were randomized to receive 2–3 doses of an experimental five-valent conjugate in infancy, followed by 23-valent polysaccharide vaccine at 18 months of age, compared to a control group who had received Hib vaccine. Carriage was measured at 24 months and children who had received three doses of conjugate had an excess of carriage of non-vaccine types (Obaro, Adegbola et al., 1996). This trial was followed by the demonstration of replacement at 9 months in a larger cohort of children who had received nine-valent conjugate in infancy (Mbelle, Huebner et al., 1999). There are a significant number of subsequent studies that have demonstrated replacement (Klugman, 2001) in carriage, and it is reasonable to assume that any vaccine able to prevent the acquisition of carriage of vaccine types will allow increased acquisition of non-vaccine types as a consequence. Indeed. the conjugate vaccines associated with greater immunogenicity (and presumably therefore greater impact on carriage of vaccine types) seem to be the very vaccines associated mostly with replacement (Klugman, 2001).

3 Replacement in Otitis Media

The relationship between conjugate pneumococcal vaccine immunogenicity and the extent of replacement in carriage is also found to some extent in otitis media. Two trials of conjugate vaccines were conducted using identical protocols in Finland (Eskola, Kilpi et al., 2001; Kilpi, Ahman et al., 2003). The vaccine conjugated to meningococcal outer membrane proteins (Kilpi, Ahman et al., 2003) was not pursued at that time for licensure because of lack of evidence of equivalent immunogenicity to the comparator, CRM-based conjugate, but although the efficacy against vaccine types was similar (56% vs. 57%), there was somewhat less evidence of replacement: 27% replacement (95% CI 6–70%) compared to the CRM conjugate (34% replacement; 95% CI 0–81%) (Eskola, Kilpi et al., 2001; Kilpi, Ahman et al., 2003). In another trial of a less immunogenic vaccine also not pursued for licensure, in which there was again similar efficacy against vaccine serotype otitis (58%), there was no evidence of replacement (−8% replacement; 95% CI −49–64%) (Prymula, Peeters et al., 2006). Although a number of reports from the USA have documented replacement of vaccine types, with nonvaccine types postconjugate vaccine introduction, these studies are confounded by a lack of a

denominator-based incidence so the extent of replacement in otitis media, postvaccine introduction, is unclear.

3.1 Replacement in Carriage and Otitis Media by Other Bacterial Species

There is little direct evidence to date that conjugate pneumococcal vaccine leads to replacement by other species. A nonsignificant increase in *Haemophilus influenzae* and *Moraxella cattarhalis* was seen in both Finnish trials (Eskola, Kilpi et al., 2001; Kilpi, Ahman et al., 2003), but not in the Czech and Slovak conjugate vaccine trial (Prymula, Peeters et al., 2006), in which trial the vaccine impact on *H. influenzae* was due to the Hemophilus protein D in the conjugate, and led to an overall increased efficacy against all otitis media (Prymula, Peeters et al., 2006). Four studies have now found a lower rate of staphylococcal carriage in HIV-uninfected children carrying pneumococci (Bogaert, van Belkum et al., 2004; Regev-Yochay, Dagan et al., 2004; McNally, Jeena et al., 2006; Madhi, Adrian et al., 2007), while the latter two studies did not find this reciprocal relationship among HIV-infected children (McNally, Jeena et al., 2006; Madhi, Adrian et al., 2007). These data suggest that at least in HIV-uninfected children, there may be an immunological mechanism that reduces pneumococcal carriage and allows the acquisition of carriage by *Staphylococcus aureus*. The only direct evidence that the pneumococcal conjugate vaccine may play a role in staphylococcal respiratory disease was an excess compared to controls in *S. aureus* isolates from draining ears of otitis-prone children who had received conjugate vaccine plus a boost with the 23v vaccine (Veenhoven, Bogaert et al., 2003).

4 Replacement in Invasive Pneumococcal Disease

There was no significant evidence of replacement disease in the randomized trials of conjugate vaccine against invasive pneumococcal disease (IPD) (Black, Shinefield et al., 2000; Klugman, Madhi et al., 2003; O'Brien, Moulton et al., 2003; Cutts, Zaman et al., 2005), suggesting that replacement only becomes significant in the community once herd immunity has led to the replacement of circulating vaccine types by nonvaccine types. Although replacement disease has been detected among IPD cases in the USA, the replacement has remained a small fraction of the disease burden prevented due to the virtual elimination from IPD of vaccine types (Whitney, Farley et al., 2003). The first evidence of replacement was found in 18–64-year-old HIV-infected adults in the USA, protected by herd immunity to a similar extent as HIV-uninfected adults (42% reduction in vaccine types compared to 47%), but showing evidence of

replacement (58% vs. 7%) (Flannery, Heffernan et al., 2006). The replacement IPD was most marked in women in whom the replacement disease completely abrogated the reduction in vaccine-type IPD (Flannery, Heffernan et al., 2006). Subsequently, replacement has been seen in both children and adults in native Alaskans, but not in nonnative Alaskans (Singleton, Hennessy et al., 2007). The replacement among native Alaskan adults has been such that among adults older than 45 years, overall IPD rates increased 43% for Alaska natives ($P = 0.03$) but declined 24% for nonnative Alaskans ($P = 0.02$) (Singleton, Hennessy et al., 2007). These data suggest that adults particularly susceptible to IPD will develop IPD when exposed to children carrying nonvaccine types, perhaps even at a greater rate than when exposed to children carrying vaccine types. There are few data on the relative invasiveness of nonvaccine types versus vaccine types, although the lesser degree of replacement with nonvaccine types in IPD compared to that observed in carriage, suggests a lesser invasiveness of these non-vaccine types at least in immunocompetent individuals. Once invasive disease occurs, a recent global study on IPD in adults was unable to detect a significant difference in outcome of IPD caused by vaccine types versus nonvaccine types (Alanee, McGee et al., 2007). However, among children in Alaska there has been an increase in the proportion, if not the absolute incidence, of IPD cases with empyema (2–13%, $P < 0.001$); IPD cases with pneumonia and bacteremia (from 40% to 57%, $P = 0.007$); and a decrease in IPD with no focus (from 54% to 40%, $P = 0.02$). The proportion of IPD cases in Alaskans younger than 5 years who were hospitalized has increased from 39% (109/279) in 1995–2000 to 62% (51/82) in 2004–2006 ($P < 0.001$) (Singleton, Hennessy et al., 2007). This observed increase in nonvaccine types has not been seen in all indigenous native groups with high rates of IPD (Giele, Moore et al., 2007) so that the risk factors for IPD due to nonvaccine types in immunized populations remain to be fully explored.

5 Genetics of Replacement Strains

The replacement strains causing IPD in children in the USA largely reflect preexisting nonvaccine clones that have expanded postconjugate vaccine introduction in 2000 (Beall, McEllistrem et al., 2006). However, there is evidence that is compatible with capsular shifts, particularly among serotype 19A strains. The expansion of ST199, previously common in serotype 19A but also found in 19F, is the least convincing example. A more striking example of potential capsular switch is the increase in ST320 among 19A strains in the USA; while globally this clone was more common in 19F than in 19A. It was not seen among 19A strains prior to vaccine introduction in the USA. The most compelling evidence of a capsular switch is the emergence of ST695; now the third most common clone among IPD cases of serotype 19A

in the USA, previously it was only found among serotype 4 pneumococci (Beall, 2007).

6 Replacement in Antibiotic-Resistant Strains

Antibiotic resistance among IPD strains in the USA has been dramatically reduced by the introduction of conjugate vaccine (Stephens, Zughaier et al., 2005; Kyaw, Lynfield et al., 2006). Most of the replacement phenomenon in the USA, as measured by the CDC surveillance program ABC, in ten states, has been due to serotype 19A (Pai, Moore et al., 2005) which has expanded from 2.5% of IPD in children less than 2 years of age in 1998–1999 to 36% of IPD in that age group in 2005, associated with an absolute increase in prevalence of that serotype (Beall, 2007). The proportion of 19A strains fully resistant to penicillin in the same time and age group among ABC sites has gone from 6.4% to 34.5% (Beall, 2007). An increase in IPD due to antibiotic-resistant serotype 19A has also been seen in Massachusetts (Pelton, Huot et al., 2007) and Texas (Messina, Katz-Gaynor et al., 2007). Antibiotic resistance has also expanded among serotype 19A causing noninvasive pneumococcal disease (Farrell, Klugman et al., 2007). Thus, the most important expansion of non-vaccine types has taken place among antibiotic-resistant strains, and therefore the factors leading to the expansion of resistance among nonvaccine types are likely to be selection by the vaccine and by antibiotic exposure. This has been shown elegantly in a prospective study of pneumococcal carriage in children with otitis media in France in which the risk of carriage of antibiotic resistance types was reduced by vaccination but increased by antibiotic exposure, with the greatest risk of carriage of resistant types among un-immunized children exposed to antibiotics (Cohen, Levy et al., 2006).

7 Conclusions

Conjugate pneumococcal disease has made a major impact on the burden of invasive disease, pneumonia, and antibiotic resistance where it has been introduced into developed countries. It has the potential to dramatically reduce the burden of infant mortality in rural developing countries without access to antibiotics and also to reduce the burden of respiratory disease among HIV-infected children. The vaccine has provided unanticipated benefits in terms of herd immunity with protection against both invasive disease and pneumonia among unvaccinated infants and adults in the USA. These benefits are tempered to some extent by the emergence of both invasive and noninvasive pneumococcal diseases due to serotypes not included in the currently available seven-valent conjugate vaccine. The extent of this replacement has been limited to date and is particularly seen among risk groups such as HIV-infected adults

and communities such as Alaskan native people at particular risk for IPD. Nonetheless, the emergence of replacement is likely to continue over time, so that the development of vaccines with greater valency as well as protein vaccine approaches to the prevention of pneumococcal disease remain an essential part of our fight against this important human pathogen.

References

Alanee, S. R., L. McGee, D. Jackson, C. C. Chiou, C. Feldman, A. J. Morris, A. Ortqvist, J. Rello, C. M. Luna, L. M. Baddour, M. Ip, V. L. Yu, and K. P. Klugman. (2007). Association of serotypes of *Streptococcus pneumoniae* with disease severity and outcome in adults: an international study. *Clin Infect Dis* **45**:46–51.

Beall, B. (2007). Vaccination with the pneumococcal 7-valent conjugate: a successful experiment but the species is adapting. *Expert Rev Vaccines* **6**:297–300.

Beall, B., M. C. McEllistrem, R. E. Gertz, Jr., S. Wedel, D. J. Boxrud, A. L. Gonzalez, M. J. Medina, R. Pai, T. A. Thompson, L. H. Harrison, L. McGee, and C. G. Whitney. (2006). Pre- and postvaccination clonal compositions of invasive pneumococcal serotypes for isolates collected in the United States in 1999, 2001, and 2002. *J Clin Microbiol* **44**:999–1017.

Black, S., H. Shinefield, B. Fireman, E. Lewis, P. Ray, J. R. Hansen, L. Elvin, K. M. Ensor, J. Hackell, G. Siber, F. Malinoski, D. Madore, I. Chang, R. Kohberger, W. Watson, R. Austrian, and K. Edwards. (2000). Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group. *Pediatr Infect Dis J* **19**:187–95.

Bogaert, D., A. van Belkum, M. Sluijter, A. Luijendijk, R. de Groot, H. C. Rumke, H. A. Verbrugh, and P. W. M. Hermans. (2004). Colonisation by Streptococcus pneumoniae and *Staphylococcus aureus* in healthy children. *Lancet* **363**:1871–2.

Cohen, R., C. Levy, F. de La Rocque, N. Gelbert, A. Wollner, B. Fritzell, E. Bonnet, R. Tetelboum, and E. Varon. (2006). Impact of pneumococcal conjugate vaccine and of reduction of antibiotic use on nasopharyngeal carriage of nonsusceptible pneumococci in children with acute otitis media. *Pediatr Infect Dis J* **25**:1001–7.

Cutts, F. T., S. M. Zaman, G. Enwere, S. Jaffar, O. S. Levine, J. B. Okoko, C. Oluwalana, A. Vaughan, S. K. Obaro, A. Leach, K. P. McAdam, E. Biney, M. Saaka, U. Onwuchekwa, F. Yallop, N. F. Pierce, B. M. Greenwood, and R. A. Adegbola. (2005). Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia: randomised, double-blind, placebo-controlled trial. *Lancet* **365**:1139–46.

Eskola, J., T. Kilpi, A. Palmu, J. Jokinen, J. Haapakoski, E. Herva, A. Takala, H. Kayhty, P. Karma, R. Kohberger, G. Siber, and P. H. Makela. (2001). Efficacy of a pneumococcal conjugate vaccine against acute otitis media. *N Engl J Med* **344**:403–9.

Farrell, D., K. Klugman, and M. Pichichero. (2007). Increased antimicrobial resistance among nonvaccine serotypes of *Streptococcus pneumoniae* in the pediatric population after the introduction of 7-valent pneumococcal vaccine in the United States. *Pediatr Infect Dis J* **26**:123–128.

Flannery, B., R. T. Heffernan, L. H. Harrison, S. M. Ray, A. L. Reingold, J. Hadler, W. Schaffner, R. Lynfield, A. R. Thomas, J. Li, M. Campsmith, C. G. Whitney, and A. Schuchat. (2006). Changes in invasive pneumococcal disease among HIV-infected adults living in the era of childhood pneumococcal immunization. *Ann Intern Med* **144**:1–9.

Giele, C., H. Moore, K. Bayley, C. Harrison, D. Murphy, K. Rooney, A. D. Keil, and D. Lehmann. (2007). Has the seven-valent pneumococcal conjugate vaccine had an impact on invasive pneumococcal disease in Western Australia? *Vaccine* **25**:2379–84.

Grijalva, C. G., J. P. Nuorti, P. G. Arbogast, S. W. Martin, K. M. Edwards, and M. R. Griffin. (2007). Decline in pneumonia admissions after routine childhood immunisation with pneumococcal conjugate vaccine in the USA: a time series analysis. *Lancet* **369**:1179–86.

Kilpi, T., H. Ahman, J. Jokinen, K. S. Lankinen, A. Palmu, H. Savolainen, M. Gronholm, M. Leinonen, T. Hovi, J. Eskola, H. Kayhty, N. Bohidar, J. C. Sadoff, and P. H. Makela. (2003). Protective efficacy of a second pneumococcal conjugate vaccine against pneumococcal acute otitis media in infants and children: randomized, controlled trial of a 7-valent pneumococcal polysaccharide-meningococcal outer membrane protein complex conjugate vaccine in 1666 children. *Clin Infect Dis* **37**:1155–64.

Klugman, K. P. (2001). Efficacy of pneumococcal conjugate vaccines and their effect on carriage and antimicrobial resistance. *Lancet Infect Dis* **1**:85–91.

Klugman, K. P., S. A. Madhi, R. E. Huebner, R. Kohberger, N. Mbelle, and N. Pierce for The Vaccine Trialists Group. (2003). A trial of a 9-valent pneumococcal conjugate vaccine in children with and those without HIV infection. *N Engl J Med* **349**:1341–8.

Kyaw, M. H., R. Lynfield, W. Schaffner, A. S. Craig, J. Hadler, A. Reingold, A. R. Thomas, L. H. Harrison, N. M. Bennett, M. M. Farley, R. R. Facklam, J. H. Jorgensen, J. Besser, E. R. Zell, A. Schuchat, and C. G. Whitney. (2006). Effect of introduction of the pneumococcal conjugate vaccine on drug-resistant *Streptococcus pneumoniae*. *N Engl J Med* **354**:1455–63.

Madhi, S. A., P. Adrian, L. Kuwanda, C. Cutland, W. C. Albrich, and K. P. Klugman. (2007). Long-term effect of pneumococcal conjugate vaccine on nasopharyngeal colonization by *Streptococcus pneumoniae* and associated interactions with *Staphylococcus aureus* and *Haemophilus influenzae* colonization in HIV infected and HIV uninfected children. *J Infect Dis* **196**:1662–6.

Madhi, S. A., L. Kuwanda, C. Cutland, and K. P. Klugman. (2005). The impact of a 9-valent pneumococcal conjugate vaccine on the public health burden of pneumonia in HIV-infected and -uninfected children. *Clin Infect Dis* **40**:1511–8.

Mbelle, N., R. E. Huebner, A. D. Wasas, A. Kimura, I. Chang, and K. P. Klugman. (1999). Immunogenicity and impact on nasopharyngeal carriage of a nonavalent pneumococcal conjugate vaccine. *J Infect Dis* **180**:1171–6.

McNally, L. M., P. M. Jeena, K. Gajee, A. W. Sturm, A. M. Tomkins, H. M. Coovadia, and D. Goldblatt. (2006). Lack of association between the nasopharyngeal carriage of *Streptococcus pneumoniae* and *Staphylococcus aureus* in HIV-1-infected South African children. *J Infect Dis* **194**:385–90.

Messina, A. F., K. Katz-Gaynor, T. Barton, N. Ahmad, F. Ghaffar, D. Rasko, and G. H. McCracken, Jr. (2007). Impact of the pneumococcal conjugate vaccine on serotype distribution and antimicrobial resistance of invasive *Streptococcus pneumoniae* isolates in Dallas, TX, children from 1999 through 2005. *Pediatr Infect Dis J* **26**:461–7.

O'Brien, K. L., L. H. Moulton, R. Reid, R. Weatherholtz, J. Oski, L. Brown, G. Kumar, A. Parkinson, D. Hu, J. Hackell, I. Chang, R. Kohberger, G. Siber, and M. Santosham. (2003). Efficacy and safety of seven-valent conjugate pneumococcal vaccine in American Indian children: group randomised trial. *Lancet* **362**:355–61.

Obaro, S. K., R. A. Adegbola, W. A. Banya, and B. M. Greenwood. (1996). Carriage of pneumococci after pneumococcal vaccination. *Lancet* **348**:271–2.

Pai, R., M. R. Moore, T. Pilishvili, R. E. Gertz, C. G. Whitney, B. Beall, and A. B. C. S. Team. (2005). Postvaccine genetic structure of *Streptococcus pneumoniae* serotype 19A from children in the United States. *J Infect Dis* **192**:1988 - 1995.

Pelton, S. I., H. Huot, J. A. Finkelstein, C. J. Bishop, K. K. Hsu, J. Kellenberg, S. S. Huang, R. Goldstein, and W. P. Hanage. (2007). Emergence of 19A as virulent and multidrug

resistant pneumococcus in Massachusetts following universal immunization of infants with pneumococcal conjugate vaccine. *Pediatr Infect Dis J* **26**:468–72.

Poehling, K. A., T. R. Talbot, M. R. Griffin, A. S. Craig, C. G. Whitney, E. Zell, C. A. Lexau, A. R. Thomas, L. H. Harrison, A. L. Reingold, J. L. Hadler, M. M. Farley, B. J. Anderson, and W. Schaffner. (2006). Invasive pneumococcal disease among infants before and after introduction of pneumococcal conjugate vaccine. *JAMA* **295**:1668–74.

Prymula, R., P. Peeters, V. Chrobok, P. Kriz, E. Novakova, E. Kaliskova, I. Kohl, P. Lommel, J. Poolman, J. P. Prieels, and L. Schuerman. (2006). Pneumococcal capsular polysaccharides conjugated to protein D for prevention of acute otitis media caused by both *Streptococcus pneumoniae* and non-typable *Haemophilus influenzae*: a randomised double-blind efficacy study. *Lancet* **367**:740–8.

Regev-Yochay, G., R. Dagan, M. Raz, Y. Carmeli, B. Shainberg, E. Derazne, G. Rahav, and E. Rubinstein. (2004). Association between carriage of Streptococcus pneumoniae and *Staphylococcus aureus* in children. *JAMA* **292**:716–20.

Singleton, R. J., T. W. Hennessy, L. R. Bulkow, L. L. Hammitt, T. Zulz, D. A. Hurlburt, J. C. Butler, K. Rudolph, and A. Parkinson. (2007). Invasive pneumococcal disease caused by nonvaccine serotypes among alaska native children with high levels of 7-valent pneumococcal conjugate vaccine coverage. *JAMA* **297**:1784–92.

Stephens, D. S., S. M. Zughaier, C. G. Whitney, W. S. Baughman, L. Barker, K. Gay, D. Jackson, W. A. Orenstein, K. Arnold, A. Schuchat, M. M. Farley, and Georgia Emerging Infections Program. (2005). Incidence of macrolide resistance in *Streptococcus pneumoniae* after introduction of the pneumococcal conjugate vaccine: population - based assessment. *Lancet* **365**:855–863.

Veenhoven, R., D. Bogaert, C. Uiterwaal, C. Brouwer, H. Kiezebrink, J. Bruin, I. J. E, P. Hermans, R. de Groot, B. Zegers, W. Kuis, G. Rijkers, A. Schilder, and E. Sanders. (2003). Effect of conjugate pneumococcal vaccine followed by polysaccharide pneumococcal vaccine on recurrent acute otitis media: a randomised study. *Lancet* **361**:2189–95.

Whitney, C. G., M. M. Farley, J. Hadler, L. H. Harrison, N. M. Bennett, R. Lynfield, A. Reingold, P. R. Cieslak, T. Pilishvili, D. Jackson, R. R. Facklam, J. H. Jorgensen, and A. Schuchat. (2003). Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. *N Engl J Med* **348**:1737–46.

WHO. (2005). World Health Report. World Health Organization, Geneva, Switzerland.

Childhood Intra-thoracic Tuberculosis

Ben J. Marais

1 Background

Tuberculosis (TB) has affected human kind for a very long time; archeological findings describe suggestive spinal changes in Neolithic man and conclusive evidence of tuberculous bone lesions have been found in mummified remains from Egypt, dating back to 3400 BC (Keers, 1978; Rubin, 1995). Hippocrates (460–377 BC) introduced the ancient Greek term for TB, *phthisis*, which became better known as consumption in the English world (Keers, 1978; Rubin, 1995). Although TB is an ancient disease it remains a major public health challenge. In fact, fuelled by poverty and rapid urbanization in developing countries, together with immune compromise resulting from human immunodeficiency virus (HIV) infection and emerging drug resistance, TB affects and kills more people today than ever before. The gravity of the situation is demonstrated by the fact that the TB epidemic continues to escalate, especially in sub-Saharan Africa, despite the declaration of a global TB emergency by the World Health Organization (WHO) in 1993.

TB is caused by *Mycobacterium tuberculosis (M. tuberculosis),* which was first identified by Robert Koch (1843–1910) in 1882. The diagnostic techniques pioneered by Koch have remained virtually unchanged since then; sputum smear microscopy remains the only diagnostic test available in most resource-limited settings. Interestingly, shortly after Robert Koch identified *M. tuberculosis* as the infectious agent that causes TB, it became apparent that infection with *M. tuberculosis* was far more common than actual TB disease. This form of latent TB infection is recognized by the presence of a positive tuberculin skin test (TST) in healthy asymptomatic individuals. A British Medical Research Council survey conducted in London in 1950 showed that 60–70% of individuals had a positive TST (were infected with *M. tuberculosis*) by 20 years of age (Bentley et al., 1954); an infection rate similar to those reported in TB-endemic areas today (Obihara

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A. Finn et al. (eds.), *Hot Topics in Infection and Immunity in Children V*,
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et al., 2005). The intriguing observation that only a small minority of people infected with *M. tuberculosis* ever develop active TB while the majority contain *M. tuberculosis* in its latent form (latent TB infection) remains largely unexplained.

In TB-endemic areas, TB control programs focus almost exclusively on adults with sputum smear-positive disease. It is commonly erroneously believed that following *M. tuberculosis* exposure children rarely develop active TB, and if they do, that the disease is mild, paucibacillary, and noninfectious. As a consequence of the misperception that children develop limited disease and therefore pose no TB transmission risk, childhood TB often fails to qualify as a public health priority in resource-limited settings. While it is true that young children contribute little to TB transmission, they frequently develop active TB following exposure and experience considerable TB-related morbidity and mortality in TB-endemic areas (Marais et al., 2006e; Chintu et al., 2002). In addition, adolescent children (>10 years of age) frequently develop sputum smear-positive adult-type TB (Marais et al., 2005b), and pose a high transmission risk particularly in congregate settings such as schools (Curtis et al., 1999).

2 Epidemiology

An estimated 8.3 million new TB cases were diagnosed in 2000, of whom 884,019 (11%) were children less than 15 years of age (Nelson and Wells, 2004). Poor countries carry the bulk of the TB disease burden, as both exposure to the organism and the risk of progression to active TB following infection are increased in these settings (Marais et al., 2005e) (Fig. 1). In addition to poverty, HIV-related immune compromise dramatically increases an individual's vulnerability to develop active TB following exposure, which explains why sub-Saharan Africa, the region worst affected by HIV, consistently reports the highest TB incidence rates in the world (Corbett, 2003; Marais et al., 2007).

TB is spread via tiny aerosol droplets that may remain suspended in the air for hours. These infectious droplets are predominantly produced by adults and adolescents with sputum smear-positive TB. However, adults with sputum smear-negative disease also pose a significant, albeit reduced, transmission risk. Pre-chemotherapy observations indicated that the transmission risk posed by a sputum smear-negative adult with pulmonary TB was reduced by 60–70% compared to a sputum smear-positive index case (Marais et al., 2004a). This is particularly relevant in settings with a high prevalence of HIV where TB is the single most common opportunistic infection. HIV-infected adults, who are predominantly of reproductive age, frequently develop sputum smear-negative TB, therefore, young children from HIV-affected households are more likely to be in contact with parents and/or caregivers with sputum smear-negative TB (Marais et al., 2007).

Following *M. tuberculosis* exposure the subsequent risk of infection depends on the infectiousness of the index case, as well as the proximity and duration of contact. According to classic teaching, TB transmission occurs mainly due to

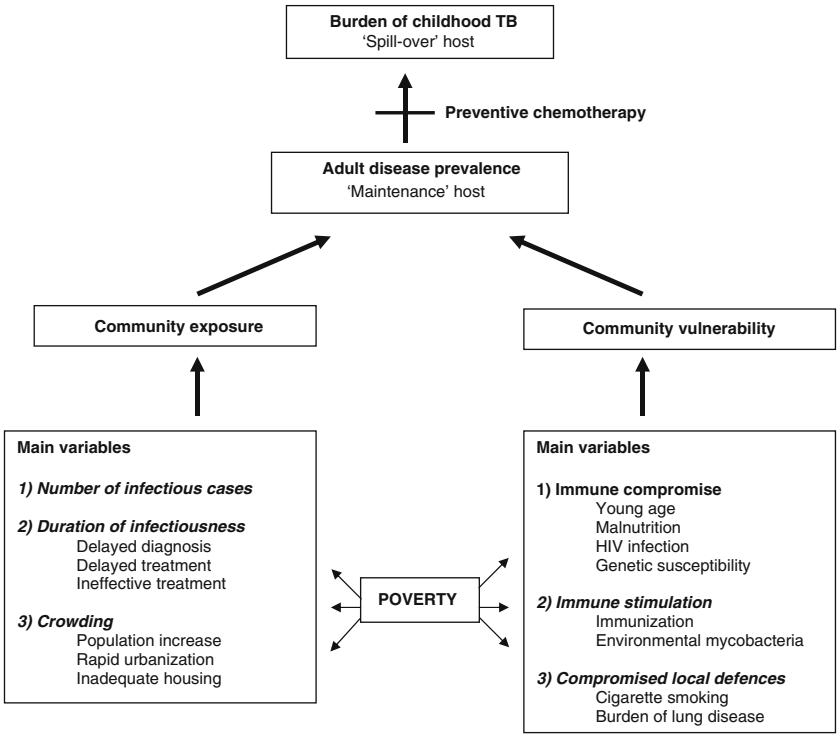


Fig. 1 Main variables that contribute to the prevalence of TB in adults and by extrapolation the burden of childhood TB
Source: Adapted from: Marais et al. (2005e)

household contact with an adult index case. Although the infection risk is highest among household contacts, in hyperendemic areas the majority of transmission occurs outside the household (Verver at al., 2004; Schaaf et al., 2003). It is important to emphasize that this is a function of the high TB prevalence within these communities and does not reduce the importance of household TB exposure, especially in young and/or vulnerable children. The natural history of TB disease demonstrates that the risk of developing active TB following infection with *M. tuberculosis* is mainly determined by the age and immune status of the child (Marais et al., 2004b).

3 Natural History of Disease

The natural history of childhood TB was meticulously documented in the pre-chemotherapy literature, especially during the period from 1920 to 1950. This represents the period when bacteriology and chest radiography was widely

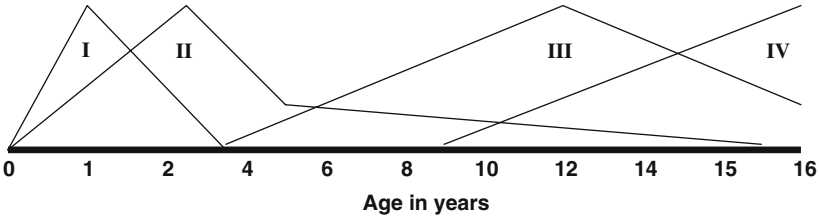
available for accurate disease diagnosis and description, but in the absence of effective treatment it was possible to record the natural progression/regression of disease. Some excellent observational studies, characterized by large cohorts of children (greater than 1000) followed for prolonged periods of time (more than 10 years) were conducted during this period (Marais et al., 2004b). The first TB drugs (para-aminosalicylic acid and streptomycin) were introduced after the World War II; more effective drugs such as isoniazid (INH) only became available in the early 1950s (Marais et al., 2004b).

A recent summary of studies of the natural history of TB concluded that the risk of developing active TB following *M. tuberculosis* infection is highest in very young (immune immature) and/or immunocompromised children (Marais et al., 2004b). In immune competent children the majority (more than 95%) who develop active TB do so within 12 months of primary infection (Marais et al., 2004b). Therefore, age at the time of exposure/infection, the immune status of the child, and the time since exposure/infection are the main determinants of a child's risk of developing active TB. Because active TB usually develops shortly after primary infection, childhood TB provides a unique epidemiological perspective; the burden of childhood TB accurately reflects the level of ongoing TB transmission within the community. In addition, due to the paucibacillary nature of childhood TB, few children acquire drug resistance. Therefore, drug-resistance patterns among child TB cases provide a reliable estimate of transmitted (primary) drug resistance within the community (Schaaf et al., 2006).

4 Intra-thoracic TB

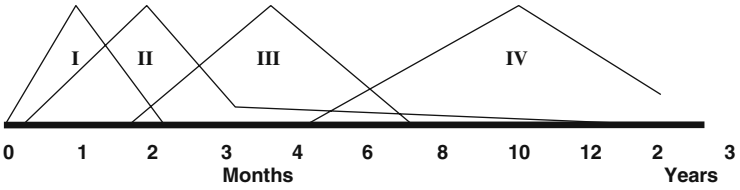
M. tuberculosis usually enters its human host via the lungs. Inhalation of an infectious droplet, with the right size to penetrate into the periphery of the lung, results in a localized area of pneumonic consolidation at the site of organism deposition. This is referred to as the primary (Ghon) focus and after an initial period of unrestrained organism multiplication, the TB bacilli drain via local lymphatics to the regional lymph nodes. The Ghon focus together with enlarged regional lymph nodes, usually situated in the subcarinal and/or perihilar area, is referred to as the primary complex (Marais et al., 2004c).

Active TB may present with a diverse spectrum of pathology (Marais et al., 2006d). The various disease manifestations show clear patterns that are related to the age at the time of primary infection (Fig. 2), and the time since infection occurred (Fig. 3) (Curtis et al., 1999; Marais et al., 2005a). Potential factors that influence the specific manifestation of intra-thoracic TB in children are shown in Fig. 4. The disease manifestations observed in immunocompromised children appear to correlate well with those seen in very young (less than 3 years of age) children with immature immune systems (Marais et al., 2004b, 2006d, 2005a). The spectrum of intra-thoracic disease manifestations are briefly summarized.



I Complicated Ghon focus and/or disseminated (miliary) disease
II Uncomplicated and complicated lymph node disease
III Pleural effusion
IV Adult-type disease

Fig. 2 Age-related manifestations of pulmonary tuberculosis in immune-competent children
Source: Adapted from: Marais et al. (2005a)



I Hypersensitivity responses and tuberculin skin test (TST) conversion
II Ghon focus and/or disseminated (miliary) disease
III Lymph node disease (<5 years of age)/ pleural or pericardial effusion (>5 years of age)
IV Adult-type disease (>10 years of age)

Fig. 3 Schematic timeline, indicating the average time when different disease manifestations occur, following primary infection with *M. tuberculosis*
Source: Adapted from the original time-line of tuberculosis described by Wallgren: Marais et al (2004b).

4.1 Lymph Node Disease

Involvement of the regional lymph nodes (subcarinal, perihilar, or paratracheal) is considered the radiological hallmark of primary infection (Figs. 5 and 6) (Leung et al., 1992). It is most commonly seen in children less than 5 years of age, probably due to exuberant lymph node responses together with the small calibre and pliability of the airways at this age (Marais et al., 2005a). On chest radiography (CXR) both antero-posterior (AP) and lateral views are required for optimal lymph node visualization, but it may remain difficult to visualize enlarged lymph nodes with certainty (Marais et al., 2004c). High-resolution computed tomography of the lung is regarded as the most sensitive method to detect hilar adenopathy (Andronikou et al., 2004), but it is important to point out

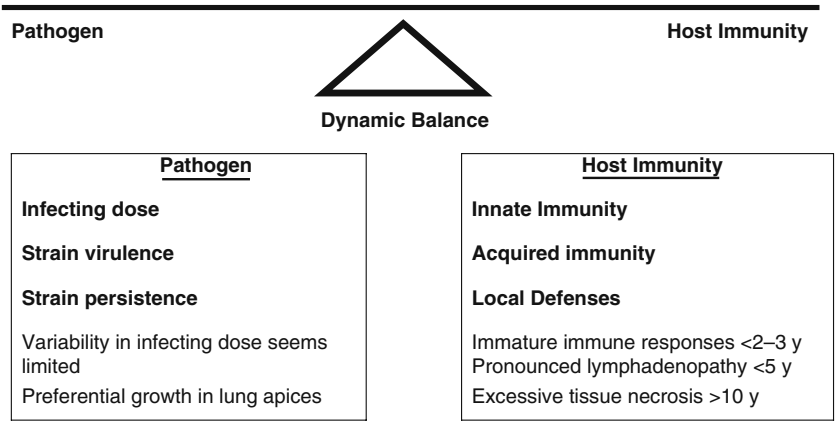


Fig. 4 Potential factors that influence the specific manifestations of intra-thoracic TB in children
Source: Adapted from: Marais et al. (2005a)

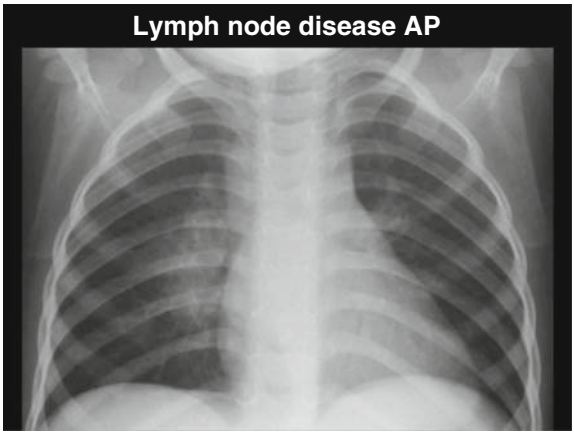


Fig. 5 Uncomplicated lymph node disease (antero-posterior view)
Source: Adapted from: Marais et al. (2004c)

that the presence of hilar adenopathy in the absence of clinical symptoms does not necessarily indicate active TB (Marais et al., 2006c).

Lymph node disease includes a range of possible lympho-bronchial pathology and involvement of adjacent anatomical structures (Marais et al., 2004b, 2004c). Airway compression is best visualized on a high kilovolt CXR and may have different radiological presentations (Marais et al., 2004c). Partial airway obstruction may cause a check-valve effect with distal hyperinflation, while total airway obstruction results in the resorption of distal air with alveolar collapse. When a caseated lymph node erupts into an airway, caseous material may be aspirated and the resulting pathology may range from caseating pneumonia to hypersensitivity-induced inflammation depending on the bacterial load and viability of the bacilli aspirated. Caseating pneumonia causes progressive parenchymal

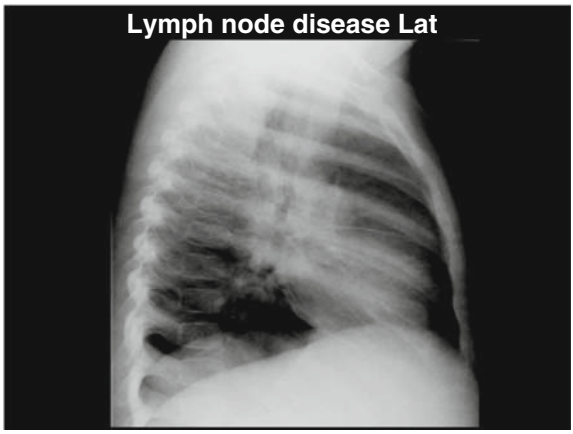


Fig. 6 Uncomplicated lymph node disease (lateral view)
Source: Adapted from: Marais et al. (2004c)

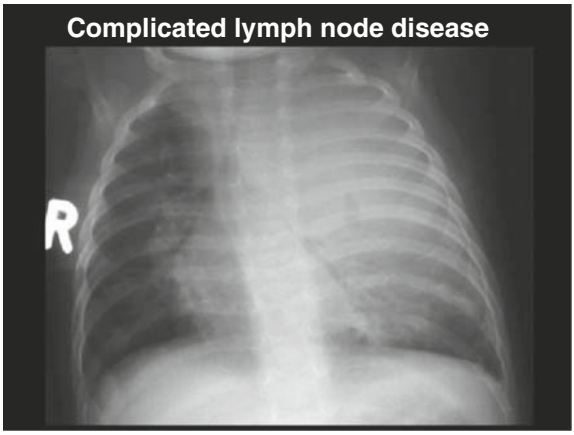


Fig. 7 Complicated lymph node disease with bronchial compression, expansile caseating pneumonia, and cavitation
Source: Adapted from: Marais et al. (2004c)

destruction; affected segments/lobes often become expansile (bulging against their anatomical boundaries), and areas of parenchymal breakdown (cavitation) may be visible on the CXR or high-resolution computed tomography scan (Fig. 7) (Goussard et al., 2004). Anatomical structures that are rarely involved include: the phrenic nerve with unilateral diaphragmatic palsy, the esophagus with the formation of a broncho- or tracheo-esophageal fistula, and/or the thoracic duct with the formation of a unilateral chylothorax (Marais et al., 2004c).

4.2 Pleural Effusion

Pleural effusions are unusual in children less than 3 years of age and tend to develop within the first 3–9 months after primary infection (Marais et al.,

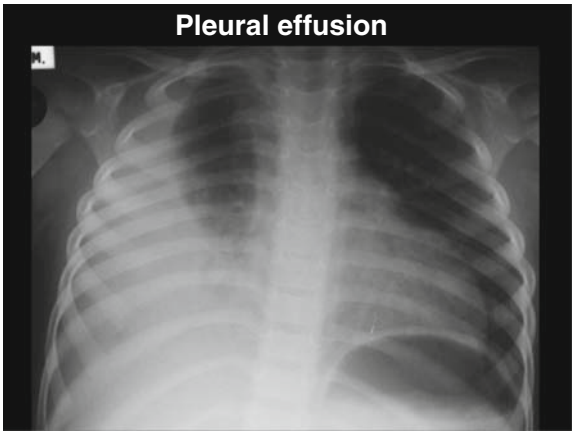


Fig. 8 Right-sided pleural effusion
Source: Adapted from: Marais et al. (2004c)

2004b). The accumulation of the typical lymphocyte-rich, straw-coloured fluid, containing very few organisms represents a hypersensitivity response. The effusion typically obliterates 30–60% of the affected hemi-thorax, although massive fluid collections may cause mediastinal shift and cardiovascular compromise (Fig. 8) (Marais et al., 2004c). A persistent loculated fluid collection may indicate the presence of a tuberculous empyema (Marais et al., 2004c).

4.3 Pericardial Effusion

A pericardial effusion usually develops when a subcarinal lymph node erupts into the pericardial space, but hematogenous spread is also possible (Marais et al., 2004c). On CXR the heart shadow is often enlarged with a suggestive globular appearance. Cardiac ultrasound is the most sensitive way to confirm the presence of a pericardial effusion (Marais et al., 2004c). Long-term sequelae include constrictive pericarditis. Reducing the development of this complication is the rationale for using adjunctive corticosteroid therapy in children with pericardial effusion (Marais et al., 2006c).

4.4 Disseminated (Miliary) Disease

Occult dissemination is not uncommon following primary infection, but it rarely progresses to disseminated disease except in very young (less than 2–3 years of age) and immunocompromised children (Marais et al., 2004c, 2006d). The typical radiological signs include the presence of uniform miliary lesions (less than 2 mm in size) that are distributed bilaterally into the periphery of the lung (Fig. 9) (Marais et al., 2004c). Diagnostic confusion often occurs in HIV-infected children in whom lymphocytic interstitial pneumonitis (LIP), malignancies, and opportunistic infections such as *Pneumocystis jiroveci* may present

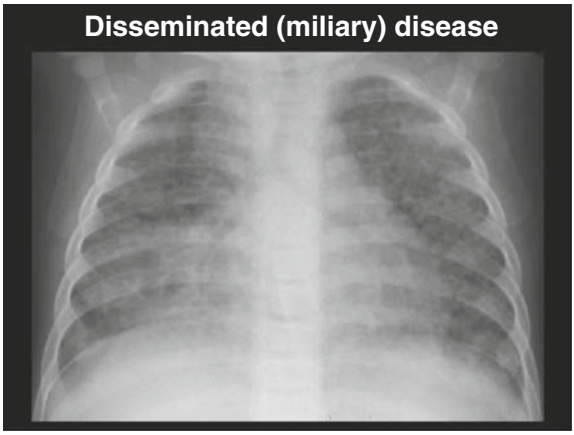


Fig. 9 Disseminated (miliary) TB disease
Source: Adapted from: Marais et al. (2004c)

with a similar radiological picture (Marais et al., 2007). Response to treatment and/or bacteriologic confirmation are the only ways to establish a more definitive diagnosis, although this is only achieved retrospectively.

4.5 Adult-Type Disease

Adult-type disease first appears from 8 to 10 years of age and becomes the dominant disease manifestation during adolescence (Marais et al., 2005a). As in adult TB, the apical and posterior segments of the upper lobe and the apical segment of the lower lobe are most commonly affected (Fig. 10). Complications include progressive cavity formation and intra-bronchial spread; these children are frequently sputum smear-positive and pose a high TB-transmission risk (Marais et al., 2005b; Marais, 2007; Weber et al., 2000).

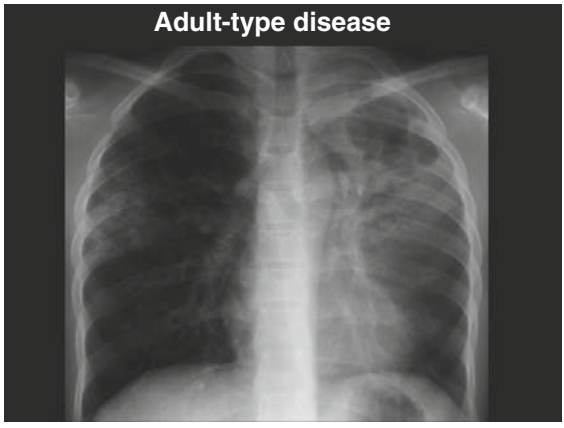


Fig. 10 Adult-type TB disease
Source: Adapted from: Marais et al. (2004c)

4.6 Immune Reconstitution Phenomena

Immune recovery may result in increased inflammation of tissues infected with *M. tuberculosis*. Immune reconstitution phenomena were first documented in the pre-chemotherapy literature following nutritional rehabilitation and/or the termination of high-dose steroid treatment (Marais et al., 2004b). Recently, immune reconstitution inflammatory syndrome (IRIS) has become an important complication to consider following the introduction of highly active antiretroviral therapy in HIV-infected children with severe immune compromise. Radiological signs include paradoxical lymph node enlargement with or without worsening airway compression due to increased inflammation surrounding diseased lymph nodes, or new alveolar consolidation with/without parenchymal breakdown due to excessive inflammation in areas of previous 'subclinical' tuberculous infiltration (Marais et al., 2007, 2006c).

5 Diagnosis

The diagnosis of childhood TB is problematic as bacteriologic confirmation is rarely achieved. Sputum smear microscopy, often the only diagnostic test available in resource-limited settings, is positive in less than 10–15% of children with TB and culture yields are also generally low (30–40%) in children with probable TB (Starke, 2003; Zar et al., 2005). However, in those with more advanced TB disease, culture confirmation is achievable in the majority of cases (60–70%) (Marais et al., 2006f). In low-burden countries the diagnosis of childhood TB is predominantly made on clinical grounds in the presence of an associated epidemiological risk. In this setting, a triad of a positive TST, a suggestive CXR, and a known infectious TB contact is usually taken to indicate active TB. This provides a fairly accurate diagnosis in nonendemic countries where exposure to *M. tuberculosis* is rare and usually well documented. However, the triad has limited value in TB-endemic areas where exposure to *M. tuberculosis* is common and often undocumented (Marais et al., 2006c). Consequently, the diagnosis of TB in these areas depends predominantly on the subjective interpretation of the CXR (Marais et al., 2005c; Theart et al., 2005) which has well-known limitations (Osborne, 1995; Du Toit et al., 2002). Recent advances in symptom-based diagnostic approaches and the use of novel T-cell assays are briefly discussed below.

5.1 Symptom-Based Approaches

5.1.1 Screening for Active TB

Most national TB guidelines regard the TST and CXR as prerequisite tests for the screening of household contacts, but these tests are rarely available in TB-endemic areas with limited resources and where access to preventive

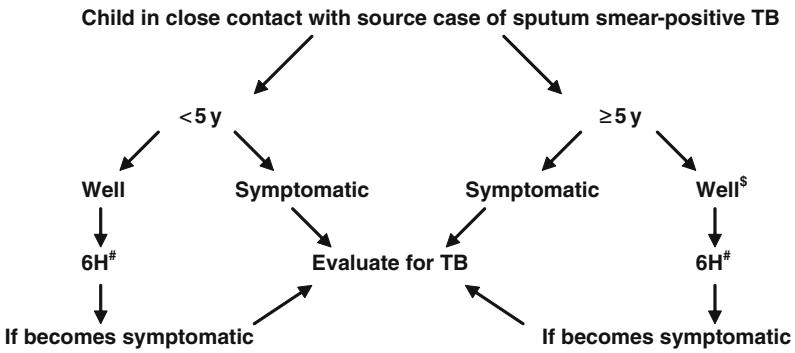


Fig. 11 Suggested approach (WHO, 2006) to contact management when chest radiography and tuberculin skin testing are not readily available
[#] Isoniazid 10mg/kg daily for 6 months.
[$]Unless the child is HIV-infected (in which case isoniazid 10mg/kg daily for 6 months is indicated).
Source: Adapted from: World Health Organization (2006).

therapy is needed most. A recent study evaluated the value of symptom-based screening compared with TST and CXR-based screening in children treated for active TB (Marais et al., 2006a). The findings suggest that simple symptom-based screening could have considerable value in resource-limited settings, given that more than 90% of children diagnosed with TB on CXR reported symptoms. Moreover, the few asymptomatic cases who received TB treatment either had no radiological signs of TB or had uncomplicated hilar adenopathy in isolation, probably reflecting recent primary infection and therefore not indicative of active TB (Marais et al., 2006c). These findings require further confirmation, but innovative approaches will be required to facilitate the provision of preventive therapy to children (at least to those at high risk of developing active TB) in resource-limited settings. The most recent WHO guidance to national TB programs on the management of TB in children, acknowledges that symptom-based screening may be the only feasible option in settings where TST and CXR are not readily available (Fig. 11) (World Health Organization, 2006).

5.1.2 Diagnosing Active TB

Due to the diagnostic limitations and the difficulty of obtaining a CXR in many TB-endemic countries, a variety of clinical-scoring systems have been developed. A critical review of these scoring systems concluded that they are severely limited by the absence of standard symptom definitions and a lack of adequate validation (Hesseling et al., 2002). Accurate symptom definition is important to differentiate TB from other common conditions. A community-based survey demonstrated that poorly defined symptoms (such as a cough of more than 3 weeks duration) are frequently reported in a random selection of healthy children and have poor

discriminatory power (Marais et al., 2005d). A follow-on study demonstrated that the use of well-defined symptoms with a persistent, nonremitting character significantly improved the diagnostic accuracy (Marais et al., 2005c).

To substantiate the findings of this small pilot study, a large prospective community-based study was recently completed that enrolled 1024 children over a 2-year period in Cape Town, South Africa (Marais et al., 2006b). The study demonstrated that well-defined symptoms offer good diagnostic accuracy in low-risk children (immune competent children more than 3 years of age). The presence of three symptoms at presentation including a persistent nonremitting cough for more than 2 weeks, documented failure to thrive during the preceding 3 months, and fatigue, provided a sensitivity of 82.3%, a specificity of 90.2%, and a positive predictive value of 82.3% for the diagnosis of pulmonary TB (Marais et al., 2006b). In addition, clinical follow-up served as a valuable tool to differentiate active TB from other common diseases in children who did not meet all three diagnostic criteria at the initial evaluation.

In high-risk groups more caution is required due to the rapidity with which disease progression may occur, but the above approach still performed reasonably well in young (less than 3 years of age) HIV-uninfected children (Marais et al., 2006b). However, it performed poorly in HIV-infected children, mainly due to poor specificity as a result of chronic symptomatology caused by other opportunistic infections or HIV-related conditions (Marais et al., 2006b). In summary, a clear distinction must be made between symptom-based screening where any current symptom, irrespective of its duration, are considered and symptom-based diagnosis where the use of well-defined symptoms is essential to improve specificity. The diagnostic use of well-defined symptoms holds definite promise in low-risk children (immune competent children more than 3 years of age) in whom TB is a slowly progressive disease. However, the diagnosis of TB in HIV-infected children remains a challenge.

The most common extra-thoracic manifestation of TB in children is cervical adenopathy. A simple clinical algorithm that identified children with persistent (more than 4 weeks) cervical adenopathy (cervical mass greater than 2 cm diameter) without a visible local cause and/or poor response to first-line antibiotics, showed excellent diagnostic accuracy in a TB-endemic area (Marais et al., 2006h). The possibility of accurate clinical diagnosis of TB cervical adenitis at primary healthcare level could dramatically improve anti-TB treatment in resource-limited settings. Clinical follow-up is essential to ensure that children who do not respond to standard anti-TB treatment are referred to establish a definitive diagnosis.

5.2 Immune-Based Approaches

5.2.1 T Cell Assays

Apart from frequent non-availability, the TST is also limited by suboptimal specificity and sensitivity, especially in HIV-infected children (Madhi et al.,

1999, Marais and Pai, 2007). An alternative to the traditional TST has emerged in the form of blood-based assays that measure interferon-gamma (IFN-γ) released by sensitized T cells after stimulation by *M. tuberculosis*-specific antigens. These antigens include early-secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), which are more specific than purified protein derivative (PPD) used in the traditional TST. These antigens are not shared with bacille Calmette-Guérin (BCG) vaccine strains and most environmental mycobacteria (Marais and Pai, 2007; Pai et al., 2004).

Two assays are currently available as commercial kits: the T-SPOT.*TB*® test (Oxford Immunotec, Oxford, UK), and the QuantiFERON®-TB Gold® assay (Cellestis Ltd, Carnegie, Australia). The QuantiFERON®-TB Gold® assay has been approved by the US Food and Drug Administration (FDA). The T-SPOT.*TB* is licensed for use in Europe and Canada, but to date has not received FDA approval. The available evidence for the use of these assays in the diagnosis of TB has been extensively reviewed (Marais and Pai, 2007; Pai et al., 2004); this is an area of active research and it remains to be seen whether these new tests will fulfill their considerable promise, particularly in children.

6 Treatment

The principles of TB treatment are: first, to reduce the organism load as rapidly as possible and second, to ensure effective eradication of persistent bacilli (Marais et al., 2006c). These principles provide the rationale behind the intensive and continuation phase of anti-TB treatment regimens. Rapid reduction of the organism load is important to reduce clinical symptoms, limit disease progression, terminate transmission, and reduce the risk of acquired drug resistance. Eradication of persistent (dormant or intermittently metabolizing) bacilli is essential to prevent future disease relapse (Marais et al., 2006c).

6.1 Preventive Chemotherapy

An important concept derived from the natural history of disease is that of risk stratification; it determines the diagnostic emphasis and guides therapeutic decision making (Marais et al., 2006c). While it is important to provide preventive chemotherapy to high-risk children with documented exposure/infection, it is less relevant in low-risk children, especially in TB-endemic areas where both primary and re-infection events are common; in these settings containment of the TB epidemic is the immediate aim and not TB eradication (Marais et al., 2006c).

INH monotherapy for 6–9 months is the best-studied preventive regimen, but poor adherence with unsupervised treatment is a serious concern and alternative preventive regimens with improved adherence require consideration (Marais et al., 2006g; Van Zyl et al., 2006). The addition of rifampicin (RMP) to

preventive regimens has numerous advantages; reducing the risk of INH monoresistance, improving organism eradication, shortening the duration of treatment, and improving adherence (Marais et al., 2006c, 2006g). INH and RMP for 3-months duration is a well-established preventive regimen that provides equivalent protection to 6–9 months of INH monotherapy (Ena and Valls, 2005). In theory the addition of pyrazinamide (PZA), a drug with strong sterilizing activity, should improve the sterilizing ability of the preventive-therapy regimen, while also shortening the required treatment duration and improving adherence (Marais et al., 2006c). This requires further evaluation.

6.2 Standard Treatment

The recommended treatment for intra-thoracic TB in children is 6 months of fully supervised treatment comprising 2 months of a three-drug (INH, RMP, PZA) regimen followed by 4 months of a two-drug (INH, RMP) regimen (World Health Organization (Regimen 3), 2006). Children with extensive lung involvement and/or visible cavitation should also receive ethambutol (EMB) during the 2-months intensive phase (WHO [Regimen 1], 2006). RMP-based regimens are effective and cheap, but child-friendly formulations are not readily available in most TB-endemic countries. Fortunately this is changing, 2007 represents the first year that the global drug facility (GDF) has made child-friendly TB treatment formulations available to resource-limited countries with a well-functioning TB-control program. A few treatment-related issues are briefly discussed below.

Current fixed-dose combination tablets provide 4–6 mg/kg of INH, which may be suboptimal, particularly in settings where the majority of the population are rapid acetylators of INH (Donald et al., 2004; Schaaf et al., 2005). In addition, the majority of INH monoresistance encountered in endemic areas is of an intermediate or low level, which underscores the importance of optimal INH dosing (Ellard et al., 1993; Schaaf et al., 2007). For these reasons and also since children are less susceptible to the toxic effects of INH than adults, a standard INH dosage of 10 mg/kg seems more appropriate in children.

In children with disseminated (miliary) disease and/or TB meningitis the penetration of individual anti-TB drugs into the cerebrospinal fluid (CSF) is an important consideration (Marais et al., 2006c). INH and PZA penetrate the CSF well (Ellard et al., 1993), while RMP and streptomycin (S) penetrate the CSF only in the presence of meningeal inflammation (ATS, 1994). Ethambutol (EMB) penetrates the CSF poorly if at all even in the presence of meningeal inflammation (ATS, 1994; Ellard et al., 1993). Ethionamide (ETH) shows good CSF penetration, which explains why its inclusion is advocated to strengthen the TBM treatment regimen (Marais et al., 2006c; Donald and Seifart, 1989).

Radiographic disease resolution may take many months despite significant symptomatic improvement; persistent radiographic signs are therefore not an indication to change treatment if there is clinical improvement. Paradoxical exacerbation of symptoms or signs may occur after TB therapy is initiated due to immune reconstitution and/or the release of bacterial toxins. Treatment should continue unaltered, although the temporary addition of corticosteroids may be considered. Adjunctive steroid therapy may be helpful when the host inflammatory response contributes to disease pathology, such as in TBM, severe lymph node compression of the airways and pericardial effusion (Marais et al., 2006c). No benefit has been demonstrated for the use of steroids in the treatment of TB pleural effusion.

6.3 Drug Resistance

The selection of drug-resistant organisms occurs primarily in patients with high bacillary loads. Due to the paucibacillary nature of childhood TB, children contribute little to the creation of drug resistance. However, they are severely affected by it. A recent survey from Cape Town recorded INH resistance in 12.4% of child isolates, and multidrug (INH and RMP) resistance in 5.3% (Schaaf et al., 2006). This is alarming as drug-resistance patterns among children provide an accurate indication of transmitted (primary) drug resistance within a community. The emergence of drug resistance pose major new challenges to TB-control efforts, although the basic principles of diagnosis and management remain similar.

In conclusion, cheap and effective TB treatment is available, but improving treatment access for children in resource-limited settings remains a major challenge. Providing access to preventive therapy for high-risk exposed and/or infected children and treatment for all children with active TB will drastically reduce the severe TB-related morbidity and mortality suffered by children in endemic areas. Ultimately the burden of childhood TB reflects the level of epidemic control achieved. There is a need expand the directly observed therapy short-course (DOTS) approach to include a greater emphasis on reducing the vulnerability of communities. The formulation of the United Nations Millennium Developmental Goals represents an important step in the right direction, but global political commitment is required to support this key initiative.

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References

American Thoracic Society. Treatment of Tuberculosis and tuberculosis infection in adults and children. Am J Respir Crit Care Med 1994; 149: 1359–1374

Andronikou S, Joseph E, Lucas S et al. CT scanning for the detection of tuberculous mediastinal and hilar lymphadenopathy in children. Pediatr Radiol 2004; 34: 232–236

Bentley FJ, Grzybowski S, Benjamin B. Tuberculosis in childhood and adolescence. The National Association for the Prevention of Tuberculosis. Waterlow and Sons Ltd, London 1954: 1–213 and 238–253

Chintu C, Mudenda V, Lucas S et al. Lung diseases at necropsy in African children dying from respiratory illnesses: a descriptive necropsy study. Lancet 2002; 360: 985–990

Corbett EL. The growing burden of tuberculosis. Global trends and interactions with the HIV epidemic. Arch Intern Med 2003; 163: 1009–1021

Donald PR, Seifart HI. Cerebrospinal fluid concentrations of ethionamide in children with tuberculous meningitis. J Pediatr 1989; 115: 483–486

Donald PR, Sirgel FA, Venter A et al. The influence of human N-acetyltransferase genotype on the early bactericidal activity of isoniazid. Clin Infect Dis 2004; 39: 1425–1430

Du Toit G, Swingler G, Iloni K. Observer variation in detecting lymphadenopathy on chest radiography. Int J Tuberc Lung Dis. 2002; 6: 814–817

Ellard GA, Humphries MJ, Allen BW. Cerebrospinal fluid drug concentrations and the treatment of tuberculous meningitis. Am Rev Resp Dis 1993; 148: 650–655

Ena J, Valls V, Short-course therapy with rifampin plus Isoniazid, compared with standard therapy with Isoniazid, for latent tuberculosis infection: a meta-analysis. Clin Infect Dis 2005; 40: 670–676

Goussard P, Gie RP, Kling S, Beyers N. Expansile pneumonia in children caused by Mycobacterium tuberculosis: clinical, radiological and bronchoscopic appearances. Pediatr Pulm 2004; 38: 451–455

Hesseling AC, Schaaf HS, Gie RP, Starke JR, Beyers N. A critical review of diagnostic approaches used in the diagnosis of childhood tuberculosis. Int J Lung Dis 2002; 6: 1038–1045

Keers RY: Pulmonary tuberculosis: A journey down the centuries. London, Baillaire-Tindall, 1978

Leung AN, Muller NL, Pineda PR, et al. Primary tuberculosis in childhood: radiographic manifestations. Radiology 1992; 182: 87–91

Madhi S, Gray G, Huebner RE et al. Correlation between CD4+ lymphocyte counts, concurrent antigen skin test and tuberculin skin test reactivity in human immunodeficiency virus type 1–infected and uninfected children with tuberculosis. Pediatr Infect Dis J 1999; 18: 800–805

Marais BJ, Gie RP, Schaaf HS et al. The clinical epidemiology of childhood pulmonary tuberculosis—A critical review of the pre-chemotherapy literature. Int J Tuberc Lung Dis. 2004a; 8: 278–285

Marais BJ, Gie RP, Schaaf HS et al. The natural history of childhood intra-thoracic tuberculosis—A critical review of the pre-chemotherapy literature. Int J Tuberc L Dis. 2004b; 8: 392–402

Marais BJ, Gie RP, Schaaf HS, Starke JR et al. A proposed radiological classification of childhood intra-thoracic tuberculosis. Ped Rad 2004c; 33: 886–894

Marais BJ, Donald PR, Gie RP, Schaaf HS, Beyers N. Diversity of disease manifestations in childhood pulmonary tuberculosis. Ann Trop Paed 2005a; 25: 79–86

Marais BJ, Gie RP, Hesseling AC, Beyers N. Adult-type pulmonary tuberculosis in children aged 10–14 years. Pediatr Inf Dis J 2005b; 24: 743–744

Marais BJ, Gie RP, Obihara CC, Hesseling AC, Schaaf HS, Beyers N. Well-defined symptoms have improved value in the diagnostics of childhood pulmonary tuberculosis. Arch Dis Child 2005c; 90: 1162–1165

Marais BJ, Obihara CC, Gie RP, Schaaf HS, Hesseling AC, Lombard C, Enarson D, Bateman E, Beyers N. The prevalence of symptoms associated with pulmonary tuberculosis in the average child from a high-burden community. Arch Dis Child 2005d; 90: 1166–1170

Marais BJ, Obihara CC, Warren RM et al. The burden of childhood tuberculosis—a public health perspective. Int J Tuberc Lung Dis 2005e; 9: 1305–1313

Marais BJ, Gie RP, Hesseling AC, Schaaf HS, Enarson DA, Beyers N. Radiographic signs and symptoms in children treated for tuberculosis: possible implications for symptom-based screening in resource-limited settings. Ped Infect Dis J 2006a; 25: 237–240

Marais BJ, Gie RP, Schaaf HS et al. A refined-symptom-based approach to diagnose pulmonary tuberculosis in children. Pediatrics 2006b; 118: e1350–e1359

Marais BJ, Gie RP, Schaaf HS, Donald PR, Beyers N, Starke J. Childhood pulmonary tuberculosis—Old wisdom and new challenges. Am J Resp Crit Care Med 2006c; 173: 1078–1090

Marais BJ, Gie RP, Schaaf HS, Hesseling AC, Enarson DA, Beyers N. The spectrum of childhood tuberculosis in a highly endemic area. Int J Tuberc Lung Dis 2006d; 10: 732–738

Marais BJ, Hesseling AC, Gie RP, Schaaf HS, Beyers N. The burden of childhood tuberculosis and the accuracy of routine surveillance data in a high-burden setting. Int J Tuberc Lung Dis 2006e; 10: 259–263

Marais BJ, Hesseling AC, Gie RP, Schaaf HS, Enarson DA, Beyers N. Bacteriologic yield in children with intra-thoracic tuberculosis. Clin Infect Dis 2006 f. 42: e69–e71

Marais BJ, van Zyl S, Schaaf HS, van Aardt M, Gie RP, Beyers N. Adherence to isoniazid preventive chemotherapy in children: a prospective community based study. Arch Dis Child 2006 g; 91: 762–765

Marais BJ, Wright C, Gie RP, Hesseling AC, Schaaf HS, Enarson D, Beyers N. Etiology of persistent cervical adenopathy in children: a prospective community-based study in an area with a high incidence of tuberculosis. Pediatr Inf Dis J 2006 h; 25: 142–146

Marais BJ. Adult-type TB in children. Expertos Invitados—Sociedad Iberoamericana de Infromacion Cientifica 2007; www.siicsalud.com

Marais BJ, Cotton M, Graham S, Beyers N. Diagnosis and management challenges of childhood TB in the era of HIV. Jnl Infect Dis 2007; 196 (Suppl 1): S76–S85

Marais BJ, Pai M. New approaches and emerging technologies in the diagnosis of childhood tuberculosis. Paediatr Respir Rev 2007; 8: 124–133

Nelson LJ, Wells CD. Global epidemiology of childhood tuberculosis. Int J Tuberc Lung Dis 2004; 8: 636–647

Obihara CC, Beyers N, Gie RP et al. Inverse association between *M. tuberculosis* infection and atopic rhinitis in children. Allergy 2005; 60: 1121–1125

Osborne CM. The challenge of diagnosing childhood tuberculosis in a developing country. Arch Dis Child 1995; 72: 369–374

Pai M, Riley LW, Colford JM. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. Lancet Inf Dis 2004; 4: 761–776

Rubin SA. Tuberculosis—Captain of all these men of death. Rad Clin N Am 1995; 33: 619–639

Schaaf HS, Michaelis IA, Richardson M et al. Adult-to-child transmission of tuberculosis: household or community contact? Int J Tuberc Lung Dis 2003; 7: 426–431

Schaaf HS, Parkin DP, Seifert HI et al. Isoniazid pharmacokinetics in children treated for respiratory tuberculosis. Arch Dis Child 2005; 90: 614–618

Schaaf HS, Marais BJ, Hesseling AC, Gie RP, Beyers N, Donald PR. Drug-resistant tuberculosis in children: a comparison of two surveys. Acta Paediatr. 2006; 95: 523–528

Schaaf HS, Victor TC, Engelke E et al. Level of isoniazid resistance in children with drug resistant tuberculosis—implications for treatment. Eur J Clin Microbiol Infect Dis 2007; 26: 203–205

Starke JR. Pediatric tuberculosis: time for a new approach. Tuberculosis 2003; 83: 208–212

Theart AC, Marais BJ, Gie RP, Hesseling AC, Beyers N. Criteria used for the diagnosis of childhood tuberculosis at primary health care level in a high-burden, urban setting. Int J Tuberc Lung Dis 2005; 9: 1210–1214

Van Zyl S, Marais BJ, Hesseling AC, Gie RP, Beyers N, Schaaf HS. Adherence to antituberculosis chemoprophylaxis and treatment in children. Int J Tuberc Lung Dis 2006; 10: 13–18

Verver S, Warren RM, Munch Z et al. Proportion of tuberculosis transmission that takes place in households in a high-incidence area. Lancet 2004; 363: 212–214

Weber HC, Beyers N, Gie RP, et al. The clinical and radiological features of tuberculosis in adolescents. Ann of Trop Paediatr 2000; 20: 5–10

World Health Organization. Guidance for national tuberculosis programmes on the management of tuberculosis in children. WHO/HTM/TB/2006.371. Geneva: Switzerland.

Zar JZ, Hanslo D, Apolles P, Swingler G, Hussey G. Induced sputum versus gastric lavage for microbiological confirmation of pulmonary tuberculosis in infants and young children: a prospective study. Lancet 2005; 365: 130–134

The Antibiotic Pipeline in Pediatrics

George H. McCracken

1 The Approval Process

Before 1970, very few antibiotics prescribed for children had specific pediatric labeling. Pharmaceutical companies avoided testing these drugs in infants and children because of the stigma associated with the early history of clinical research, liability issues, and difficulty of working with infants and children as research subjects. As a result Dr Harry Shirkey, a pediatrician with a clinical pharmacy background, coined the term 'orphan drugs' for those essential drugs, like antibiotics, that were used commonly in pediatrics, but had not been thoroughly evaluated, especially for appropriate dosage and potential toxicity (Shirkey, 1968).

It was not until 20 years later that the Food and Drug Administration (FDA) initiated several labeling efforts to remedy this situation (Roberts et al., 2003).

2 Pediatric Labeling Efforts in the United States

In 1994 the Pediatric Rule was enacted to encourage companies to perform complete clinical pharmacologic studies of potentially useful drugs in infants and children, but the effort was less successful than anticipated (Table 1). In 1997 the FDA Modernization Act was enacted that again encouraged companies to assess drugs in infants and children and offered them an incentive, a 6-month extension of the patent on the drug, to do so. In 1998 the Pediatric ('Final') Rule made it mandatory for companies to test drugs in pediatric patients, if those drugs were considered potentially useful in these younger patients. The exclusivity incentive for pediatric clinical pharmacology studies was renewed in 2003 by the Pediatric Research Equity Act.

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Table 1 Pediatric labeling efforts in the United States

- 1968 Therapeutic orphan concept
- 1994 Pediatric Rule
- 1997 FDA Modernization Act
- 1998 Pediatric ('Final') Rule
- 2003 Pediatric Research Equity Act
- 2007 Renewal of the BPCA

3 Antibiotic-Approval Mechanism in the United States

The number of antibacterial agents approved by the FDA since 1983 has fallen from 16 in the 5-year period of 1983–1987 to six in the comparable period of 2003–2007 (Fig. 1).

Advances in antibiotic development have been most often made utilizing current, successful antibiotic structures to enhance pharmacokinetic properties, antibacterial activity, and/or safety. By contrast, new viable molecular entities are more difficult to discover and riskier to develop. Targets for antibiotic development are increasingly being linked to new knowledge of bacterial function (Evans and McLeod, 2003).

The drug-approval process by the FDA can be time consuming and expensive. As a generalization, for every 5000 drugs that enter the preclinical discovery phase, only approximately five proceed to phase I–III clinical trials and of those, one is licensed for use.

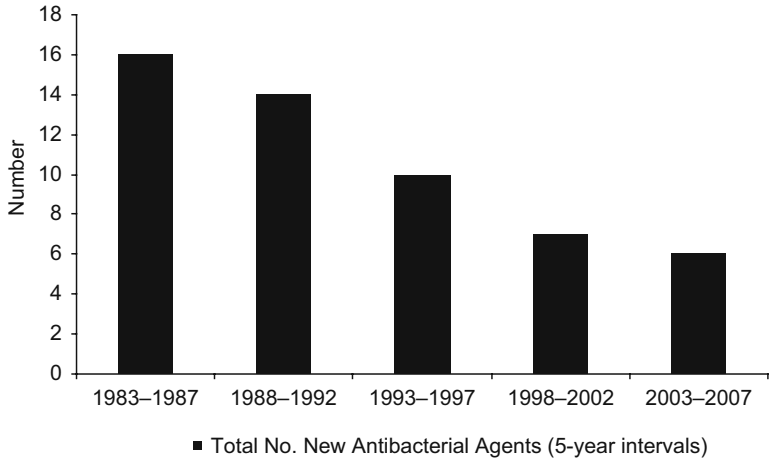

Fig. 1 Antibacterial agents approved by the FDA, 1983–2007

4 Drug Development in the United States Food and Drug Administration

For antibiotics that have potential usefulness in pediatrics, phase I studies usually commence in older children during or after phase III studies of that same agent in adults (Table 2). As data become available for the pharmacokinetics, tolerance, and early safety of the antibiotic in children, younger study subjects are recruited, the lower age limit being 6 months to 2 years depending on the class of antibiotic and the frequency of the targeted infectious disease in infancy and early childhood. For approval of exclusivity for an antibiotic, the manufacturer must respond to a written request from the FDA for pediatric studies. Examples of the exclusivity process are the studies of ciprofloxacin therapy in children 1 year and older with complicated urinary tract infections and of levofloxacin for acute pneumonia in infants and children. Exclusivity can be granted without approval of the antibiotic for a specific therapeutic indication; the latter is dependent on the interest of the manufacturer and the results of a review of submitted efficacy and safety data by the FDA.

Phase III clinical studies have recently become more complicated with a reevaluation of the non-inferiority (i.e., no worse than the control by a certain margin) criterion of evaluating a new antibiotic for clinical effectiveness. Whereas previously it was acceptable to use non-inferiority testing for determining effectiveness of a new drug for treatment for many conditions, the FDA has now decided that this study design approach is only acceptable for serious or life-threatening diseases. By contrast, antibiotics that are intended for treatment of nonserious, self-limited infections, such as acute otitis media, cannot use the non-inferiority design because of the drift-effect toward comparability in clinical efficacy of the investigational agent and the comparator, a previously approved antibiotic, for this self-limited infection. Rather, the FDA will now request that the pharmaceutical company demonstrate that the efficacy of their new antibiotic is either superior to placebo or superior to the standard treatment (e.g., amoxicillin-clavulanate for acute otitis media) for that condition. This new criterion has raised the bar considerably and could discourage some companies from pursuing new antimicrobial agents for such conditions.

Table 2 Drug development in the United States Food and Drug Administration

		Clinical Trials			
	Discovery Preclinical	I	II	III	FDA
Years	6.5	1.5	2	3–4	1–2
Test Population	Lab animals	20–100 HVs	100–500 PVs	1000–5000 PVs	
Success	5000 cpds	5 enter clinical trials			1

HVs = healthy volunteers; PVs = patient volunteers.

5 New Antibacterial Agents in Development

In the past decade we have seen the emergence of multidrug-resistant bacteria causing diseases that have challenged the clinician with regard to selection of effective antimicrobial therapy. These bacterial pathogens include community-acquired methicillin-resistant *Staphylococcus aureus* (Ca-MRSA), ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli*, vancomycin-resistant *Enterococcus faecium,* and multidrug resistant *Acinetobacter* sp. To counter this challenge the pharmaceutical industry has developed several new agents that are in various stages of testing or have been recently approved for use in adults. These include the anti-MRSA cephalosporins, ceftobiprole, and ceftaroline, the lipopeptide or lipoglycopeptide agents for multidrug-resistant Gram positive pathogens, like daptomycin, telavancin, and dalbovancin, the carbapenems, like doripenem, and finally, the glycylcycline agent, tigecycline. Other than doripenem, all of these antimicrobials have anti-MRSA activity and the cephalosporins and lipopeptides are rapidly bactericidal, an obvious advantage over vancomycin, clindamicin, and linezolid for treatment of life-threatening MRSA infections. These agents will soon undergo or are currently engaged in phase I and II clinical studies in pediatric patients to define the pharmacokinetics, appropriate dosages, and initial safety profile. These studies in infants and children can be slow and difficult, but the data are critical for the appropriate use of the drug, whether or not a specific indication for use is sought by the company.

For some new antimicrobial agents, long-term follow-up evaluations are indicated to be certain that unexpected adverse events are not associated with their use. For example, because fluoroquinolones cause cartilage toxicity in juvenile animals and have occasionally been associated with musculoskeletal effects, such as severe myalgia or Achilles tendon rupture in adults, it was necessary for companies to assess the long-term effects in children of their fluoroquinolone compounds if they wished to establish an indication for use in this age group. These studies must be carefully designed to avoid observer bias (i.e., parents reporting aches and pains in muscles and joints of their children) during a period when the children are active in sports and other physical activities. The parents and investigators must be blinded as to which drug (fluoroquinolone vs. comparator) was given to the child and some evidence of objective musculoskeletal abnormalities should accompany the subjective musculoskeletal complaints. To date, these criteria have not been stringently meet resulting in uncertainty whether the fluoroquinolones are safe to prescribe to children for treatment of self-limited infections.

6 The Future for Antibiotics in Pediatrics

There are fewer than 10 new antibiotics that will enter phase 1 and 2 clinical evaluation in pediatrics in the next few years. Although any drug approved for use in adults can be prescribed to infants and children, it is critical for pediatricians to have as complete information on the pharmacokinetics and safety as

possible in order to dose the agent properly according to age, weight, and medical condition. There are many targets for development of new antimicrobials and a critical need for appropriate and reasonable incentives for development of these agents in pediatric patients. Antibiotic stewardship programs are essential to limit emergence of resistance that results in shortening the life span of antibiotics.

References

Evans, WE, McLeod, HL. Pharmacogenomics-drug disposition, drug targets and side effects. N Engl J Med 2003; 348:538.

Roberts, R, Rodriquez, W, Murphy, D, Crescenzi, T. Pediatric drug labeling. J Amer Med Assoc 2003; 290:905.

Shirkey, HC. Thearapeutic orphans. J. Pediatr 1968; 2:119.

Paediatric Drug Development and Clinical Trials

E. David G. McIntosh

1 Introduction

Children have featured in clinical research for a number of centuries, albeit in a relatively unregulated way. Edward Jenner utilized children in his work on smallpox in the late 1700s. Joseph Lister (1827–1912) utilized children in his work on wound infections and Louis Pasteur first tried his rabies vaccine, in 1885, on a child bitten by a rabid dog (Barfield and Church, 2005). In the early twentieth century, children at the Hebrew Orphan Asylum were fed diets known to induce scurvy and rickets so that these diseases could be better understood. Between 1958 and 1960, residents with learning difficulties at Willowbrook State School were deliberately exposed to hepatitis. European children had to wait until the beginning of the twenty-first century for their right to participate in clinical research to be enshrined in legislation.

2 The 'New' European Paediatric Legislation

Regulation (EC) No 1901/2006 of the European Parliament and of the Council of December 12, 2006 on medicinal products for paediatric use was published in the Official Journal on December 27, 2006, and entered into force on January 26, 2007. This paediatric regulation created a new type of marketing authorisation, the paediatric use marketing authorisation (PUMA) as an incentive for the development of off-patent medicines for children.

The key elements of the legislation are:

- mandatory paediatric research and submission of the resulting data at the time of the application for marketing authorisation for new products, and for line extensions for products protected by a supplementary protection certificate (SPC) unless a waiver or deferral is granted;

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- research is to be completed in accordance with a paediatric investigation plan (PIP), submitted, and agreed with the paediatric committee (PDCO) at an early stage of product development; and
- rewards are available (subject to conditions)
  - Products eligible for SPC gain 6-month extension of their SPC,
  - Other products gain 10-year data exclusivity protection specific to the paediatric use data, and
  - Orphan drugs gain an additional 2 years of market exclusivity.

The scope of the legislation is as follows:

The 'paediatric population' is defined in the Regulation as the population aged between birth and 18 years of age

- The Regulation covers medicinal products for human use within the meaning of Directive 2001/83/EC (as amended). In other words, the Regulation covers all human medicines regardless of registration pathway (centralized, mutual recognition, decentralized or national);
- Within its scope, the Regulation further distinguishes three main categories of products:
  - Products in development (unauthorized),
  - Authorized products still covered by patents or SPCs, and
  - Authorized products not covered by patents or SPCs (generics); and
- The different measures contained in the Regulation are designed in order to stimulate paediatric research on all three categories.

There is also the intention that research into the paediatric use of medicinal products which are not protected by a patent or SPC should be financed under Community research programmes: "Funds for research into medicinal products for the paediatric population shall be provided for in the Community budget in order to support studies relating to medicinal products or active substances not covered by a patent or a supplementary protection certificate" (e.g., the 7th Framework Programme for Research: http://ec.europa.eu/research/fp7/index_en.cfm).

According to the legislation, the PUMA is a "marketing authorization granted in respect of a medicinal product for human use which is not protected by an SPC under Regulation (EEC) No 1768/92 or by a patent which qualifies for the granting of the SPC, covering exclusively therapeutic indications which are relevant for use in the paediatric population, or subsets thereof, including the appropriate strength, pharmaceutical form or route of administration for that product".

With regard to orphan medicinal products, under Regulation (EC) No 141/2000 of the European Parliament and of the Council of December 16, 1999 on orphan medicinal products, medicinal products designated as orphan medicinal products gain 10 years of market exclusivity on the granting of a marketing authorization for the orphan indication. As such products are frequently not

patent protected and the reward of SPC extension cannot be applied. Where they are patent protected, such an extension would provide a double incentive. Therefore, for orphan medicinal products, instead of an extension of the SPC, the 10-year period of orphan market exclusivity is extended to 12 years if the requirement for data on use in the paediatric population is fully met.

The duties of the PDCO are to:

- evaluate and approve PIPs;
- evaluate requests for waivers and deferrals;
- check compliance with PIPs;
- make ad hoc assessments of paediatric quality, safety, and efficacy at the request of the committee for medicinal products for human use or of national health authorities; and
- prepare an inventory of paediatric use, therapeutic needs, and priorities for research within 3 years. The inventory is intended to be an information source for physicians and patients. The inventory is intended to help pharmaceutical companies to identify business development opportunities

We believe that, under the legislation, there is likely to be a focus on 'new' drug development. Paediatric expertise in designing and executing clinical trials is less than adult expertise, and there may be considerable recourse to the free scientific advice offered by the regulatory agency. More paediatricians are likely to be employed in the pharmaceutical industry and clinical trial networks will be developed to take advantage of economies of scale. More trials enrolling both adults and children in same trial will be designed and cost–benefit analyses will be performed to assess the pros and cons of performing paediatric clinical trials. There may be difficulties harmonizing FDA and European Union requirements, and there may be some movement 'away' from Europe. There may be some recourse to deferrals and waivers.

3 Extrapolation

The European Medicines Agency has published a guideline on the role of pharmacokinetics in the development of medicinal products in the paediatric population document EMEA/CHMP/EWP/147013/2004 (London June 28, 2006). Population pharmacokinetic (PK) analysis, using non-linear mixed effects models, is deemed an appropriate methodology for obtaining PK information in paediatric trials. The mean and variances are estimated, and information from all individuals is merged, making it possible to use sparse sampling schemes. The population approach may replace conventionally designed PK studies with rich sampling; simulations or theoretical optimal design approaches should be considered.

PK information may be used to extrapolate clinical efficacy and safety from adult to paediatric patients as well as between paediatric patients at different

ages, provided that data from adults are considered relevant. If similar exposure in adult and paediatric patients can be assumed to produce similar efficacy, then PK data alone would be sufficient. If not, then paediatric PK/PD (biomarker) data can be used. PK information from one indication can be extrapolated to another indication if it can be assumed that the diseases and commonly used concomitant medications are not affecting the PK of the drug.

4 When Should 'Small' Studies Maximizing the Use of PK Be Performed?

See Table 1.

5 When Should a Full Drug-Development Programme Be Performed?

Whilst extrapolation may be useful in some circumstances, there will be other circumstances when a full drug-development programme will be necessary (Table 2).

6 Developing Off-Patent Medicines for Paediatric Use

EU member states will perform a survey of all existing uses of medicinal products in children, including off-label, within 2 years of the enactment of the legislation and a final EMEA inventory should be compiled by the third-year (2009). Updates of paediatric needs will be performed by the PDCO on the basis of inventory. It is expected that formulations and dosages may need to be adapted and that development of child-friendly formulations will need to occur. Full PK and dose-finding studies may need to be performed.

Table 1 When should 'small' studies maximizing the use of PK be performed?

Extrapolation acceptable from the same indication, different indication, other children, adults
Paediatric variant of adult disease, e.g. epilepsy
When PK differs markedly between age bands: perform PK in each age band
When death is imminent/expected – no time for full study
When unpredictable relapses and remissions
When disease manifestations occur much later, e.g. genetic predisposition, prophylaxis
Some conditions associated with congenital malformations
When many confounding factors – premature newborns
When no defined/agreed biomarker or surrogate marker
When consent for participation in full study unlikely
When good PK/PD modelling or population PK available

Table 2 When should a full drug-development programme be performed?

Particular indications and situations	Paediatric only indication.
	Cancer – when effectively all patients need to be studied.
	Most infectious diseases – antibiotics, antivirals, etc.
	Highly heterogeneous manifestations and variable diagnostic criteria.
	When no proven treatment exists.
	Vaccines.
Situations were safety is a prime consideration	Unpredictable safety concerns.
	Drug interactions anticipated.
Statistical considerations	When one can use placebo to gain full statistical power.
	When statistical power is required – non-inferiority, equivalence, superiority.
	When there are large numbers of patients and/or the disease is common.
PK or PD	PK measurements not possible.
	Unpredictable PK eg high intra- and inter-individual variability.
	Extensive metabolism.
	When there is a defined/agreed biomarker and/or surrogate marker.
Measurements	When collection of blood samples not possible.
	When blood samples not transportable, e.g. samples deteriorate.
When there is a clear public health need	For example, antibiotics for resistance organisms.

7 US Legislation

The Best Pharmaceuticals for Children Act (S.838) (2002) was designed to improve the safety and efficacy of patented and off-patent medicines for children. It continued the exclusivity provisions for paediatric drugs as mandated under the FDA Modernization Act of 1997, in which market exclusivity of a drug is extended by 6 months, and in exchange the manufacturer carries out studies of the effects of drugs when taken by children. The provisions both clarified aspects of the exclusivity period and amended procedures for generic drug approval in cases when paediatric guidelines are added to the labelling.

The bill also created a research fund to pay for studies on older, 'off-patent' drugs, which are not eligible for the paediatric testing incentive. In addition, the bill required the FDA to disseminate quickly the information gathered from paediatric studies and created a new Office of Pediatric Therapeutics at the agency. Under the Best Pharmaceuticals for Children Act of 2002, government agencies worked with experts in paediatrics and paediatric research to develop and prioritize a list of off-patent drugs for which paediatric studies were urgently needed. Four such listings were published in the Federal Register from January 2003–January 2005. The National Institute of Child Health and

Human Development (NICHD) and the FDA also initiated the Newborn Drug Development Initiative (NDDI). This is a multiphase programme to determine gaps in knowledge concerning neonatal pharmacology and clinical trial design, and to explore novel study designs for use in newborns, with the ultimate goal of increasing knowledge about the safety and efficacy of drugs used to treat newborns.

Under the US legislation, there have been 139 paediatric exclusivity label changes to December 2007 (http://www.fda.gov/cder/pediatric/labelchange.htm). The Best Pharmaceuticals for Children Act and the Pediatric Research Equity Act were re-authorized and amended as the H.R 2589: Improving Pharmaceuticals for Children Act of 2007.

8 Economic Return of Clinical Trials Performed Under the US Paediatric Exclusivity Programme

From programmes conducted which resulted in paediatric exclusivity, nine drugs covering nine different indications that had in fact been granted paediatric exclusivity were studied (Li et al., 2007). From the final study reports submitted to the FDA between 2002 and 2004, the key elements of the clinical trial design and study operations were obtained, and the cost of performing each study was estimated and converted into estimates of after-tax cash outflows. Three-year market sales were obtained and converted into estimates of after-tax cash inflows based on 6 months of additional market protection. The net economic return (cash inflows minus outflows) and net return-to-costs ratio (net economic return divided by cash outflows) for each product were then calculated.

The distribution of net economic return for 6 months of exclusivity varied substantially among products. Net economic return ranged from –$8.9 million (anti-infective) to + $507.9 million. Net return-to-cost ratio ranged from –0.68 to 73.63. The authors concluded that the economic return for paediatric exclusivity is variable. In addition to accruing benefits to the population under study, it is also possible for paediatric clinical trials to generate returns on investment, although this is dependent on the indication under study.

9 Safety

Guideline EMEA/CHMP/EWP/147013/2004 notes that a paediatric development programme is often restricted in terms of the number of patients included. Therefore, the safety assessment for systemically as well as locally acting drugs often has to be extrapolated from data obtained in adults or from a different target group within the paediatric population. The guideline goes on to note that the underlying assumption that the exposure-adverse event relationship is similar in adult and paediatric patients should not be taken for granted. The

possibility of a marked difference in incidence of side effects should be considered, taking into account the preclinical and clinical pharmacology of the drug and, if possible, known effects of substances with a similar pharmacological profile. PK data may be important for identification of 'sub-groups' in which exposure differs from the overall study population to a clinically relevant extent.

10 Conclusions

The Best Pharmaceuticals for Children Act is the basis of legislation in the USA. This legislation has recently been amended and re-authorized. There may be financial incentives for pharmaceutical companies undertaking studies of both patented and off-patent medicines in paediatrics although this of course should not be the primary motivation. The primary motivation should be to perform high-quality clinical trials in paediatrics, especially where there is an unmet medical need. Whilst the 'new' European legislation addresses both patented and off-patent medicines, research activity may well focus on 'patented' medicines. An inventory of needs will assist in identifying potential off-patent candidates that could be studied in paediatrics. The Paediatric Investigation Plan is the documentation required in the EU, the body responsible is the PDCO and the licence itself is called a Paediatric Use Marketing Authorisation (PUMA). Pharmacokinetics and extrapolation have an important role in paediatric clinical trials, and may enable smaller paediatric clinical trials to be performed. However, the gold standard, as with adults, will continue to be the randomized, controlled clinical trial.

References

Barfield RC, Church C. Informed consent in pediatric clinical trials. Current Opinion in Pediatrics 2005; 17: 20–24.

Li et al. Economic return of clinical trials performed under the pediatric exclusivity program. JAMA 2007; 297: 480–488.

Influenza in Pregnancy: The Case for Prevention

Shelly McNeil, Beth Halperin, and Noni MacDonald

1 Introduction

Influenza viruses are the most common cause of serious respiratory morbidity throughout the world with annual attack rates of 10–40% during each 5–6-week winter outbreak in temperate climates. In tropical climes, the outbreaks have a less distinct and more variable seasonal pattern but the attack rates are similar. Certain populations are well recognized to be at higher risk for serious disease with influenza (Table 1) either due to age (<2 years or >65 years) or to underlying medical conditions or pregnancy (CDC, 2007a; NACI, 2007). Influenza immunization is recommended for all these high-risk groups in North America (CDC, 2007a; NACI, 2007).

While observational and anecdotal data first documented the risk of severe disease with influenza in pregnant women, more recent cohort and case control data have corroborated and defined the higher risk (Black et al., 2004; MacDonald et al., 2004; Neuzil et al., 1998; Dodds et al., 2007; Schanzer et al., 2007; Mortimer, 2006; Department of Health UK, 2006). Despite this, pregnancy as a risk factor for influenza morbidity appears underappreciated and immunization rates remain low in this risk population (Black et al., 2004; Neuzil et al., 1998; Dodds et al., 2007; NACI, 2007). This chapter focuses on influenza during pregnancy; the epidemiology in healthy pregnant women and in those with comorbidities, the safety and efficacy of influenza vaccine in pregnancy, vaccine uptake rates, barriers to vaccine uptake, and potential strategies for improving uptake in this high-risk population.

2 Epidemiology of Influenza

In temperate climates, influenza occurs almost every winter while in more tropical climes there is less seasonality to the outbreaks. During a typical influenza season, the influenza outbreak is associated with a surge in physician

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Table 1 People at high risk of influenza-related complications (CDC, 2007; NACI, 2007)

Adults and children with chronic health conditions requiring medical follow-up or hospital care:
• Cardiac or pulmonary disorders (including asthma, cystic fibrosis, and bronchopulmonary dysplasia)
• Diabetes mellitus and other metabolic disorders
• Cancer, immunodeficiency, or immunosuppression
• Renal disease
• Anemia or hemoglobinopathy
• Conditions that compromise management of respiratory secretions
• Children and adolescents requiring long-term acetylsalicylic acid therapy
Residents of nursing homes or chronic care facilities
People ≥65 years
Children <2 years (influenza vaccine recommended >6 months)
Pregnant women, including those with selected high-risk conditions, and healthy pregnant women

office visits, hospitalizations for acute respiratory illness, and deaths due to pneumonia and influenza (Glezen and Couch, 1978; Glezen et al., 1991; Wong et al., 2006) (Fig. 1). The very young (<2 years of age) and the very old (>65 years of age) are particularly vulnerable to more severe disease.

Even in temperate climates, influenza is unpredictable in terms of severity and specific timing of the outbreak in a community. Influenza A viruses undergo continual, typically gradual, genetic evolution (antigenic drift); the degree of drift in the virus from one season to the next is the primary factor influencing the severity risk during an outbreak. With little change (drift) in the circulating strain compared to a previous year, the seasonal outbreak or 'epidemic' is typically not severe in terms of attack rate or complications, owing to the fact that much of the population has some degree of protective immunity.

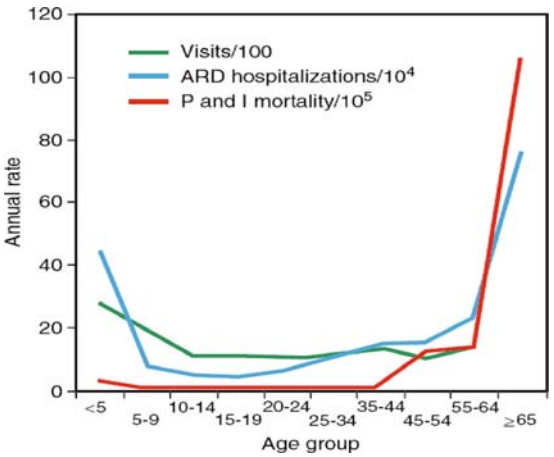

Fig. 1 Typical pattern and rates of medically attended illnesses during interpandemic influenza seasons
Reprinted from Principles and Practices of Infectious Diseases 6th Edition. Edited by Mandell, Douglas and Dohlin. Chapter 162, Influenza Virus, Treanor JJ. Page 2062, Figure 2. Copyright 2005, with permission from Elsevier Inc 2005. Data from Glezen et al. (1991).

As the degree of antigenic drift increases, so too does the attack rate and severity of illness associated with seasonal outbreaks. Antigenic shift, resulting in the introduction of an entirely new influenza strain to which the population has no protective immunity, is a rare event with the potential to cause a worldwide 'pandemic' associated with very high attack rates and large numbers of complications and deaths.

In the past century, three pandemics have occurred, each differing in etiologic Influenza A strain, epidemiology, and severity; the 1918 'Spanish flu' (H1N1), the 1957 'Asian flu' (H2N2) and the1968 'Hong Kong flu' (H3N2) (Kilbourne, 2006). The 1918 pandemic was particularly devastating, infecting an estimated one-third of the world's population with a mortality rate of over 2.5 % compared to <0.1% in the other pandemics (Taubenberger and Morens, 2006). Persons <35 years of age in 1918 had an unusually high influenza incidence and in contrast to interpandemic influenza patterns, absolute influenza deaths were higher in those <65 years of age than in those over 65 years of age (99% excess influenza related deaths in <65 years of age). Thus, instead of a typical U-shaped mortality curve by age for pneumonia and influenza seen with interpandemic influenza, the curve was a **W** (Fig. 2) (Taubenberger and Morens, 2006).

While annual interpandemic influenza causes high absenteeism from work and schools, as well as an increase in hospitalization and excess deaths, particularly in those aged ≥65 years (Glezen and Couch, 1978; Glezen et al., 1991; Wong et al., 2006) (Fig. 2), the effect of a particular strain is variable and hard to predict (Glezen

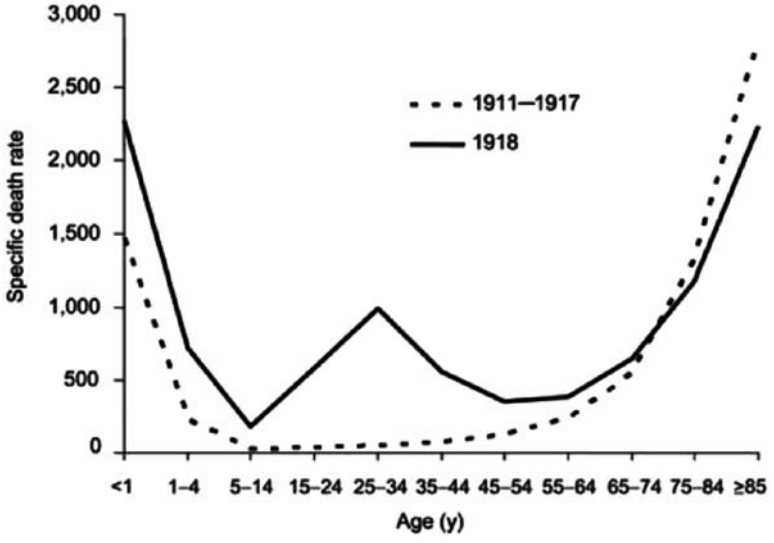

Fig. 2 U- and W-shaped combined influenza and pneumonia mortality, by age at death per 100,000 persons in each group
From Taubenberger and Morens, (2006). The Centers for Diseases Control and Prevention.

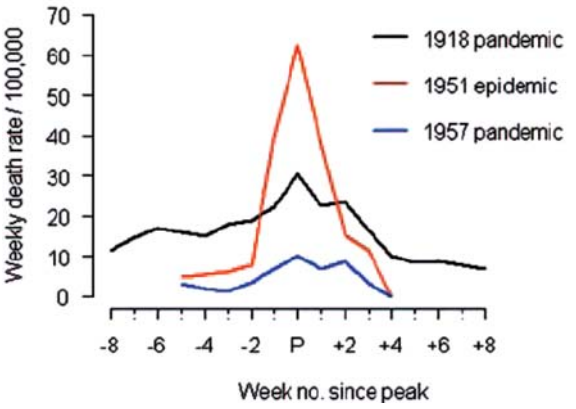

Fig. 3 Comparison of 1918 and 1957 pandemic influenza weekly death rate per 100,000 to epidemic influenza in 1951 in Liverpool, England
From Viboud et al. (2006). The Centers for Diseases Control and Prevention.

and Couch, 1978; Viboud et al., 2006). For example, excess death rates can vary nearly fourfold among different A/H3N2 seasons in the same country even after adjusting for population aging (Simonsen et al., 2005). Similarly, with some strains in a given locale, reported excess death rates have been higher than even in pandemics, e.g. 1951 A/H1N1 in Liverpool, England (Fig. 3) (Viboud et al., 2006).

Both the 1957 and 1968 influenza pandemics occurred when genetic mixing between human influenza A virus and avian influenza A viruses occurred, resulting in an antigenic shift in the virus and the ability of an avian influenza virus to infect humans. Thus, there is reason for concern that the current outbreak of a novel avian influenza virus (AH5N1) could lead to the next human pandemic. The first human cases of avian influenza (AH5N1) occurred in Hong Kong in 1997. Subsequent outbreaks in humans have been small but all have been associated with a mortality rate of over 50% (World Health Organization, 2007). (http://www.who.int/csr/disease/avian_influenza/country/cases_table_2007_07_11/en/index.html). While avian influenza AH5N1 is clearly able to infect humans, the strain has not yet evolved to allow efficient human-to-human transmission (Poland et al., 2007), a trait critical to allow this strain to lead to a human pandemic. However, should this virus develop the ability to readily infect and spread among humans, the resulting pandemic may be particularly devastating given the predilection of this virus for the young, a pattern reminiscent of the 1918 'Spanish flu'.

3 Epidemiology of Influenza in Pregnant Women

3.1 Pandemic Influenza

Contemporary reports of the 1918–1919 pandemic noted that previously healthy pregnant women were especially vulnerable to severe influenza (Harris, 1919; Bland, 1919). In a study by Harris in Maryland and in four large cities in

the United States, 50% of the influenza cases among 1350 pregnant women were complicated by pneumonia and 50% of the pregnant women who developed pneumonia died (Harris, 1919). While the mortality rate was 'somewhat greater' during the third trimester, it was over 40% in all trimesters (Harris, 1919). Furthermore, pregnancy was interrupted by spontaneous abortion or stillbirth in 26% of women with uncomplicated influenza and in 52% of those with pneumonia. Among women who died, abortion or premature labor occurred in 62%. Similarly high mortality rates as those observed by Harris were also described in Philadelphia where 155 of 337 (46%) pregnant women with influenza died (Bland, 1919). While recognizing that these data may be skewed toward observation of more severe cases and restricted by geography, the 24.5% influenza death rate for pregnant women in England in 1918–1919 adds credence to these findings (Local Government Board, 1919) as does the observation of a dramatic plunge in live births in England and Wales in the first half of 1919 (Registrar General's Report, 1919) likely due to increased still births and spontaneous abortions with severe influenza in pregnant women during this pandemic.

The 1957–1958 pandemic (AH2N2) was less severe than in 1918–1919. Again contemporary reports show the heightened risk of severe disease for pregnant women (Greenberg et al., 1958; Freeman and Barno, 1959). When 8–10% of the total population of New York City fell ill in October and November 1957, most were under 20 years of age (Greenberg et al., 1958). Of the 216 who died, 43% (93) were under 50 years of age. Among these 93 deaths, 40% were pregnant women or persons with rheumatic heart disease (15 pregnant women, 15 rheumatic heart disease, and 7 pregnant women with rheumatic heart disease). Of the 47 deaths of women of childbearing age due to influenza, almost half were pregnant. A similar serious impact on pregnant women was noted in Minnesota where influenza accounted for 19.2 % of all deaths in pregnant women in 1957–1958 (Freeman and Barno, 1959).

The data from these two influenza pandemics clearly show that pregnant women are at increased risk for severe disease during a pandemic. The question then arises—what about interpandemic influenza? Are pregnant women at increased risk for more severe illness than nonpregnant women of childbearing age?

3.2 Interpandemic Influenza

3.2.1 Mortality

While there are case reports and a 30-year case series of fatal influenza in pregnant women during interpandemic years in the literature (Ramphal et al., 1980; Kort et al., 1986; McKinney et al., 1990; Schoenbaum and Weinstein, 1979) the rarity of these events suggests that fatalities are unusual. This is supported by Widelock and colleagues in New York City who noted that a

higher than average rate of maternal deaths occurred with the 1957 Asian influenza pandemic but that the risk of maternal mortality during influenza season was not raised in the three subsequent interpandemic influenza seasons (Widelock et al., 1963). Interpretation of the impact of avian influenza on pregnant women is harder to assess as so few pregnant women have been infected and the overall mortality rate for avian influenza is so high. There is one report with a fatal outcome in China (Shu et al., 2006).

3.2.2 Morbidity

Several small studies have shown an increased morbidity with influenza in pregnant women. A retrospective case control study of 5518 pregnant women in Oregon in the latter half of the1970s found a significantly increased rate of use of medical services for acute respiratory disease in 3 of 5 influenza seasons compared to nonpregnant controls (23.7/1000 vs. 10.2/1000) with an influenza-attributable rate of hospitalization for pregnant women of 2/1000 (Mullooly et al., 1986). Two small prospective studies, one in Newcastle, England, and the other in the Eastern Townships of Quebec in Canada noted that influenza infection was common in pregnancy and was associated with more health services utilization but that complications were infrequent (Irving et al., 2000; Tuyishime et al., 2003). Of note, in the Newcastle study the incidence of any single fetal, medical, or obstetric complication was not significantly increased between cases and controls but when aggregated, significantly more cases than controls suffered a fetal, medical, or obstetrical complication (106/181 vs. 73/180, $p < 0.001$) (Irving et al., 2000).

More substantial morbidity evidence for pregnant women with interpandemic influenza is provided by a retrospective 19-year (1974–1993) nested case control cohort study in a Tennessee Medicaid population (Neuzil et al., 1998). When 4369 pregnant women with a first study event (hospitalization or death from a cardiopulmonary condition) were compared to 21,845 population controls between 15 and 44 years, pregnant women with and without underlying comorbidity were at increased risk for hospitalizations in influenza season compared to controls (odds ratio 1.44 (95% CI 0.97–2.15) for women at 14–20 weeks gestation and 4.67 (95% CI 3.42–6.39) for women 37–42 weeks gestation compared to postpartum women) (Neuzil et al., 1998). Of every 10,000 women in their third trimester without risk factors for severe influenza, 25 were hospitalized with influenza-like morbidity during the influenza season. Those with underlying comorbidities had more cardiopulmonary hospitalizations during influenza seasons compared to those without (i.e., 110/10,000 women-months vs. 22/10,000).

A subsequent 9-year (1985–1993) matched cohort study, also in the Tennessee Medicaid population, demonstrated that pregnant women with asthma, a known risk factor for more severe disease with influenza, accounted for half of all respiratory-related hospital admissions for influenza (Hartert et al., 2003). Among pregnant women with asthma in this Medicaid population, 6%

required hospitalization during influenza season. In this study, there was no significant increase in preterm deliveries or low birth-weight outcomes attributable to maternal influenza during pregnancy.

While both of these Tennessee Medicaid population studies provided good evidence of the increased risk of more serious illness with influenza in pregnancy, concerns were raised about whether these data could be generalized to broader populations such as those with a wider range of socioeconomic backgrounds and those with universal access to healthcare (NACI, 2004; MacDonald et al., 2004). The Tennessee Medicaid study population was comprised predominantly of young African-American women of lower socioeconomic class, a population not reflective of the general population in many industrialized countries.

The recent 13-year (1990–2002) retrospective population-based cohort study involving 134,188 pregnant women in Nova Scotia, Canada, addresses this limitation by including all pregnant women in the province during that period, regardless of socioeconomic status or geographic location (Dodds et al., 2007). In this study, each woman acted as her own control with the risk for severe illness with influenza during pregnancy compared to the risk in the year prior to pregnancy. Being pregnant during the influenza season, particularly if in the third trimester, was associated with significantly increased hospitalization rates (non delivery related) and increased physician office visits compared to rates in the year before pregnancy both in women with and in women without medical comorbidities (Dodds et al., 2007; McNeil et al., 2007) (Table 2, Fig. 4). Pregnant women with an underlying medical comorbidity (10.1% study population) experienced an excess of 39.2 hospital admissions per 10,000 woman-months in the third trimester compared with the year before pregnancy (rate ratio 7.9; 95% CI 5.0–12.5) while healthy pregnant women experienced an excess of two hospital admissions per 10,000 woman-months (rate ratio 5.1; 95% CI 3.6–7.3). These findings held true even when adjusted for socioeconomic class, maternal age, smoking status, and number and ages of other children in the household. The observed rate of excess hospitalizations among pregnant women with comorbidities in Nova Scotia, Canada, is lower than that seen in the Tennessee Medicaid population noted above (39.2/10,000 women-months vs. 110/10,000 women-months) and may reflect differences in the population demographics and in access to outpatient care for management of both underlying medical conditions and complications of influenza.

A recent population-based sampling study that is more representative of the American population than that studied by Neuzil and colleagues, done by the Center for Disease Control and Prevention utilizing 1998–2002 data, noted that a diagnosis of respiratory illness was included in 3.4 per 1000 hospitalizations of pregnant women (Cox et al., 2006). While overall 88% of these hospitalizations were also for delivery of a child, during influenza seasons 70% of admissions for acute respiratory illness did not include the delivery (Cox et al., 2006b). The portion of nondelivery hospitalizations with respiratory illness was 22.3/1000 during the influenza seasons compared to only 11.7/1000 for the rest of the years (Cox et al., 2006). Factors associated with a higher odds ratio of being hospitalized with respiratory illness not related to delivery were: (1) underlying medical

Table 2 Hospitalization admissions because of respiratory illness during influenza season in year prior to pregnancy and during pregnancy by presence of comorbidities

Period	Women with no comorbidity			Women with ≥1 comorbidity		
	No. of admissions during influenza season	Rate per 10,000 woman-months	Rate ratio (95% C1)*	No. of admissions during influenza season	Rate per 10,000 woman-months	Rate ratio (95% C1)*
Year before pregnancy	49	1.4	1.0	23	5.7	1.0
Pregnancy						
First trimester	22	2.4	1.7 (1.0–2.8)	17	16.3	2.9 (1.5–5.4)
Second trimester	30	3.0	2.1 (1.3–3.3)	22	19.4	3.4 (1.9–6.0)
Third trimester	76	7.4	5.1 (3.6–7.3)	49	44.9	7.9 (5.0–12.5)

Reprinted from Dodds et al. (2007) Canadian Medical Association.
Note: Cl = confidence interval.
*Rate ratio of admissions during pregnancy compared with admissions in the year before pregnancy.

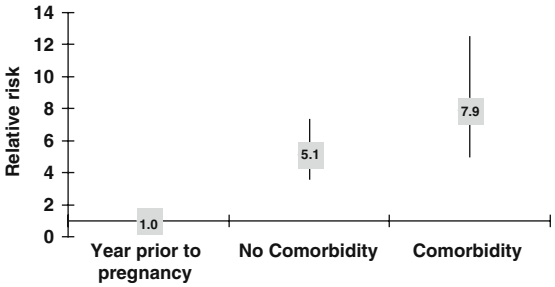

Fig. 4 Relative risk of third trimester hospitalization for respiratory illness during influenza season among women with and without comorbidities compared with the influenza season in the year prior to pregnancy (RR (95% CI)) in a 13-year (1990–2002) population cohort in Nova Scotia, Canada
Reprinted from McNeil et al. (2007)., with permission from Society of Obstetricians and Gynaecologists of Canada.

condition associated with increased risk of severe influenza (OR 3.2, 95% CI 3.0–3.5), (2) Medicaid/Medicare as the primary expected payer (OR 1.2, 95% CI 1.1–1.3), and (3) hospitalization in a rural area (OR 1.2, 95% CI 1.1–1.4) (Cox et al., 2006). Furthermore, during influenza seasons, hospitalized pregnant women with respiratory illness also had longer lengths of stay (3.88 days vs. 2.65 days; $p<0.001$) and higher odds of having delivery complications than pregnant women hospitalized during noninfluenza seasons.

A comparable analysis of all admissions for acute respiratory conditions among pregnant women in Canada between 1994 and 1999 showed that 1 of every 1000 pregnant women in Canada experienced an influenza-attributable admission annually and that 60% of admissions among previously healthy pregnant women were attributable to influenza (Schanzer et al., 2007).

Thus, there are now robust population-based data that show that pregnant women are at increased risk for more hospitalizations and physician office visits during interpandemic influenza seasons regardless of their trimester of pregnancy. This risk increases in the third trimester and is markedly increased if there is also an underlying comorbidity. As Table 3 indicates, when the rates of excess hospitalizations in influenza season for pregnant women with and without comorbidities are compared to the rates in groups well accepted to be at high risk for serious influenza (very young, elderly, and children and adults with comorbidities), the rates are similar and well above the rates for healthy adults between 15 and 44 years (McNeil et al., 2007; CDC, 2007a; Mullooly et al., 2007; Izurieta et al., 2000; Neuzil et al., 2000; Wong et al., 2006).

3.3 Impact of Influenza in Pregnancy on the Fetus

In the 1918 pandemic influenza, as noted above, pregnant women with influenza had an increased risk of spontaneous abortion and stillbirth (Harris, 1919)

Table 3 Excess hospitalizations in influenza seasons for different populations with known comorbidities for serious influenza

Population	Rate of Hospitalization per 100,000
Healthy children under 6–23 mo	90–1038 Izurieta et al., 2000 Neuzil et al., 2000
Adults 15–44 years without comorbidities	23–25 Mulhooly et al., 2007, CDC, 2007
Adults 15–44 years with comorbidities	56–110 Mulhooly et al., 2007, CDC, 2007
Women of childbearing age no comorbidity (Canada)	6 (Schanzer et al., 2007)
Women of childbearing age with asthma (Canada)	110 (Schanzer et al., 2007)
Healthy pregnant women, all trimesters (Canada)	156 (Schanzer et al., 2007)
3rd trimester pregnant women no comorbidity (Nova Scotia, Canada)	68 (Dodds et al., 2007)
3rd trimester pregnant women with comorbidity (Nova Scotia, Canada)	1210 (Dodds et al., 2007)
Adults $\geq$65 years	125–228 (Simonsen et al., 2000)

but there is no contemporary comment on excess fetal anomalies. The 1963 report of Widelock and colleagues from their New York City observation of pandemic and interpandemic influenza in pregnancy did not reveal any increase in prematurity, fetal death, or congenital malformations (Widelock et al., 1963). In 1986, an investigation of a cluster of spontaneous abortions and stillbirths in the United Kingdom revealed a higher incidence of influenza-like illness among cases than controls and cases were more likely to have serological evidence of influenza infection, predominately A H3N2 (Stanwell-Smith et al., 1994). In contrast, the much larger Tennessee Medicaid cohort study did not find any increase in prematurity or low birth-weight infants attributable to influenza in pregnancy (Hartert et al., 2003). Among women in the 13-year cohort study performed Nova Scotia, Canada, no increase in preterm deliveries were observed during influenza seasons compared to noninfluenza seasons (McNeil et al., 2006).

Prospective studies examining the potential for adverse fetal outcomes using serological confirmation of maternal influenza infection are sparse (Laibl and Sheffield, 2005). A study in Nottingham England between May 1993 and July 1994 noted above failed to show any difference in congenital malformations when cases were compared to controls (Irving et al., 2000). While there have been reports of an association between neural tube defects (Lynberg et al., 1994; Li et al., 2007), reduction limb defects (Aro et al., 1984), anophthalmos (Busby et al., 2005), and cleft lip (Leck, 1971) with influenza in pregnancy, other studies have not found associations, e.g. anencephaly (Saxen et al., 1990). Several studies using the Hungarian Case-Control Surveillance of Congenital Abnormalities data suggest that the association between multiple congenital abnormalities and influenza may be an association with high fever, and not with the virus itself (Czeizel et al., 2007; Acs et al., 2005). The timing of the infection and the fever may be critical to the impact. The evidence for transplacental passage of

influenza from the pregnant mother to the fetus is sparse (Yawn et al., 1971; McGregor et al., 1984; Ramphal et al., 1980).

Beyond congenital anomalies and fetal wastage, there are epidemiological reports suggesting that influenza infection in pregnancy may be a factor in the development of psychopathology or cancer in the offspring. For example, epidemiology studies supported by rodent models have suggested that influenza infection during the second trimester – a period when fetal neuronal cells are migrating – may be associated with an increased incidence of schizophrenia (Brown, 2006; Meyer et al., 2006; Fatemi et al., 2005). Similarly, maternal fever in pregnancy, such as fever that occurs with influenza, has also been suggested as a factor in the development of autism, a hypothesis also supported by animal models (Edwards, 2006; Previc, 2007; Fatemi et al., 2005). With respect to childhood cancer, a maternal history of influenza infection in pregnancy has also been associated with an increased risk of acute lymphoblastic leukemia (Kwan et al., 2007). Unfortunately, these types of epidemiological association studies are often limited in value due to selection bias and recall bias reporting (Voldsgaard et al., 2002) making interpretation difficult (Laibl and Sheffield, 2005).

Thus overall, beyond the evidence in pandemics of increased spontaneous abortion and stillbirths, the evidence for adverse outcomes such as specific fetal malformations with maternal influenza infection in pregnancy is not strong. The evidence for influenza- associated high maternal fever as a cause of adverse fetal outcome is modest but plausible if the fever occurs at critical times in fetal development.

4 Prevention of Influenza

The measures available for prevention of influenza include immunoprophylaxis with killed (i.e., trivalent inactivated influenza vaccine (TIV)) or attenuated (live attenuated influenza vaccine (LAIV)) vaccine (CDC, 2007a; NACI, 2007), infection control procedures (CDC, 2005; CDC, 2007b) and chemoprophylaxis with oseltamivir or zanamivir (CDC, 2007a). While infection control practices such as hand washing are recommended for all, this is not a successful means of preventing influenza in the community. Similarly, influenza chemoprophylaxis is not realistic given the length and vagaries of the influenza season, the cost, and the size of the population at risk. Thus, immunoprophylaxis through immunization is the most feasible option.

4.1 Influenza Immunization

Of the two influenza vaccines available, TIV and LAIV, only TIV is recommended in pregnant women. TIV composition varies from year to year, with the influenza strains (2 A strains and 1 B strain) in the vaccine selected to match, as

closely as possible, the strains causing the seasonal outbreak. The influenza strains for the vaccine are grown in eggs. In healthy young adults, receipt of inactivated influenza vaccine is not associated with higher rates of systemic symptoms such as fever, headache, malaise, or myalgia when compared to controls (CDC, 2007a). However, a small increase in reported 'body aches' (25.1% versus 20.8%) among healthy adults receiving TIV compared to placebo has been observed in some studies (American Lung Association Asthma Clinical Research Centers, 2001). In some years (especially 1976 with swine influenza), TIV has been associated with slightly increased rates of Guillian Barré Syndrome—approximately one excess case per million people immunized, mostly in recipients over 25 years of age (CDC, 2007a). Even with this very small risk, the potential benefits of influenza vaccine far outweigh its risk. While significant hypersensitivity reactions can occur, these are also rare. TIV is not recommended for those who have a history of serious hypersensitivity reaction to previous influenza vaccine or have a documented IgE-mediated hypersensitivity to eggs or egg proteins. TIV is considered safe, effective, and cost effective in healthy adolescent and adult populations (Muennig and Khan, 2001).

4.2 Influenza Immunization During Pregnancy

In determining the place of influenza vaccine in a public health strategy to prevent influenza in pregnancy, factors such as (1) burden of disease—including risk of exposure and severity of illness, (2) vaccine characteristics – including side effects and efficacy, (3) acceptability, (4) feasibility, and (5) equity need to be assessed (Erickson et al., 2005).

In annual community outbreaks of pandemic influenza, the attack rates in the general population vary from 10% to 40%. Thus, exposure of pregnant women to influenza is probable during an annual outbreak. In the prospective study by Tuyishime and colleagues in the Eastern Townships of Quebec in Canada, 64% of the 531 pregnant woman cohort reported an influenza-like illness during the 10-week study period (15 February to 30 April 2002) when influenza was known to be circulating in the community. As discussed above, even healthy pregnant women are at significantly increased risk for more severe disease (hospitalization) (Fig. 4) than when not pregnant (McNeil et al., 2007). As noted, the high rates of influenza-related hospital admissions in the third trimester are similar to the rates for other at-risk groups for whom annual influenza immunization is recommended (McNeil et al., 2007) (Table 3). Thus, although the impact on the fetus of maternal influenza is not clear, the risk of the pregnant woman becoming infected during an influenza outbreak is high and the risk of more severe illness is now well documented.

With respect to the safety of TIV in pregnancy, this vaccine appears to be safe (CDC, 2007a; NACI, 2007; Englund, 2003). In the 1950s and 1960s, influenza vaccine was routinely given to pregnant women in the United States without reports of serious adverse events or increase in adverse outcomes in their offspring (Englund, 2003). A 7-year long-term follow-up study of the offspring of pregnant women who received 2291 doses of TIV (640 in first trimester) showed no increase in fetal malformations and no association with cognitive or neurological disorders or childhood cancers compared to those whose mothers did not receive TIV (Heinonen et al., 1973; Englund, 2003). No serious acute adverse events were reported in the pregnant women. A more recent, albeit small, study of TIV in 252 pregnant women has also not shown serious adverse events or differences in the offspring at 6 months of age (Munoz et al., 2005). A recent review of the passive reports to the vaccine adverse event reporting system (VAERS) in the United States showed no unexpected vaccine-adverse events in pregnant women who had received TIV and no increase in miscarriages (Pool and Iskander, 2006). With respect to mild adverse events, Yeager and colleagues in a study of 319 pregnant women reported a rate of 5.3% (Yeager et al., 1999) similar to reported rates in young healthy adults. Thus, TIV appears to be safe to use in pregnancy (CDC, 2007a; NACI, 2007) although more substantial large-population data would be helpful.

With respect to effectiveness, the data in pregnant women are limited. Several small studies have demonstrated that pregnant women respond to TIV with antibody titers similar to those seen in nonpregnant women (Sumaya and Gibbs, 1979; Murray et al., 1979; Englund et al., 1993). Vaccine-specific IgG placental transmission of influenza antibodies to the infants has been documented but not transmission of specific T lymphocyte response(s) or production of infant IgM anti-influenza antibody (Englund et al., 1993). In contrast, a recent study by Rastogi and colleagues using a different set of assays suggests that B and T cell responses might occur in the fetus with maternal immunization (Rastogi et al., 2007).

With respect to efficacy, the data are limited. A study in a large managed-care organization population in the United States did not show an impact on hospitalization and outpatient visit rates for influenza immunization of pregnant women (Black et al., 2004). However, the rate of immunization for the pregnant women was very low (range 4.7 to a maximum of 11.9%; Black et al., 2004); the women were not randomized to receive vaccine, and the overall rate of hospitalization was low. The study was not sufficiently powered to detect benefit, should it have occurred. A retrospective matched cohort study in four managed-care organizations examining the impact of maternal immunization on infant hospitalization; emergency and outpatient visits for acute respiratory illness also did not demonstrate benefit (visit rates 15.4 vs. 17.1 per 100 person months (IRR, 0.90; 95% CI, 0.80–1.20)) (France et al., 2006). Again the rates of immunization in the pregnant women were low, the women were not randomized to receive vaccine and adverse outcomes were rare in both groups. In addition, this study is hampered by short study time frames

(average influenza season 9.5 weeks each year) and a mismatch between the vaccine and the circulating strains. Both of these factors may have minimized impact. In direct contrast, a preliminary report of a randomized controlled trial of maternal third trimester TIV vaccine versus pneumococcal vaccine in 340 pregnant women in an impoverished area in Dacca, Bangladesh, noted a major benefit with maternal influenza immunization (Steinhoff et al., 2006). Third trimester maternal influenza immunization was associated with a sharp decrease in maternal and infant respiratory illness with fever (mothers decrease by 1.7/100 maternal months; infants decrease by 9.4/100 infant months) such that 103 respiratory illnesses with fever could be averted for each 170 doses of TIV administered (Steinhoff et al., 2006). Of note in this more tropical climate, influenza is not seasonal but occurs during most of the year which means exposure is more prolonged and hence benefit of influenza immunization may be easier to demonstrate.

An American cost-effectiveness analysis using literature-based estimates of costs and probabilities noted that universal influenza vaccination in a pregnant population would be cost saving relative to providing supportive care alone if given in a routine prenatal visit (Roberts et al., 2006).

4.3 Influenza Immunization in Pregnancy Recommendations by Country

As shown in Table 4, despite the evidence of increased severity of illness with influenza in pregnancy and the availability of a vaccine that appears to be safe and is likely to be as effective as in the nonpregnant population of similar age, the recommendation for universal immunization of all women who will be pregnant during the influenza season is still not the norm even in industrialized countries. Furthermore, influenza vaccine uptake in pregnant women in countries where immunization has been recommended for a number of years are still low—generally less than 10% (Naleway et al., 2006) and for pregnant women with comorbidities distressingly low (6.7%) (Dodds et al., 2007).

Table 4 Influenza immunization recommendations for pregnant women in selected countries

Country	Recommendations	Notes
Canada	All* pregnant women	
United States	All* pregnant women	
Australia	All* women who will be in the 2nd or 3rd trimester of pregnancy during influenza season	Immunization during all trimesters acceptable
United Kingdom	Pregnant women with comorbidities	

*includes both healthy pregnant women and women with comorbidities.

5 Barriers to Influenza Immunization of Pregnant Women

Although the inactivated influenza vaccine is considered safe at all stages of pregnancy (Munoz et al., 2005; Englund, 2003; CDC, 2007a; NACI, 2007), several reports have shown that concerns about safety continue to be a factor in the acceptance of this vaccine (Halperin et al., 2006; Naleway et al., 2006; Silverman and Greif, 2001). In a survey of 662 pregnant women in Nova Scotia, Canada, designed to explore key factors that influence acceptance of influenza vaccination in pregnancy, Halperin and colleagues found that 30% of women surveyed felt they should avoid all vaccines during pregnancy and 41% believed it was safer to wait until after the first trimester (Halperin et al., 2006). Of note, the survey statement "influenza vaccine is safe in all stages of pregnancy" was answered correctly by only 36% of women. In addition, women who were less accepting of influenza vaccine in all stages of pregnancy had lower overall knowledge scores. This concern about the safety of influenza vaccine in pregnancy is consistent with findings from other studies. Silverman and Greif (2001) in a prospective survey administered during influenza season to postpartum women and obstetricians in a high-volume urban medical center in metropolitan Los Angeles found that 44% of the 242 postpartum women interviewed believed that all vaccines should be avoided during pregnancy.

Healthcare-provider recommendation has also emerged as a strong factor in determining whether this vaccine will be accepted in pregnancy (Halperin et al., 2006; Silverman and Greif, 2001; Yeager et al., 1999). In the Nova Scotia, Canada, study, women whose doctor discussed influenza vaccination during pregnancy were more knowledgeable and more accepting of the vaccine in any stage of pregnancy (Halperin et al., 2006). However, despite 61% of respondents stating that they would accept the vaccine while pregnant if their doctor recommended it, and 54% citing their doctor/nurse as their primary source of vaccine information, only 20% said that their doctor had discussed influenza vaccination during their pregnancy. This is consistent with Silverman and Greif 's survey in Los Angeles that found that only 22% of the women had discussed influenza vaccine with their physician during pregnancy and only 8% had been vaccinated. However, 56% of the women surveyed during the postpartum period said they would have accepted influenza vaccination during pregnancy if their physician had recommended it (Silverman and Greif, 2001).

Lack of information about influenza vaccine during pregnancy has been identified as a provider-perceived barrier. In the survey by Silverman and Greif in Los Angeles, 12% of the 113 providers surveyed said they believed all vaccines should be avoided during pregnancy (Silverman and Greif, 2001). Providers who were more knowledgeable about influenza vaccine were more likely to initiate discussions about vaccination with their pregnant patients than were providers with less knowledge about the vaccine. Physician knowledge that influenza during pregnancy is associated with increased morbidity compared to that in nonpregnant adults was associated with a significantly higher

rate of discussing influenza vaccine with their patients. Another survey of obstetricians and family physicians conducted in Santa Monica, California, in 2001 regarding knowledge of indications and contraindications to influenza vaccination in pregnancy found that 63.5% of the 74 family physicians and obstetricians surveyed (FP 36/57; OB 11/17) incorrectly identified the first trimester as a contraindication to influenza vaccination (Wallis et al., 2004). Although this study was limited in the number of physicians surveyed, it demonstrates the need for effective educational interventions targeting perceived provider and patient barriers to vaccination during pregnancy.

6 Next Steps to Improve Influenza Vaccine Uptake in Pregnant Women

Concerted efforts must be directed at ensuring that pregnant women, particularly those with medical comorbidities, have the opportunity to benefit from protection with influenza vaccine. Pregnant women need to be recognized as a priority risk group for influenza immunization in National Guidelines and all pregnant women need to have access to publicly funded influenza vaccine. If the work of Steinhoff and colleagues in Bangladesh is corroborated, then UNICEF and the World Health Organization need to support wide use of influenza vaccine in pregnancy in developing countries.

Although the reasons for the failure of healthcare providers in industrialized countries to offer influenza vaccine during pregnancy are complex, compliance with current recommendations in many countries may be reduced due to targeted rather than universal programs. For pregnant women, nonuniversal, targeted immunization recommendations require assessment of both maternal health status and timing of anticipated delivery in relation to the local influenza season in order to determine whether an individual pregnant woman should be immunized. Broadening the immunization recommendation to include all pregnant women enables easier identification of the target population. In Ontario, Canada, implementation of a universal influenza immunization program led not only to increased immunization coverage rates among healthy children and adults who would not previously have been offered vaccine, but also higher uptake rates among high-risk adolescents and adults in all age groups (Kwong et al., 2006).

The importance of healthcare providers recommending influenza vaccine to pregnant women cannot be overstated as the research studies noted above highlight the importance of this for maternal decision making about accepting the vaccine. Thus, healthcare providers who care for pregnant women need to be well educated about the risks associated with influenza in pregnancy and the safety and the efficacy of influenza vaccine to ensure that they feel comfortable in encouraging their pregnant patients to receive the vaccine. Many healthcare providers for pregnant women are not traditionally involved in advising

patients about vaccines (e.g., obstetricians, midwives, prenatal care nurses, and doulas) so specific programs to educate these groups will need to be developed.

In most industrialized countries, public health recommendations for preventative health interventions are promoted primarily through the dissemination of information to healthcare providers through continuing professional development activities. Systematic reviews have found that traditional, didactic continuing medical education (CME) presentations while improving knowledge are ineffective in changing physician behaviour (Davis et al., 1999; Mazmanian et al., 2002). Patient-mediated strategies, those in which the patient asks the physician for a test or treatment, are a more effective way of changing physician behaviour, improving physician performance by a median of 21% (Grimshaw et al., 2004). Empowering pregnant women with the information they need to understand the risks of influenza during pregnancy and the safety and benefits of the influenza vaccine can encourage women to ask their care provider about influenza vaccine to protect themselves and their baby. This partnership between women and their healthcare providers holds promise for improving influenza vaccine uptake. Further work is needed to determine optimal strategies for improving the delivery of information on influenza to pregnant women. If influenza immunization programs are expanded to target pregnant women in developing countries, work will need to be done to tailor programs to best fit with the resources and opportunities.

Education of healthcare providers and pregnant women about the need and benefits of influenza vaccine in pregnancy is not enough. Access to and delivery of vaccine to pregnant women will require strategic delivery programs. Care plans that ensure that influenza vaccine is offered to all pregnant women; capacity to provide influenza vaccine at the point-of-care, and clearly defined alternative sites for access to influenza vaccines will need to be developed.

7 Areas for Future Research

Many questions remain unanswered about influenza in pregnancy. Long-term studies evaluating the relationship between maternal influenza and the development of diseases such as autism, schizophrenia, and malignancy in offspring are critical to an understanding of the etiology of these diseases and the potential to prevent them through immunization. Given ongoing concerns of providers and the public about the safety of influenza immunization during pregnancy, well-designed, prospective trials including large numbers of women at all stages of pregnancy should be undertaken to further address safety concerns. Trials evaluating the impact of maternal immunization on incidence and severity of influenza-associated illness in infants less than 6 months of age, a group for whom immunization is not an option are needed. Further work in

developing countries is needed to verify if influenza immunization of pregnant women does have the huge impact noted by Steinhoff and colleagues (Steinhoff et al., 2006). If so, then research is needed on best practice to ensure influenza immunization is provided to these pregnant women given the vagaries of healthcare delivery in many developing countries. Similarly, in industrialized countries, careful evaluation of immunization program delivery in a variety of settings is needed to inform best practices, particularly best practice in reaching vulnerable or marginalized pregnant women and those with medical comorbidities.

8 Conclusions

There is now compelling evidence that influenza poses a risk of morbidity among pregnant women much higher than among their nonpregnant peers. The rates for serious illness are similar or higher to those observed in other recognized high-risk populations for whom influenza immunization is routinely recommended. Mounting data demonstrates the safety of the inactivated influenza vaccine at all stages of pregnancy. While further studies examining the efficacy of the vaccine in this population are needed, immunogenicity studies suggest that pregnant women will benefit from immunization and recent evidence suggests that immunization of pregnant women may provide tremendous benefit to newborns either by placental antibody transfer or prevention of infection in mothers, or both. Despite these data, there remains a dismally low rate of influenza immunization of pregnant women, even for women with comorbidities. In order to decrease influenza morbidity for pregnant women it is vital that National Immunization Guidelines recognize all pregnant women among risk groups for increased morbidity due to influenza and that influenza immunization programs provide influenza vaccine to all pregnant women at no cost. A concerted effort must be made to educate healthcare providers of pregnant women about influenza morbidity, influenza vaccine safety in pregnancy, and the positive influence that physician recommendation of vaccine has on vaccine uptake. Public health and other organizations must develop public awareness campaigns to educate pregnant women about the risks of influenza and the safety and benefits of vaccine, and must develop effective strategic delivery programs that enhance access to and uptake of influenza vaccine in pregnancy. For developing countries, if the preliminary work showing a big benefit of influenza vaccine in pregnancy both for the mother and her infant is confirmed by future research, then UNICEF and the World Health Organization must work to make influenza vaccine available to these pregnant women. Finally, national pandemic influenza preparedness plans must specifically identify pregnant women among priority groups for influenza immunization and address strategies to reach these women during a pandemic.

References

Acs N, Banhidy F, Puho E, Czeizel AE (2005). Maternal influenza during pregnancy and risk of congenital abnormalities in offspring. *Birth Defects Res A Clin Mol Teratol*, *73*: *989–996*.

American Lung Association Asthma Clinical Research Centers (2001). The safety of inactivated influenza vaccine in adults and children with asthma. *N Engl J Med*, *345*: *1529–1536*.

Aro T, Haapakoski J, Heinonen OP (1984). A multivariate analysis of the risk indicators of reduction limb defects. *Int J Epidemiol*, *13*: *459–464*.

Black SB, Shinefield HR, France EK, Fireman BH, Platt ST, Shay D (2004). Vaccine Safety Datalink Workgroup: Effectiveness of influenza vaccine during pregnancy in preventing hospitalizations and outpatient visits for respiratory illness in pregnant women and their infants. *Am J Perinatol*, *21*: *333–339*.

Bland PB (1919). Influenza in its relation to pregnancy and labor. *Am J Obstet Dis Women Child*, *79*: *184–197*.

Brown A (2006). Prenatal infection as a risk factor for schizophrenia. *Schizophr Bull*, *32*: *200–202*.

Busby A, Dolk H, Armstrong B (2005). Eye anomalies. Seasonal variation and maternal viral infections. *Epidemiology*, *16*: *317–322*.

Centers for Disease Control and Prevention (2007a). Prevention and control of influenza: recommendations of the advisory committee on immunization practices. *MMWR*, *56(RR-6)*: *1–25*.

Centers for Disease Control and Prevention (2007b). Guidance for prevention and control of influenza in the peri- and postpartum settings. www.cdc.gov/flu/professionals/infectioncontrol/peri-post-settings.htm.

Cox S, Posner SS., McPheeters M, Jamieson DJ, Kourtis AP, Meikle S (2006). Hospitalizations with respiratory illness among pregnant women during influenza season. *Obstet Gynecol*, *107*: *1315–1322*.

Cox S, Posner SF, McPheeters M, Jamieson DJ, Kourtis AP, Meikle S (2006). Influenza and pregnant women: hospitalization burden, 1998–2002. *J Womens Health*, *15*: *891–893*.

Czeizel AE, Puho EH, Acs N, Banhidy F (2007). High fever-related maternal diseases as possible causes of multiple congenital abnormalities: a population-based case-control study. *Birth Defects Res A Clin Mol Teratol*, *79*: *544–551*.

Davis D, O'Brien MAT, Wolf FM, Freemantle N, Mazmanian P, Taylor-Vaisey A (1999). Impact of formal continuing medical education. Do conferences, workshops, rounds and other traditional continuing education activities change physician behaviour or health care outcomes? *JAMA*, *282*: *867–874*.

Department of Health, United Kingdom (2006). Immunization against infectious disease- the Green Book. Chapter 19. Influenza, ed. R.M. Salisbury D, Noakes K. 2006, London, UK: TSO (The Stationery Office) www.dh.gov.uk/en/Policyandguidance/Healthandsocialcaretopics/Greenbook/index.htm.

Dodds L, McNeil SA, Fell DB, Allen VM, Coombs A, Scott J, MacDonald N (2007). Impact of influenza exposure on rates of hospital admissions and physician visits because of respiratory illness among pregnant women. *CMAJ*, *176*: *463–468*.

Edwards MJ (2006). Review: hyperthermia and fever during pregnancy. *Birth Defects Res A Clin Mol Teratol*, *76*: *507–516*.

Englund J (2003). Maternal immunization with inactivated influenza vaccine: rationale and experience. *Vaccine*, *21*: *3460–3464*.

Englund JA, Mbawuike IN, Hammill H, Holleman MC, Baxter BD, Glezen WP (1993). Maternal immunization with influenza or tetanus toxoid vaccine for passive antibody protection in young infants. *J Infect Dis*, *168*: *647–656*.

Erickson LJ, De Wals P., Farand L (2005). An analytical framework for immunization programs in Canada. *Vaccine*, *23*: *2470–2476*.

Fatemi SH, Pearce DA, Brooks AI, Sidwell RW (2005). Prenatal viral infection in mouse causes differential expression of genes in brains of mouse progeny: a potential animal model for schizophrenia and autism. *Synapse*, *57*: *91–99*.

France EK, Smith-Ray R, McClure D, Hambidge S, Xu S, Yamasaki K, Shay D, Weintraub E, Fry AM, Black SB, Shinefeld HR, Mullooly JP, Jackson LA, and the Vaccine Safety Datalink Team (2006). Impact of maternal influenza vaccination during pregnancy on the incidence of acute respiratory illness visits among infants. *Arch Pediatr Adolesc Med*, *160*: *1277–1283*.

Freeman DW, Barno A (1959). Deaths from Asian Influenza Associated with Pregnancy. *Amer J Obst Gynec*, *78*: *1172–1175*.

Glezen WP, Couch RB (1978). Interpandemic influenza in the Houston area, 1974–1976. *N Engl J Med*, *298*: *587–592*.

Glezen WP, Keitel WA, Taber LH, Piedra PA, Clover RD, Couch RB (1919). Age distribution of patients with medically-attended illnesses caused by sequential variants of influenza A/H1N1: comparison to age-specific infection rates, 1978–1989. *Am J Epidemiol, 133(3): 296–304*.

Greenberg M, Jacobziner H, Pakter J, Weisl BA(1958). Maternal mortality in the epidemic of Asian influenza, New York City 1957. *Amer J Obst and Gynec*, *76*: *897–902*.

Grimshaw JM, Thomas RE, MacLennan G, Fraser C, Ramsay CR, Vale L, Whitty P, Eccles MP, Matowe L, Shirran L, Wensing M, Dijkstra R, Donaldson C (2004). Effectiveness and efficiency of guideline dissemination and implementation strategies. *Health Technol Assess*, *8(6): iii–iv, 1–72*.

Halperin BA, MacKinnon-Cameron D, McNeil S (2006). Survey of knowledge, attitudes, and behaviour regarding influenza vaccination in pregnancy and childhood. *International Conference on Women and Infectious Diseases (ICWID). Atlanta, USA March 17–19, 2006 (Abstract #38)*. www.womenshealthconf.org

Harris JW (1919). Influenza occurring in pregnant women. A statistical study of thirteen hundred and fifty cases. *Jour A.M.A*, *72*: *978–980*.

Hartert TV, Neuzil KM, Shintani AK, Mitchel EF Jr., Snowden MS, Wood LB, Dittus RS, Griffin MR (2003). Maternal morbidity and perinatal outcomes among pregnant women with respiratory hospitalizations for influenza season. *Am J Obstet Gynecol*, *189*: *1705–1712*.

Heinonen OP, Shapiro S, Monson RR, Hartz SC, Rosenberg L, Slone D (1973). Immunization during pregnancy against poliomyelitis and influenza in relation to childhood malignancy. *Int J Epidemiol*, *2*: *229–235*.

Irving WL, James DK, Stephenson T, Laing P, Jameson C, Oxford JS, Chakraverty P, Brown DWG, Boon ACM, Zambon MC (2000). Influenza virus infection in the second and third trimesters of pregnancy: a clinical and seroepidemiological study. *BJOG*, *107*: *1282–1289*.

Izurieta HS, Thompson WW, Kramarz P, Shay DK, Davis RL, DeStefano F, Black S, Shinefield H, Fukuda K (2000). Influenza and the rates of hospitalization for respiratory disease among infants and young children. *N Engl J Med*, *342*: *232–239*.

Kilbourne E (2006). Influenza pandemics of the 20th century. *Emerg Iinfect Dis*, *12*: *9–14*.

Kort BA, Cefalo RC, Baker VV(1986). Fatal influenza in pregnancy. *Am J Perinatol*, *2*: *179–182*.

Kwan ML, Metayer C, Crouse V, Buffler PA (2007). Maternal illness and drug/medication use during the period surrounding pregnancy and risk of childhood leukemia among offspring. *Am J Epidemiol*, *165*: *27–35*.

Kwong JC, Sambell C, Johansen H, Stukel TA, Manuel DG (2006). The effect of universal influenza immunization on vaccination rates in Ontario. *Health Rep*, *17*: *31–40*.

Laibl VR, Sheffield JS (2005). Influenza and pneumonia in pregnancy. *Clin Perinatol*, *32*: *727–738*.

Leck I (1971). Further tests of the hypothesis that influenza in pregnancy causes malformations. *HSMHA Health Rep*, *86*: *265–269*.

Li Z, Ren A, Liu J, Pei L, Zhang L, Guo Z, Li Z (2007). Maternal flu or fever, medication use and neural tube defects: a population based case control study in northern China. *Birth Defects Res A Clin Mol Teratol*, *79*: *295–300*.

Local Government Board (1919). 48th Annual Report 1918–1919. Supplement containing the report of the medical department. Section 2. Influenza. *London*: *Her Majesty's Stationery Office*; *7–32*.

Lynberg MC, Muin JK, Lu X, Cocian T (1994). Maternal flu, fever and the risk of neural tube defects: a population-based case control study. *Am J Epidemiol*, *140*: *244–255*.

MacDonald NE, McNeil S, Allen VM, Scott J, Dodds L (2004). Influenza vaccine programs and pregnancy: a need for more evidence. *J Obstet Gynaecol Can*, *26*: *961–963*.

Mazmanian PE, Davis DA (2002). Continuing medical education and the physician as learner: guide to the evidence. *JAMA*, *288*: *1057–1060*.

McGregor JA, Burns JC, Levin MJ, Burlington B, Meiklejohn G (1984). Transplacental passage of influenza A/Bangkok (H3N2) mimicking amniotic fluid infection syndrome. *Am J Obstet Gynecol*, *149*: *856–859*.

McKinney WP, Volkert P, Kaufman J (1990). Fatal swine influenza pneumonia during late pregnancy. *Arch Intern Med*, *150*: *213–215*.

McNeil S, Dodds L, Fell D, Coombs A, Scott J, Allen V, Halperin B, MacDonald N (2006). Impact of influenza on respiratory-related hospitalizations and pre-term births in pregnant women: from evidence to public health practice in Canada, 83rd Canadian Pediatric Society Annual Meeting, June 13–17, 2006, St. John's, *NL Paediatr Child Health, 11 Suppl B: Abstract# 19B*.

McNeil SA, Dodds L, Allen VM, Scott J, Halperin B, MacDonald N (2007). Influenza vaccine programs and pregnancy: new Canadian evidence for immunization. *JOGC*, *29*: *674–676*.

Meyer U, Schwendener S, Feldon J, Yee B (2006). Prenatal and postnatal maternal contributions in the infection model of schizophrenia. *Exp Brain Res 173*: *243–257*.

Mortimer P (2006). Influenza-related death rates for pregnant women. Emerging infectious diseases. *Emerg Infect Dis*, *12*: *1805–1806*.

Muennig PA, Khan K (2001). Cost-effectiveness of vaccination versus treatment of influenza in healthy adolescents and adults. *CID*, *33*: *1879–1885*.

Mullooly JP, Barker W, Nolan TF (1986). Risk of acute respiratory disease among pregnant women during influenza A epidemics. *Public Health Rep*, *101*: *205–211*.

Mullooly JP, Bridges CB, Thompson WW, Chen J, Weintaub E, Jackson LA, Black S, Shay DK, Vaccine Data Safety Adult Working Group (2007). Influenza- and RSV-associated hospitalizations among adults. *Vaccine*, *25*: *846–855*.

Munoz FM, Greisinger AJ, Wehmanen OA, Mouzoon ME, Hoyle JC, Smith FA, Glezen WP (2005). Safety of influenza vaccination during pregnancy. *Am J Obstet Gynecol*, *192*: *1098–1106*.

Murray DL, Imagawa DT, Okada DM, St. Geme JW Jr. (1979). Antibody response to monovalent A/New Jersey/8/76 influenza vaccine in pregnant women. *J Clin Microbiol*, *10*: *184–187*.

Naleway AL, Smith WJ, Mullooly JP (2006). Delivering influenza vaccine to pregnant women. *Epidemiol Rev*, *28*: *47–53*.

National Advisory Committee on Immunization (2004), Statement on influenza vaccination for the 2004–2005 season. *CCDR*,*30 (ACS-3)*: *1–32*.

National Advisory Committee on Immunization (2007). Statement on influenza vaccination for the 2007–2008 season. *CCDR*, *33 (ACS-7)*: *1–38*.

Neuzil KM, Reed GW, Mitchel EF, Simonsen L, Griffin MR (1998). Impact of influenza on cardiopulmonary hospitalizations in pregnant women. *Am J Epidemiol*, *148*: *1094–1102*.

Neuzil KM, Mellen BG, Wright PF, Mitchel EF Jr, Griffin MR (2000). The effect of influenza on hospitalizations, outpatient visits, and courses of antibiotics. *N Engl Med*, *324*: *225–231*.

Poland GA, Jacobson RM, Targonski PV (2007). Avian and pandemic influenza: an overview. *Vaccine*, *25*: *3057–3061*.

Pool V, Iskander J (2006). Safety of influenza vaccination during pregnancy. *Am J Obstet Gynec*, *194*: *1200–1201*.

Previc FH (2007). Prenatal influences on brain dopamine and their relevance to the rising incidence of autism. *Med Hypotheses*, *68*: *46–60*.

Ramphal R, Donnelly WH, Small PA Jr (1980). Fatal influenzal pneumonia in pregnancy: failure to demonstrate transplacental transmission of influenza virus. *Am J Obstet Gynecol*, *138*: *347–348*.

Rastogi D, Wang C., Mao X, Lendor C, Rothman PB, Miller RL (2007). Antigen-specific immune responses to influenza vaccine in utero. *J Clin Invest*, *117*: *1637–1646*.

Registrar General's Report 1918/1919 (1919). Eighty-second annual report of the registrar-general of births, deaths, and marriages in England and Wales (1919). *Her Majesty's Stationery Office*. *p. xxviii*.

Roberts S, Hollier LM, Sheffield J, Laibl V, Wendel GD Jr (2006). Cost-effectiveness of universal influenza vaccination in a pregnant population. *Obstet Gynecol*, *107*: *1323–1329*.

Saxen L, Holmberg PC, Kurpa K, Kuosma E, Pyhala R (1990). Influenza epidemics and anencephaly. *AJPH 80*: *473–475*.

Schanzer DL, Langley JM, Tam TWS (2007). Influenza-attributed hospitalization rates among pregnant women in Canada 1994–2000. *JOGC*, *29*: *622–629*.

Schoenbaum SC, Weinstein L (1979). Respiratory infection in pregnancy. *Clin Obstet Gynecol*, *22*: *293–300*.

Shu Y, Yu H, Li D (2006). Lethal avian influenza A (H5N1) infection in a pregnant woman in Anhui Province, China. *N Engl J Med*, *354*: *1421–1422*.

Silverman NS, Greif A (2001). Influenza vaccination during pregnancy: patients' and physicians' attitudes. *J Reprod Med*, *46*: *989–994*.

Simonsen L, Fukuda K, Schonberger LB, Cox NJ (2000). The impact of influenza epidemics on hospitalizations. *J Infect Dis*, *181*: *831–837*.

Simonsen L, Reichert TA, Viboud C, Blackwelder WC, Taylor RJ, Miller MA (2005). Impact of influenza vaccination on seasonal mortality in the US elderly population. *Arch Intern Med*, *165*: *265–272*.

Stanwell-Smith R, Parker AM, Chakraverty P, Soltanpoor N, Simpson CN (1994). Possible association of influenza A with fetal loss: investigation of a cluster of spontaneous abortions and stillbirths. *Commun Dis Rep CDR Review*, *4*: *R28–R32*.

Steinhoff MC, Zaman K, Roy E, Arifeen SE, Rahman M, Raqib R, Shahid N (2006). Incidence of vaccine preventable influenza illness in 0–5 month-old infants. *Infectious Disease Society of America. Toronto Canada. October 2006: Abstract LB-6*.

Sumaya CV, Gibbs RS (1979). Immunization of pregnant women with influenza A/New Jersey/76 virus vaccine: reactogenicity and immunogenicity. *J Infect Dis*, *140*: *141–146*.

Taubenberger JK, Morens DM (2006). 1918 Influenza: the mother of all pandemics. *Emerg Infect Dis*, *12*: *15–22*.

Tuyishime J-de-D, DeWals P, Moutquin J-M, Frost E (2003). Influenza-like illness during pregnancy: results from a study in the Eastern Townships, Province of Quebec. *JOGC*, *25*: *1020–1025*.

Viboud C, Tam T, Fleming D, Miller MA, Simonsen L (2006). 1951 influenza epidemic, England and Wales, Canada, and the United States. *Emerg Infect Dis*, *12*: *661–668*.

Voldsgaard P, Schiffman J, Mednick S, Rodgers B, Christensen H, Bredkjaer S, Schulsinger F (2002). Accuracy of retrospective reports of infections during pregnancy. *Int J Methods Psychiatr Res*, *11*: *184–186*.

Wallis DH, Chin JL, Sur DKC (2004). Influenza vaccination in pregnancy: current practices in a suburban community. *J Am Board Fam Pract*, *17*: *287–291*.

Widelock D, Csizmas L, Klein S (1963). Influenza, pregnancy and fetal outcome. *Public Health Rep*, *78(1)*: *1–11*.

Wong CM, Yang L, Chan KP, Leung GM, Chan KH, Guan Y, Lam TH, Hedley AJ, Peiris JSM (2006). Influenza-associated hospitalization in a subtropical city. *PLOS Medicine*, *3*: *485–492*.

World Health Organization (2007). Cumulative number of confirmed human cases of Avian Influenza A/(H5N1) Reported to WHO: July 11, 2007. www.who.int/csr/disease/avian_influenza/country/cases_table_2007_07_11/en/index.html.

Yawn DH, Pyeatte JC, Joseph JM, Eichler SL, Garcia-Bunuel R (1971). Transplacental transfer of influenza virus. *JAMA*, *216*: *1022–1023*.

Yeager DP, Toy EC, Baker B III (1999). Influenza vaccination in pregnancy. *Am J Perinatol*, *16*: *283–286*.

Febrile Neutropenia in Children with Cancer

Stéphane Paulus and Simon Dobson

1 Introduction

Febrile neutropenia (FN) is a common complication in children treated for malignancies. In this review, we describe recent advances in several aspects of this topic at the crossroads between oncology and infectious diseases. Although a large amount of information on the subject comes from the adult literature, we have tried to integrate information from paediatric series when this data were available.

2 Definitions

A recent article has highlighted the lack of an agreed definition of fever, and the variability amongst units caring for children with cancer (Phillips B., et al., 2007). The Infectious Diseases Society of America 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer defines fever as a single oral temperature of 38.3°C, or 38.0°C twice at least 1 h apart (Hughes et al., 2002). Neutropenia is defined as an absolute neutrophil count (ANC) of less than 500 cells/μL, or less than 1000 cells/μL in a patient anticipated to rapidly become severely neutropenic after a course of chemotherapy.

3 Microbiology and Antimicrobials in Febrile Neutropenia

Febrile neutropenic patients are at high risk of bacterial sepsis. Prompt initiation of empiric antimicrobial therapy has been critical in reducing mortality and morbidity in those patients (Viscoli, 2005). A variety of empiric antibiotic regimens can be used for FN. While published guidelines are useful, they cannot

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replace a good knowledge of local resistance patterns. Regular surveillance of the local microbiological data is necessary to inform the best choice of first-line empiric therapy (Ammann et al., 2004).

3.1 Common Pathogens

Blood cultures are positive in 20–30% of paediatric or adult patients with FN (Chamberlain et al., 2005; Hann et al., 1997). This is a conservative estimate in view of the frequently inadequate blood volume drawn for blood cultures in paediatric patients (Connell et al., 2007) and the difficulty in recovering some organisms, which can be fastidious to culture (e.g. yeast). Most patients will be colonized by the infecting organisms, usually after initial admission to the hospital, before invasive disease becomes apparent (Walsh et al., 2005). This is important because knowledge of local bacterial flora on a particular unit can inform empiric antibiotic choices. Organisms will then either invade an injured mucosal barrier secondary to chemotherapy (translocation from the oral mucosa or gut) or access the blood stream via the skin, through central venous catheters or skin breakdown. Gut translocation represents a major route of infection, and some authors have advocated the use of non-absorbable antibiotics in order to eradicate the carriage of potential pathogen in the gut (Paulus et al., 2005). However, there is ongoing debate in the literature about the usefulness of this approach and its impact on the potential emergence of multi-resistant organisms.

The microbiology of FN is a constantly changing picture. In the last decade, the focus has shifted towards an increased prevalence of Gram positive organisms, which now represent about 70% of blood cultures of patients with FN (Hughes et al., 2002). In order of frequency organisms recovered are: coagulase negative staphylococcus, viridans group streptococcus (VGS), *Staphylococcus aureus*, and *Enterococcus faecalis*. Gram negative organisms are responsible for most of the mortality associated with sepsis in oncology patients. Most commonly isolated are *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter* spp. Although usually described as a classical pathogen in the context of FN, *Pseudomonas aeruginosa* prevalence is highly variable from institution to institution. Anaerobic organisms are more commonly associated with intraabdominal sepsis and typhlitis (inflammation of the caecum seen post chemotherapy). Finally, infections with fungal pathogens, such as *Candida spp*. and *Aspergillus* spp., play an important role in high-risk patients (HSCT, AML) with persistent severe FN.

3.2 Empiric Therapy Choices

To cover the range of pathogens encountered in the setting of FN, the prompt administration of broad spectrum antibiotic therapy is necessary. The use of

monotherapy versus combination therapy with an aminoglycoside has been much debated in the literature. A Cochrane Collaboration review, evaluating 46 randomized controlled trials, which included 7642 adult and paediatric patients, showed no significant benefit for combination therapy in terms of survival or treatment failure, while adverse events were more common with combination treatment (Paul et al., 2003). The report recommends the adoption of monotherapy with a broad spectrum beta-lactam as the standard of care for the treatment of FN in adult patients. In another recent review summarizing studies and meta-analyses on the empirical antibiotic therapy in high risk patients, different authors draw a similar conclusion regarding the preference for monotherapy (Glasmacher et al., 2005). The paediatric data is more limited at this time, but the gathering evidence seems to show similar efficacy using monotherapy as combination with an aminoglycoside (Agaoglu et al., 2001; Ariffin et al., 2006; Duzova et al., 2001; Hung et al., 2003).

Regimens using a cephalosporin, usually ceftazidime with or without an aminoglycoside, has shown efficacy in the past (Granowetter et al., 1988). Ceftazidime has good activity against *P. aeruginosa* and most Gram negative bacilli. However, its lack of reliable activity for Gram positive organisms such as *Streptococcus* spp. and *S. aureus* has been a growing concern in an era of increasing infections with those organisms (Hughes et al., 2002). Ceftriaxone has also been used for empiric cover in FN with success (Ariffin et al., 2001; Charnas et al., 1997). It is to be used with caution because, although it has better coverage of *Streptococcus* spp., ceftriaxone lacks any activity against *P. aeruginosa*. Additionally, cephalosporins do not have any activity against *Enterococcus* spp.

Cefipime, a fourth-generation cephalosporin has an expanded spectrum of activity for Gram positive pathogens, with reliable activity for *S. aureus* and VGS while displaying enhanced Gram negative cover, being resistant to Amp-C type 1 β-lactamases produced by an increasing number of Enterobacteriaceae. It lacks activity against *Enterococcus faecalis*. Cefepime monotherapy has been reported as a feasible option for treatment of childhood cancer patients with FN (Ariffin et al., 2006; Chastagner et al., 2000). In a clinical trial comparing cefipime with ceftazidime monotherapy, it was shown that the addition of vancomycin was required less frequently with cefipime (Owens et al., 2000). Cefipime monotherapy also showed a quicker defervescence, shorter hospitalization, and lower therapy cost when compared with combination of ceftazidime and amikacin (Corapcioglu and Sarper, 2005). However, the Food and Drug Administration (FDA) issued a caution in late 2007 on the use of cefipime in this setting as possibly having a higher all cause mortality than other beta-lactam antibiotics.

The carbapenems imipenem and meropenem have excellent in vitro activity for Gram positive organisms, Gram negative organisms, and anaerobes. They are active agents against *P. aeruginosa* and are resistant to β-lactamase-producing organisms. However, there are now increasing concerns regarding the emergence of new carbapenemases in some Gram negative bacilli. There is little

difference between meropenem and imipenem. Meropenem has better in vitro activity against Gram negatives with minimal inhibitory concentrations (MIC) tenfold lower for most Gram negative organisms compared with imipenem (Chambers, 2005). Conversely, imipenem has better in vitro activity for Gram positive organisms. Meropenem has potential advantages over imipenem with regard to gastrointestinal toxicities and reported lower threshold for the onset of seizure in seriously ill patients with imipenem use (Walsh et al., 2005). Meropenem has been well studied as an agent for children with FN (Cometta et al., 1996; Duzova et al., 2001; Fleischhack et al., 2001) and has demonstrated clinical superiority to ceftazidime and amikacin in a randomized clinical trial (Hung et al., 2003).

The combination of a β-lactam antibiotic with a β-lactamase inhibitor has been increasingly popular for use in children. Examples are piperacillin/tazobactam and ticarcillin/clavulanate. These antibiotics are well suited in the context of FN as they possess a wide spectrum of activity on most Gram positive, negative, and anaerobic organisms. It is important to note that ticarcillin/clavulanate is less active in vitro than piperacillin/tazobactam against *Streptococcus spp*., *E. faecalis*, *Klebsiella* spp., and *Pseudomonas* spp. (Blondell-Hill, 2006). Piperacillin/tazobactam display a good safety profile and has been shown to be an effective agent in children with FN (Corapcioglu et al., 2006; Fouyssac et al., 2005; Le Guyader et al., 2004).

4 Practice Surveys

There are a great variety of approaches when it comes to dealing with febrile neutropenic children not only around the globe, but also from institution to institution within a single country. Definition of fever, empiric regimen of choice, and risk stratification may vary from centre to centre. The lack of unifying approach is of concern and there have been calls for more standardization in the way in which the diagnosis and treatment of children with FN is approached (Phillips R. et al., 2007). Three practice surveys from the UK, Australasia, and Canada have recently been published and illustrate this issue. On the other hand, this variability of practice should allow for comparisons to be made in future collaborative studies.

A UK survey published in 2007 (Phillips B. et al., 2007) reports on the heterogeneity of the approach for the management of FN. A questionnaire was sent to all of the 21 United Kingdom Children's Cancer Study Group (UKCCSG) assessing local policies and protocols for the management of FN. The definition of fever used in these centres ranged from a persistent temperature of $>37.5^{\circ}$C to a single reading of $>39.0^{\circ}$C. Neutropenia was defined as either an ANC <1, <0.75, or <0.5 cells/μL depending on the unit. A variety of antibiotic combinations were used, the most common consisting of a piperacillin containing antibiotic together with an aminoglycoside compound. Indications for modification of the empiric regimen varied greatly and few centres had a defined endpoint for treatment with an antifungal. Finally, risk stratification

was undertaken in 11 centres, with six using a policy of reduced intensity therapy in 'low-risk' patient. The UKCCSG is currently working towards the development of a common strategy in the approach of FN patients in the UK.

In a prospective audit undertaken in Australia and New Zealand (Chamberlain et al., 2005), authors reported on the variability in treatment approaches to children with FN. They looked at the management of all cases of FN in nine centres for a period of 2 months. There are no published guidelines for the management of FN in either country. They report 127 episodes of FN, of which a positive blood culture was documented in 30%. There were 18 different first-line antibiotic combinations used, the most popular being a combination of ticarcillin/clavulanic acid and gentamicin. Vancomycin was the most common addition to the empiric regimen. The median length of stay in the hospital was 6 days. Six out of nine centres had a protocol for early discharge in low-risk patients, most commonly on daily ceftriaxone and tobramycin intravenously. Two deaths were recorded in that study period, neither linked to an infectious aetiology.

Reporting on the Canadian experience (Boragina et al., 2007), investigators have focused on the different approach in centres regarding risk stratification and the possible management of patients in an outpatient setting. The survey included 17 centres, 14 of which did offer modified treatment for children considered low risk. The most common antibiotic regimens were a two-drug combination with an aminoglycoside and either an antipseudomonal penicillin or ceftazidime. Four centres had protocols for entirely outpatient management of patients meeting the local criteria of low risk. They used ceftriaxone $\pm$ tobramycin every 24 h. These centres report a high success rate with this approach with more than 80% of patients being successfully treated as outpatients. However, the lack of agreed consensus on the definition of low risk at presentation leads to most Canadian centres being still reluctant to use strict outpatient management. This chapter underlines the necessity to have large prospective observational studies to derive and validate low-risk criteria, followed by a multicentred clinical trial assessing alternative and traditional treatment in children.

In the following section we will review criteria that have been developed by authors in the adult and paediatric literature to try and establish a risk stratification of FN patients.

5 Defining Low-Risk and High-Risk Patients

A focus of recent attention has been the possibility of distinguishing between children at high risk of developing bacterial sepsis, where an aggressive approach is required, and those at low risk, who could be managed at home. In adults, there exists an accepted scoring system for risk prediction, and a number of studies have helped define a low-risk approach to the management of FN using the MASCC (Multinational Association for Supportive Care in Cancer) risk index

(Innes and Marshall, 2007; Klastersky et al., 2000). This validated index is based on seven independent risk factors present at the onset of FN. These include age, clinical symptoms and severity (hypotension), type of cancer, previous fungal infection, and chronic pulmonary obstructive disease. No such consensus on risk prediction has been reached in children. However, several studies have attempted to develop a risk stratification based on history, physical findings, and laboratory values. Table 1 summarizes recent studies which have looked at risk factors for serious bacterial infection in children with FN.

Other authors have tried to identify particular laboratory markers which would bring high sensitivity and specificity to the question of predicting serious bacterial infection in FN. C-reactive protein (CRP), interleukins (IL-6, IL-8), and procalcitonin (PCT) have all been used either alone or in combination to try to predict the presence or absence of sepsis. In a study of 56 children with a known malignancy who presented with fever and neutropenia, Stryjewski et al. reported that combined CTpr (PCT precursor) >500 pg/ml at 24 h combined with IL-8 >20 pg/ml at 48 h after admission predicted sepsis with 94% sensitivity and 90% specificity (Stryjewski et al., 2005). In a study involving 68 episodes of FN, investigators have reported on the superiority of IL-6 and PCT over CRP (Kitanovski et al., 2006). PCT and IL-6 had both an excellent negative predicting value of 97.3% and 95.6%, respectively, on the day after presentation with FN. Different authors have used a combination of CRP, IL-8, and monocyte chemotactic protein 1-α (MCP-1-α) measured within 24 h of the onset of fever. MCP-1-α had the best specificity (92.3%) and positive predicative value (95%) (El-Maghraby et al., 2007). It appears that the combination of two or three markers for sepsis holds some promise to help stratify risk in FN patients. However, prospective randomized studies are necessary. One unanswered question is how sensitive does a combination of tests needs to be if management is to be based on the result? In FN patients the stakes are high and both parents and doctors are likely to be reluctant to rely on laboratory results alone.

6 Outpatient Management for FN

Children who present with FN and classified as low risk for complication have been increasingly managed with early discharge, or entirely as outpatients with daily re-evaluation. This treatment philosophy has several potential advantages:

- Convenience for children and their families
- Improved quality of life
- Reduction of the incidence of nosocomial infections
- Reduction in the prolonged use of potent wide-spectrum antibiotics
- Reduction in antibiotic-related toxicity
- Reduction of the economic impact of admission to the hospital

Table 1 Studies identifying risk factors for the prediction of sepsis in children with febrile neutropenia. ANC: absolute neutrophil count, CVL: central venous line, URTI: upper respiratory tract infection, NPV: negative predictive value

Study	Number of episodes	High-risk criteria after multivariate analysis	OR (95% CI) or relative risk*	P value	Comments
(Klaassen et al., 2000)	227 (156 children) Prospective	Bone marrow disease	3.7 (1.4–9.9)	0.008	Validation of the model in 136 episodes showed an incidence of a serious infection in 12% in low risk vs. 25% in high-risk group
		Unwell on examination	2.3 (1.1–4.9)	0.030	
		ANC $<0.1 \times 10^9$/L	2.7 (1.1–6.7)	0.031	
		Peak oral temperature >39°C	2.2 (1.1–4.6)	0.033	
(Santolaya et al., 2001)	447 (257 children) Prospective	CRP > 90 mg/l	4.2* (3.6–4.8)	n/a	Invasive bacterial Infection present in 75% if three criteria, 100% if four criteria
		Hypotension	2.7* (2.3–3.2)		
		Relapse of Leukaemia	1.8* (1.7–2.3)		
		Platelets < 50,000/mm^3	1.7* (1.4–2.2)		
		Recent chemotherapy (<7 days)	1.3* (1.1–1.6)		
(Ammann et al., 2003)	285 (111 children) Retrospective	Bone marrow involvement	6.4 (2.6–15.2)	n/a	Development of a risk score based on this logistic regression model showed a NPV of 91% for the development of sepsis
		No clinical viral infection	3.0 (1.4–6.2)		
		CRP > 50 mg/l	2.4 (1.4–3.9)		
		Leukocyte count $<0.5 \times 10^9$/l	2.0 (1.3–3.0)		
		Presence of CVL	1.9 (1.0–3.6)		
		High haemoglobin level	0.6 for low Hb		
		Pre-B cell leukaemia	0.5 for other dx		
(Rondinelli et al., 2006)	283 Retrospective	Age <5 years	1.8 (1.0–3.4)	0.049	Development of a score to predict severe bacterial infection with stratification of risk of severe infection from low, intermediate (13-fold) and high (50-fold)
		CVL	2.8 (1.5–5.5)	0.001	
		Clinical focus of infection	16.6 (7.0–39.9)	0.001	
		Absence of URTI	5.1 (1.7–15)	0.001	
		Haemoglobin <7 g/dl	2.0 (1.2–3.6)	0.021	

*relative risk rather than odds ratios (OR).

In a report that describes the acceptance of outpatient therapy by doctors and families, Quezada et al. find that there are multiple barriers to the implementation of such protocols. They point out that the medical-exclusion criteria usually adopted for such treatment are stringent resulting in only between one-quarter to a third of patients being eligible for outpatient management. Social barriers such as communication issues (language), distance from the hospital, or reluctance from parents or physicians to pursue the strategy can also prevent outpatient management. Nevertheless, this practice has been increasingly popular in the last decade and is the subject of several publications, which are detailed in Table 2. Typical practice consists of a short course of observation (1–24 h) or hospitalization followed by outpatient management with either an intravenous agent such as ceftriaxone or oral ciprofloxacin, along with daily re-evaluation. Although a multicentre trial on risk stratification is necessary to further assess the safety and efficacy of outpatient management in children, it seems to be a reasonable approach at this time in a defined subset of patients at low risk for bacterial sepsis.

7 Imaging

The yield of routine chest X-rays is low in asymptomatic neutropenic patients, but an initial X-ray at presentation with FN provides a baseline to further examination (Walsh et al., 2005). It also might reveal some subtle indication of an infectious pneumonic process, which could lead to further imaging using high-resolution CT (HRCT) and possible indication for a broncho-alveolar lavage (BAL).

Patients with persistent FN are at increased risk for invasive fungal disease (IFI) and are usually started on antifungal therapy at 5–7 days of fever. In adult practice, the standard of care is now to perform a CT of the chest (+ /– sinuses) at the time of starting antifungal therapy. This practice leads to an earlier diagnosis of IFI, in particular with moulds, such as invasive pulmonary aspergillosis (IPA). In IPA, patients characteristically develop a 'halo-sign' (Fig. 1) on CT early in the first week of the disease (Caillot et al., 1997). In a study of patients with IPA, 95% of subjects had characteristic halo-sign lesions on HRCT when chest X-ray showed either normal (29%) or non-specific findings (71%) (Hauggaard et al., 2002). It is important to stress that the halo-sign is only present in the first week in IPA, and then progresses to be a non-specific infiltrate if the CT is performed at a later stage. Caillot and colleagues have also demonstrated the benefit of using early CT, along with early surgery and antifungals in IPA. Using this approach, they report a cure rate of about 84%, compared with a success rate of 40–50% usually reported in the literature (Caillot et al., 1997, 2001).

By contrast, the paediatric literature has fewer reports on the use of early CT in FN. In a retrospective review of CT in 109 episodes of prolonged FN in

Table 2 Studies describing the outpatient management of febrile neutropenic children. ANC: absolute neutrophil count, NPV: negative predictive value

Study	No. of episodes (% of total FN episodes)	Criteria for low risk	Treatment	Success rate (complete management as outpatient)
(Aquino et al., 2000) Oral ciprofloxacin	45 (28%)	>1 year malignancy in remission ANC $>0.1 \times 10^9$/l >7 days since last chemotherapy Reliable parents	Ceftazidime single dose (with observation 2–24 h), then ciprofloxacin po until afebrile for 24 h	89% success rate 5 readmissions for: non-compliance (2), herpes zoster (1), bacteriaemia (2), all uncomplicated No death
(Mullen, 2003) Oral ciprofloxacin vs. IV ceftazidime	73 (25–30%)	>2 years living <1 h away Excludes: myeloablative treatment, induction treatment, severe mucositis, dehydration, pneumonia, enterocolitis, shock	Ceftazidime single dose (observation 3–16 h), then randomized to ciprofloxacin po or ceftazidime IV. Continued until afebrile ×48 h & ANC $>0.5 \times 10^9$/l	86% success rate (No statistical difference between two groups) four episodes of uncomplicated bacteriaemia, three of which treated as outpatients No death
(Santolaya et al., 2004) Ceftriaxone + Teicoplanin IV followed by oral cefuroxime	161 (41%)	CRP <90 mg/l Normal Blood pressure Not Relapse of Leukaemia Platelets >50,000/mm3 No recent chemotherapy (<7 days)	Randomization to ambulatory vs. inpatient All received Ceftriaxone/ teicoplanin IV × 3 days with stepdown to cefuroxime oral	95% vs. 94% success rate 11 low risk episodes had invasive bacterial infection, 1 patient died in inpatient group of sepsis to pseudomonas after deterioration on day 3

Table 2 (continued)

Study	No. of episodes (% of total FN episodes)	Criteria for low risk	Treatment	Success rate (complete management as outpatient)
(Oude Nijhuis et al., 2005) No antibiotics	36 (18%) Adult and children (42%)	No clinical signs of sepsis No signs of local bacterial infection IL-8 <40–60 ng/L	No antibiotic treatment If IL-8 remains low at 24 h and remained stable, discharged home if afebrile >12 h	100% success rate No treatment failure in low risk group NPV 100%
(Petrilli et al., 2007) Oral gatifloxacin	201 (n/a)	>3 years solid tumour or leukaemia/lymphoma in remission Excludes HSCT, severe co-morbidities, poor clinical status	Gatifloxacin oral until afebrile for 2 days and ANC $>0.5 \times 10^9$/l	86.6% Success rate fever and clinical status deterioration in 12% Three episodes of bacteriaemia No death

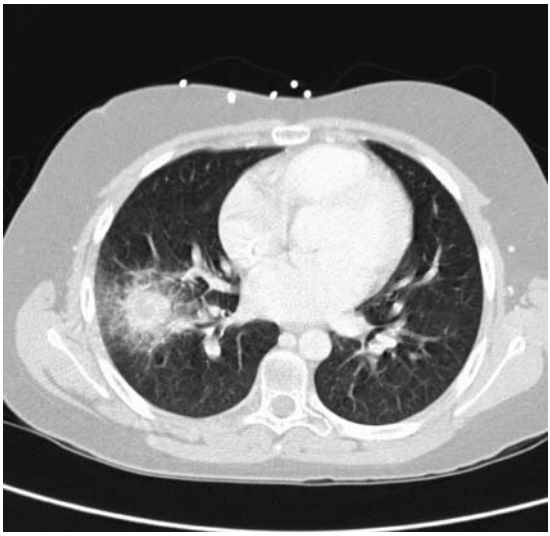

Fig. 1 CT scan of the chest in a 12-year-old patient with AML and a history of long-standing neutropenia with 5 days of fever. The lesion in the right lung displays the characteristic 'halo-sign' feature of a macronodule surrounded by an area with a ground glass appearance. The patient was treated with intravenous followed by oral voriconazole with good clinical response

paediatric patients, investigators from the University of Washington, Seattle, emphasize the diagnostic utility of performing chest CT (Archibald et al., 2001). In this review, 49% of children had CT abnormalities, which led to the modification of therapy in a third of patients. In a single institution review of 10 years of invasive aspergillosis in children, investigators from the UK document the finding on imaging in 27 patients with documented *Aspergillus* infection (Thomas et al., 2003). The X-ray finding had great variability including segmental and multilobar consolidations, perihilar infiltrates, and nodular masses. CT examinations were available in eight cases, and had been performed late in the disease. None had halo-signs and two children had presence of cavitating nodules. The authors acknowledge that this is a reflection of the late stage of the disease in which the patients were scanned in an era where CT was not so readily available. At the conclusion of their article, they recommend performing CT early (at 5–7 days of fever) in order to find the characteristic halo-sign.

We believe that the reluctance to CT high risk patients for IPA because of exposure to radiation must be balanced with the improved success rates in diagnosing and treating this illness in the early stages. Confirmation of the diagnosis by percutaneous biopsy has shown great specificity and susceptibility in paediatric patients (Hoffer et al., 2001).

8 The Role of Respiratory Viruses

Respiratory infections due to viruses are ubiquitous in children. The role played by viral infections in the context of FN is not well established. Direct immunofluorescence techniques (DFA) performed on naso-pharyngeal washings

(NPW) can identify most common respiratory viruses. This allows for a rapid and reliable diagnosis of viral URTI and has been a useful tool for paediatricians looking after immunocompetent children. Using this technique, reports have described that between 25% and 37% of patients with FN have viral respiratory infections (Arola et al., 1995; Tager et al., 2006). However, studies looking at the role played by viruses in FN are limited and no large trial has been published to date. In addition, little is known about the interaction of viral pathogens with colonizing bacteria in the respiratory tract of FN patients. New molecular diagnostic techniques now available for testing of BAL or NPW might lead to further information on the prevalence and role of viruses in FN. The usual technique to collect NPW involves flushing a small volume of saline into a nostril with the head tipped back followed by the insertion of a soft narrow bore catheter into the nasopharynx and applying suction. There is reluctance to do this in oncology patients, not least because of the fear of inducing bleeding in thrombocytopenic children. Alternative techniques of nasal swabbing or of tipping nasally inserted saline back into a paper cup have been used in patients with some degree of success (Heikkinen et al., 2002).

9 Emerging Pathogens

9.1 Viridans Group Streptococcus

VGS are alpha-haemolytic Gram positive cocci belonging that are part of the normal flora of the oral cavity, upper respiratory tract, and gastrointestinal system. In the last 15 years, VGS has become a leading cause of bacteriaemia and sepsis in the immunocompromised host, particularly in children undergoing chemotherapy for AML and post haematopoietic stem cell transplantation (HSCT). In two large multi-institutional studies of children with AML, VGS represented 22–25% of all bacteriaemic events (Gamis et al., 2000; Lehrnbecher et al., 2004). In a review of 36 cases of VGS bacteriaemia from 1991 to 2000 at St. Jude Hospital, Memphis, TN, a recrudescence of cases linked to a change of protocol using increased doses of cytosine arabinoside was noted (Okamoto et al., 2003). High-dose cytosine arabinoside has since been linked to the development of VGS sepsis by other authors (Lehrnbecher et al., 2004; Paganini et al., 2003). It is unclear if this is secondary to a direct effect of this chemotherapeutic agent or is the indirect result of the development of mucositis and the prolonged period of neutropenia induced by this agent. Patients with VGS bacteriaemia often present with septic shock and acute respiratory distress syndrome (ARDS). In the report by Okamoto et al., patients were febrile for a median of 15 days; 64% of patients were admitted to ICU, 33% experienced hypotension, and 28% had ARDS. A higher-than-expected proportion of these patients (18%) subsequently developed an IFI (one of whom died of IPA) during the same febrile episode. No patients died

primarily of VGS-associated septic shock. The prolonged duration of the fever and the associated symptoms are not clearly explained, as most of the blood cultures become rapidly negative. Some authors have suggested a role for an inflammatory response triggered by the organism leading to a degree of cytokine release dysregulation (Ihendyane et al., 2004). However, no causative toxin has been demonstrated to be present in VSSS.

The increase in VGS septic episodes has led to an increase in the use of vancomycin as part of the empiric antibiotic regimen in FN. VGS resistance to penicillin is variable reported from 4 to 14%, with intermediate susceptibility reported between 14 and 64% (Bruckner and Gigliotti, 2002). Amongst β-lactam antibiotics, ceftazidime has been reported as having the least activity in vitro, with mean MIC reported 15-folds higher than penicillin (Kennedy et al., 2001). It is therefore a reasonable proposition to start vancomycin empirically in AML patients who present with sepsis, before identification and susceptibilities are fully known.

9.2 β-Lactamase-Producing Organisms

β-Lactamase-producing organisms are an increasing concern in oncology-related infections. This occurs either through plasmid transfer, such as in extended spectrum β-lactamase-producing organisms (ESBL) or though induction of a chromosomally encoded β-lactamase such as in class C cephalosporinase (AmpC). These two mechanisms constitute the most clinically relevant mechanisms of resistance in Gram negative bacilli. ESBL are mostly found in *E. coli* and *K. pneumoniae*. In a study looking at the prevalence of ESBL in *K. pneumoniae* bloodstream infection in a children's oncology unit in Malaysia, authors describe a prevalence of ESBL-producing bacteria in up to 50% of clinical isolates (Ariffin et al., 2000). Predisposing factors were recent exposure to a third-generation cephalosporin-containing regimen and a hospital stay of 2 weeks or more. AmpC-producing Enterobacteriaciae, such as *Serretia* spp. or *Acinetobacter* spp., are inducible and can even develop resistance while the patient is on large-spectrum antibiotics. There is variability between antibiotics regarding their potential to induce this AmpC enzyme, as well as their lability to its production. First-generation cephalosporin ceftazidime and carbapenems are good inducers, but only carbapenems are not labile to the enzyme produced. The incidence of these various resistant organisms is variable from institution to institution and knowledge of local microbiological data is key in making decisions about appropriate empiric antimicrobial therapy protocols.

9.3 Vancomycin-Resistant Enterococcus

Vancomycin-resistant enterococci (VRE) have been a concern in an era where some institutions have been using vancomycin or teicoplanin as part of an

empiric regimen protocol. Other risk factors include duration of neutropenia and antibiotic therapy, with ceftazidime or amikacin in particular (Nourse et al., 1998). Outbreaks with VRE have been described in paediatric oncology units with associated deaths (Gray and George, 2000). Patient-to-patient transmission on an oncology unit is an important recognized factor in the development of outbreaks. Therefore, barrier isolation associated with a restricted used of glycopeptides have been key in decreasing colonization of patients at risk (Nourse et al., 1998; Schuster et al., 1998). The emergence of VRE also illustrates the difficulty of trading off the risk to the individual patient and society (or at least the oncology unit). A balance is required to give maximal benefit of broad-spectrum coverage to the individual and the wider benefit to the unit as a whole by limiting the use of unnecessary agents and therefore avoid the spread of organisms such as VRE.

10 Best Practice

In this final section of the chapter we will try to offer a practical way to approach patients with FN (Fig. 2). A standardized approach starts with a clear and accepted definition of fever and neutropenia. We define fever as an oral temperature of 38.3°C, or 38.0°C twice at least 1 h apart. Neutropenia is defined as an absolute neutrophil count (ANC) of less than 1000 cells/μL. Many patients are 'high risk' for the development of sepsis and invasive bacterial disease and are not eligible for outpatient therapy. Those are usually patients undergoing intense chemotherapy such as with AML on relapse protocols or post-HSCT. A recent episode of proven bacterial sepsis, expected neutropenia for more than 1 week, severe mucositis or the clinical suspicion of typhlitis also exclude patients for management as outpatients. Inclusion criteria for 'low risk' management usually include a clinically well child with viral symptoms, a CRP < 10 mg/L (other markers such as IL-8 or PCT can be used) and most importantly reliable parents with easy access to the hospital. The use of DFA on NPW samples can also help the clinician to determine if the episode has a viral aetiology. It is possible to manage low-risk patients with daily re-evaluation and intravenous ceftriaxone every 24 h (or oral ciprofloxacin) until blood cultures are negative at 48–72 h.

In moderate and high-risk patients, one approach is to admit to the hospital for therapy with a broad-spectrum agent such as piperacillin-tazobactam, cefipime, or meropenem. The addition of an aminoglycoside to the empiric regimen is still commonplace in paediatric practice, but as discussed earlier, there is now little evidence to support its use. If an aminoglycoside is used, the choice of agent depends on the local microbiological data. In institutions where *Enterococcus spp*. is a predominant pathogen, gentamicin is considered a better agent owing to its synergistic properties when given along with β-lactams. If *P. aeruginosa* infections are predominant, the use of tobramycin may be a better

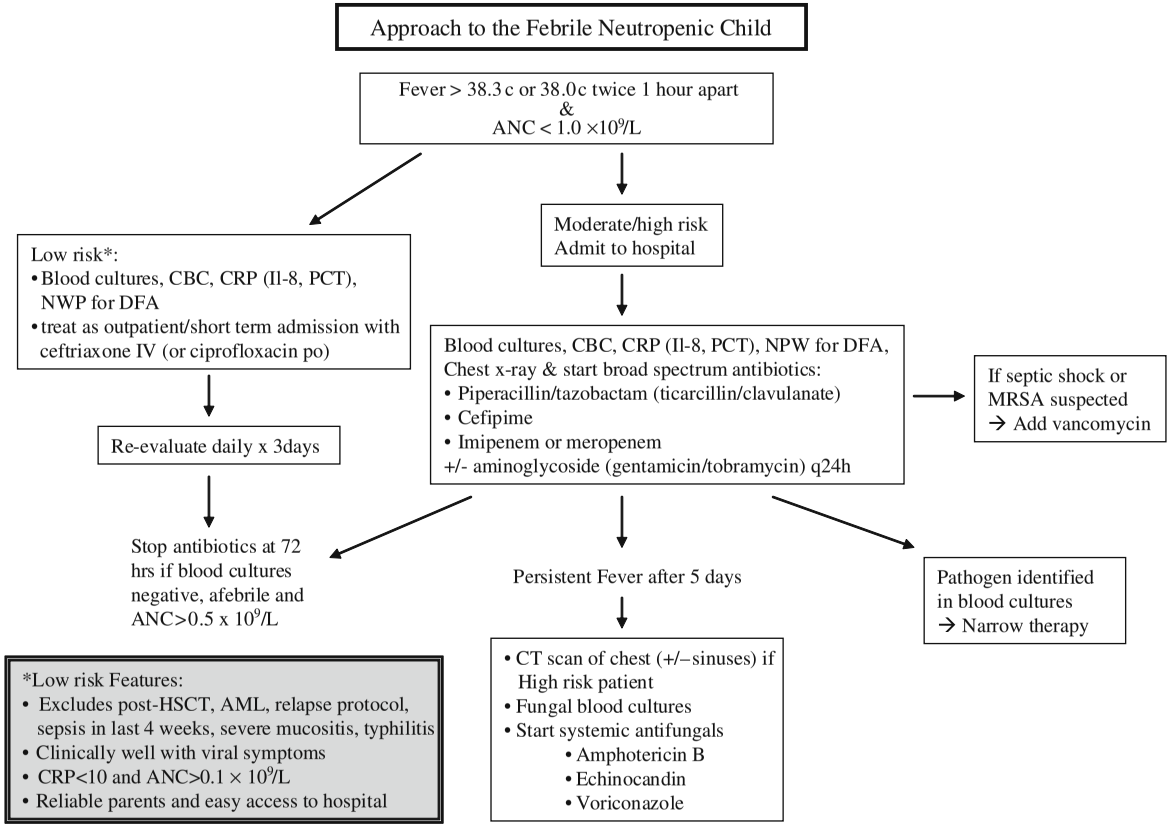

Fig. 2 General approach to the child with febrile neutropenia. ANC: absolute neutrophil count, CRP: C-reactive protein, PCT: procalcitonin, IL-8: intraleukin-8, NPW: nasopharyngeal wash

choice. In patients presenting with septic shock, especially in patients with AML (at risk for VGS sepsis), or in patients with suspected MRSA sepsis (e.g. previously colonized or with tunnel infection), the empiric addition of vancomycin is recommended.

The approach to management of suspected IFI is beyond the scope of this review. However, it is important to mention that patients with FN for 5 days or more should be assessed for the possibility of invasive fungal disease. Fungal blood culture should be drawn and a HRCT of the chest (+/– sinuses) should be performed in high risk patients (AML, ALL relapse, Post HSCT) prior to the start of antifungal therapy with either amphotericin B, a wide-spectrum triazole, or an echinocandin. Finally, it is important to remind the clinician of the necessity, upon identification of an aetiological pathogen, to review, and, if possible, narrow the spectrum of antibiotic therapy.

11 Summary

- There is a need for increased consensus in the definition of fever and neutropenia, the approach to risk stratification (including outpatient therapy and early discharge) and choices of empiric antimicrobial therapy in children.
- There has been an increased incidence of Gram positive infection in FN patients, in particular with VGS in patient with AML. However, Gram negative bacteria are still responsible for most of the mortality associated with FN.
- Piperacillin/tazobactam, cefipime, or meropenem are all effective first-choice antimicrobial monotherapy in FN. There is no good evidence for adding an aminoglycoside compound to the initial empiric therapy regimen.
- Following local microbiological data is of utmost importance in choosing the right empiric antimicrobial regimen for a particular institution.
- Outpatient management of a well-defined subset of low-risk patient for bacterial invasive infection with intravenous ceftriaxone or oral ciprofloxacin and daily re-evaluation is possible.
- Early CT of the chest (after 5–7 days of FN) in high-risk patients is essential to make a prompt diagnosis of pulmonary aspergillosis and improve outcome.

References

Agaoglu L., Devecioglu O., Anak S., Karakas Z., Yalman N., Biner B., Eryilmaz E., Goksan B., Unuvar A., Agirbasli H., Can M., Bilgen H.,Gedikoglu G. (2001) Cost-effectiveness of cefepime plus netilmicin or ceftazidime plus amikacin or meropenem monotherapy in febrile neutropenic children with malignancy in Turkey. *J Chemother* **13**: 281–287

Ammann R. A., Aebi C., Hirt A., Luthy A. R. (2004) Fever in neutropenia in children and adolescents: evolution over time of main characteristics in a single center, 1993–2001. *Support Care Cancer* **12**: 826–832

Ammann R. A., Hirt A., Luthy A. R., Aebi C. (2003) Identification of children presenting with fever in chemotherapy-induced neutropenia at low risk for severe bacterial infection. *Med Pediatr Oncol* **41**: 436–443

Aquino V. M., Herrera L., Sandler E. S., Buchanan G. R. (2000) Feasibility of oral ciprofloxacin for the outpatient management of febrile neutropenia in selected children with cancer. *Cancer* **88**: 1710–1714

Archibald S., Park J., Geyer J. R., Hawkins D. S. (2001) Computed tomography in the evaluation of febrile neutropenic pediatric oncology patients. *Pediatr Infect Dis J* **20**: 5–10

Ariffin H., Ai C. L., Lee C. L., Abdullah W. A. (2006) Cefepime monotherapy for treatment of febrile neutropenia in children. *J Paediatr Child Health* **42**: 781–784

Ariffin H., Arasu A., Mahfuzah M., Ariffin W. A., Chan L. L., Lin H. P. (2001) Single-daily ceftriaxone plus amikacin versus thrice-daily ceftazidime plus amikacin as empirical treatment of febrile neutropenia in children with cancer. *J Paediatr Child Health* **37**: 38–43

Ariffin H., Navaratnam P., Mohamed M., Arasu A., Abdullah W. A., Lee C. L., Peng L. H. (2000) Ceftazidime-resistant Klebsiella pneumoniae bloodstream infection in children with febrile neutropenia. *Int J Infect Dis* **4**: 21–25

Arola M., Ruuskanen O., Ziegler T., Salmi T. T. (1995) Respiratory virus infections during anticancer treatment in children. *Pediatr Infect Dis J* **14**: 690–694

Blondell-Hill E., Bug and Drugs (4th Ed.), p. 37, Capital Health, Edmonton, AB (2006)

Boragina M., Patel H., Reiter S., Dougherty G. (2007) Management of febrile neutropenia in pediatric oncology patients: A Canadian survey. *Pediatr Blood Cancer* **48**: 521–526

Bruckner L., Gigliotti F. (2002) Alpha-hemolytic streptococcal infections among immunocompromised hosts: Increasing incidence, severity and antibiotic resistance. *Pediatr Infect Dis J* **21**: 343–345

Caillot D., Casasnovas O., Bernard A., Couaillier J. F., Durand C., Cuisenier B., Solary E., Piard F., Petrella T., Bonnin A., Couillault G., Dumas M., Guy H. (1997) Improved management of invasive pulmonary aspergillosis in neutropenic patients using early thoracic computed tomographic scan and surgery. *J Clin Oncol* **15**: 139–147

Caillot D., Couaillier J. F., Bernard A., Casasnovas O., Denning D. W., Mannone L., Lopez J., Couillault G., Piard F., Vagner O., Guy H. (2001) Increasing volume and changing characteristics of invasive pulmonary aspergillosis on sequential thoracic computed tomography scans in patients with neutropenia. *J Clin Oncol* **19**: 253–259

Chamberlain J. D., Smibert E., Skeen J., Alvaro F. (2005) Prospective audit of treatment of paediatric febrile neutropenia in Australasia. *J Paediatr Child Health* **41**: 598–603

Chambers H.; "Other B-Lactams Antibiotics" in Principles and Practice of Infectious Diseases p. 313, Elsevier Churchill Livingstone (2005)

Charnas R., Luthi A. R., Ruch W. (1997) Once daily ceftriaxone plus amikacin vs. three times daily ceftazidime plus amikacin for treatment of febrile neutropenic children with cancer. Writing Committee for the International Collaboration on Antimicrobial Treatment of Febrile Neutropenia in Children. *Pediatr Infect Dis J* **16**: 346–353

Chastagner P., Plouvier E., Eyer D., Plesiat P., Lozniewski A., Sommelet D. (2000) Efficacy of cefepime and amikacin in the empiric treatment of febrile neutropenic children with cancer. *Med Pediatr Oncol* **34**: 306–308

Cometta A., Calandra T., Gaya H., Zinner S. H., de Bock R., Del Favero A., Bucaneve G., Crokaert F., Kern W. V., Klastersky J., Langenaeken I., Micozzi A., Padmos A., Paesmans M., Viscoli C., Glauser M. P. (1996) Monotherapy with meropenem versus combination therapy with ceftazidime plus amikacin as empiric therapy for fever in granulocytopenic patients with cancer. The International Antimicrobial Therapy Cooperative Group of the European Organization for Research and Treatment of Cancer and

the Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto Infection Program. *Antimicrob Agents Chemother* **40**: 1108–1115

Connell T. G., Rele M., Cowley D., Buttery J. P., Curtis N. (2007) How reliable is a negative blood culture result? Volume of blood submitted for culture in routine practice in a children's hospital. *Pediatrics* **119**: 891–896

Corapcioglu F., Sarper N. (2005) Cefepime versus ceftazidime plus amikacin as empirical therapy for febrile neutropenia in children with cancer: A prospective randomized trial of the treatment efficacy and cost. *Pediatr Hematol Oncol* **22**: 59–70

Corapcioglu F., Sarper N., Zengin E. (2006) Monotherapy with piperacillin/tazobactam versus cefepime as empirical therapy for febrile neutropenia in pediatric cancer patients: A randomized comparison. *Pediatr Hematol Oncol* **23**: 177–186

Duzova A., Kutluk T., Kanra G., Buyukpamukcu M., Akyuz C., Secmeer G., Ceyhan M. (2001) Monotherapy with meropenem versus combination therapy with piperacillin plus amikacin as empiric therapy for neutropenic fever in children with lymphoma and solid tumors. *Turk J Pediatr* **43**: 105–109

El-Maghraby S. M., Moneer M. M., Ismail M. M., Shalaby L. M., El-Mahallawy H. A. (2007) The diagnostic value of c-reactive protein, interleukin-8. and monocyte chemotactic protein in risk stratification of febrile neutropenic children with Hematologic malignancies. *J. Pediatr Hematol Oncol* **29**: 131–136

Fleischhack G., Hartmann C., Simon A., Wulff B., Havers W., Marklein G., Hasan C., Bode U. (2001) Meropenem versus ceftazidime as empirical monotherapy in febrile neutropenia of paediatric patients with cancer. *J Antimicrob Chemother* **47**: 841–853

Fouyssac F., Salmon A., Mansuy L., Schmitt C., Bordigoni P., Chastagner P. (2005) Treatment of febrile neutropenia episodes in children, with a piperacillin-tazobactam and netilmicin combination. *Medecine Et Maladies Infectieuses* **35**: 357–362

Gamis A. S., Howells W. B., DeSwarte-Wallace J., Feusner J. H., Buckley J. D., Woods W. G. (2000) Alpha hemolytic streptococcal infection during intensive treatment for acute myeloid leukemia: a report from the Children's cancer group study CCG-2891. *J Clin Oncol* **18**: 1845–1855

Glasmacher A., von Lilienfeld-Toal M., Schulte S., Hahn C., Schmidt-Wolf I. G., Prentice A. (2005) An evidence-based evaluation of important aspects of empirical antibiotic therapy in febrile neutropenic patients. *Clin Microbiol Infect* **11 Suppl 5**: 17–23

Granowetter L., Wells H., Lange B. J. (1988) Ceftazidime with or without vancomycin vs. cephalothin, carbenicillin and gentamicin as the initial therapy of the febrile neutropenic pediatric cancer patient. *Pediatr Infect Dis J* **7**: 165–170

Gray J. W., George R. H. (2000) Experience of vancomycin-resistant enterococci in a children's hospital. *J Hosp Infect* **45**: 11–18

Hann I., Viscoli C., Paesmans M., Gaya H., Glauser M. (1997) A comparison of outcome from febrile neutropenic episodes in children compared with adults: results from four EORTC studies. International Antimicrobial Therapy Cooperative Group (IATCG) of the European Organization for Research and Treatment of Cancer (EORTC). *Br J Haematol* **99**: 580–588

Hauggaard A., Ellis M., Ekelund L. (2002) Early chest radiography and CT in the diagnosis, management and outcome of invasive pulmonary aspergillosis. *Acta Radiol* **43**: 292–298

Heikkinen T., Marttila J., Salmi A. A., Ruuskanen O. (2002) Nasal swab versus nasopharyngeal aspirate for isolation of respiratory viruses. *J Clin Microbiol* **40**: 4337–4339

Hoffer F. A., Gow K., Flynn P. M., Davidoff A. (2001) Accuracy of percutaneous lung biopsy for invasive pulmonary aspergillosis. *Pediatr Radiol* **31**: 144–152

Hughes W. T., Armstrong D., Bodey G. P., Bow E. J., Brown A. E., Calandra T., Feld R., Pizzo P. A., Rolston K. V. I., Shenep J. L.,Young L. S. (2002) 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer. *Clin. Infect. Dis.* **34**: 730–751

Hung K. C., Chiu H. H., Tseng Y. C., Wang J. H., Lin H. C., Tsai F. J., Peng C. T. (2003) Monotherapy with meropenem versus combination therapy with ceftazidime plus

amikacin as empirical therapy for neutropenic fever in children with malignancy. *J Microbiol Immunol Infect* **36**: 254–259

Ihendyane N., Sparrelid E., Wretlind B., Remberger M., Andersson J., Ljungman P., Ringden O., Normark B. H., Allen U., Low D. E., Norrby-Teglund A. (2004) Viridans streptococcal septicaemia in neutropenic patients: role of proinflammatory cytokines. *Bone Marrow Transplant* **33**: 79–85

Innes H., Marshall E. (2007) Outpatient therapy for febrile neutropenia. *Curr Opin Oncol* **19**: 294–298

Kennedy H. F., Gemmell C. G., Bagg J., Gibson B. E., Michie J. R. (2001) Antimicrobial susceptibility of blood culture isolates of viridans streptococci: relationship to a change in empirical antibiotic therapy in febrile neutropenia. *J Antimicrob Chemother* **47**: 693–696

Kitanovski L., Jazbec J., Hojker S., Gubina M., Derganc M. (2006) Diagnostic accuracy of procalcitonin and interleukin-6 values for predicting bacteremia and clinical sepsis in febrile neutropenic children with cancer. *Eur J Clin Microbiol Infect Dis* **25**: 413–415

Klaassen R. J., Goodman T. R., Pham B., Doyle J. J. (2000) "Low-risk" prediction rule for pediatric oncology patients presenting with fever and neutropenia. *J Clin Oncol* **18**: 1012–1019

Klastersky J., Paesmans M., Rubenstein E. B., Boyer M., Elting L., Feld R., Gallagher J., Herrstedt J., Rapoport B., Rolston K., Talcott J. (2000) The Multinational Association for Supportive Care in Cancer risk index: A multinational scoring system for identifying low-risk febrile neutropenic cancer patients. *J Clin Oncol* **18**: 3038–3051

Le Guyader N., Auvrignon A., Vu-Thien H., Portier E., Tabone M. D., Leverger G. (2004) Piperacillin-tazobactam and netilmicin as a safe and efficacious empirical treatment of febrile neutropenic children. *Support Care Cancer* **12**: 720–724

Lehrnbecher T., Varwig D., Kaiser J., Reinhardt D., Klingebiel T., Creutzig U. (2004) Infectious complications in pediatric acute myeloid leukemia: analysis of the prospective multi-institutional clinical trial AML-BFM 93. *Leukemia* **18**: 72–77

Mullen C. A. (2003) Ciprofloxacin in treatment of fever and neutropenia in pediatric cancer patients. *Pediatric Infectious Disease Journal* **22**: 1138–1142

Nourse C., Murphy H., Byrne C., O'Meara A., Breatnach F., Kaufmann M., Clarke A., Butler K. (1998) Control of a nosocomial outbreak of vancomycin resistant Enterococcus faecium in a paediatric oncology unit: risk factors for colonisation. *Eur J Pediatr* **157**: 20–27

Okamoto Y., Ribeiro R. C., Srivastava D. K., Shenep J. L., Pui C. H., Razzouk B. I. (2003) Viridans streptococcal sepsis: clinical features and complications in childhood acute myeloid leukemia. *J Pediatr Hematol Oncol* **25**: 696–703

Oude Nijhuis C., Kamps W. A., Daenen S. M., Gietema J. A., van der Graaf W. T., Groen H. J., Vellenga E., Ten Vergert E. M., Vermeulen K. M., de Vries-Hospers H. G., de Bont E. S. (2005) Feasibility of withholding antibiotics in selected febrile neutropenic cancer patients. *J Clin Oncol* **23**: 7437–7444

Owens R. C., Owens C. A., Holloway W. J. (2000) Reduction in Vancomycin consumption in patients with fever and neutropenia [abstract 458]. *Clin. Infect. Dis.* **31**: 291

Paganini H., Staffolani V., Zubizarreta P., Casimir L., Lopardo H., Luppino V. (2003) Viridans streptococci bacteraemia in children with fever and neutropenia: a case-control study of predisposing factors. *Eur J Cancer* **39**: 1284–1289

Paul M., Soares-Weiser K., Grozinsky S., Leibovici L. (2003) Beta-lactam versus beta-lactam-aminoglycoside combination therapy in cancer patients with neutropaenia. *Cochrane Database Syst Rev* **3**: CD003038

Paulus S. C., van Saene H. K. F., Hemsworth S., Hughes J., Ng A., Pizer B. L. (2005) A prospective study of septicaemia on a paediatric oncology unit: A three-year experience at The Royal Liverpool Children's Hospital, Alder Hey, UK. *Eur J Cancer* **41**: 2132–2140

Petrilli A., Altruda Carlesse F., Alberto Pires Pereira C. (2007) Oral gatifloxacin in the outpatient treatment of children with cancer fever and neutropenia. *Pediatr Blood Cancer* **49**: 682–686

Phillips B., Selwood K., Lane S. M., Skinner R., Gibson F., Chisholm J. C. (2007) Variation in policies for the management of febrile neutropenia in United Kingdom Children's Cancer Study Group centres. *Arch Dis Child* **92**: 495–498

Phillips R., Skinner R., Chisholm J. C. (2007) Treating low-risk febrile neutropenia: Jenny's story. *Arch Dis Child* **92**: 7–8

Rondinelli P. I. P., Ribeiro K. D. B., de Camargo B. (2006) A proposed score for predicting severe infection complications in children with chemotherapy-induced febrile neutropenia. *J Pediatr Hematol Oncol* **28**: 665–670

Santolaya M. E., Alvarez A. M., Aviles C. L., Becker A., Cofre J., Cumsille M. A., O'Ryan M. L., Paya E., Salgado C., Silva P., Tordecilla J., Varas M., Villarroel M., Viviani T., Zubieta M. (2004) Early hospital discharge followed by outpatient management versus continued hospitalization of children with cancer, fever, and neutropenia at low risk for invasive bacterial infection. *J Clin Oncol* **22**: 3784–3789

Santolaya M. E., Alvarez A. M., Becker A., Cofre J., Enriquez N., O'Ryan M., Paya E., Pilorget J., Salgado C., Tordecilla J., Varas M., Villarroel M., Viviani T., Zubieta M. (2001) Prospective, multicenter evaluation of risk factors associated with invasive bacterial infection in children with cancer, neutropenia, and fever. *J Clin Oncol* **19**: 3415–3421

Schuster F., Graubner U. B., Schmid I., Weiss M., Belohradsky B. H. (1998) Vancomycin-resistant-enterococci – colonization of 24 patients on a pediatric oncology unit. *Klin Padiatr* **210**: 261–263

Stryjewski G. R., Nylen E. S., Bell M. J., Snider R. H., Becker K. L., Wu A., Lawlor C., Dalton H. (2005) Interleukin-6, interleukin-8, and a rapid and sensitive assay for calcitonin precursors for the determination of bacterial sepsis in febrile neutropenic children. *Pediatr Crit Care Med* **6**: 129–135

Tager F. M., Zolezzi R. P., Folatre B. I., Navarrete C. M., Rojas P. J. (2006) Respiratory virus infections in children with acute lymphoblastic leukemia and febrile neutropenia: a prospective study. *Rev Chilena Infectol* **23**: 118–123

Thomas K. E., Owens C. M., Veys P. A., Novelli V., Costoli V. (2003) The radiological spectrum of invasive aspergillosis in children: a 10-year review. *Pediatr Radiol* **33**: 453–460

Viscoli C., Castagnola E. "Prophylaxis and Empirical Therapy for Infection in Cancer Patients" in Principles and Practice of Infectious Diseases (6th Ed), pp. 3442–3462, Elsevier Churchill Livingstone (2005).

Walsh T. J., Groll, A., Gonzalez C., Pizzo, P. "Infectious Complications in Pediatric Cancer Patients" in Principles and Practice of Pediatric Oncology (5th Ed.), pp. 1269–1329, Lippincott Williams & Wilkins (2005)

The PANDAS Syndrome

Michael E. Pichichero

1 Introduction

PANDAS is an acronym for Pediatric Autoimmune Neurologic Disorders Associated with Streptococcus coined by Swedo et al. (1998) to describe a syndrome of symptoms to include a variable combination of obsessions, compulsions, tics, hyperactivity, inattention, and mild choreiform movements (Table 1). These symptoms affect children with a fluctuating course following Group A Streptococcal (GAS) tonsillo-pharyngitis and may reach sufficient severity to qualify for a diagnosis of obsessive–compulsive disorder (OCD), Tourette syndrome (TS), chronic tic disorder, attention-deficit/hyperactivity disorder (ADHD), or other diagnostic entities. Although estimating the frequency of PANDAS is difficult, some investigators believe that it may account for 10% or more of cases of childhood-onset OCD (Giuliano et al., 1998) and tic/TS (Cardona and Orefici, 2001). Growing clinical interest has spurred attempts to better define the PANDAS syndrome and develop new treatments for PANDAS. In this chapter, the author will give a historical overview of the syndrome, describe the proposed pathogenesis, heritability, neuroimaging findings, clinical presentation and differential diagnosis, treatment, and provide a perspective to evaluate new data generated on this topic.

2 Historical Overview

Delineation of the PANDAS syndrome grew initially from the observed similarities between the symptoms of TS or OCD and the psychiatric and neurologic symptoms associated with Sydenham's chorea (SC), a sequela of acute rheumatic fever (ARF). ARF is an autoimmune disorder following GAS infection that is characterized by carditis, arthritis, SC, erythema

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Table 1 PANDAS

Pediatric Autoimmune Neuropsychiatric Disorder Associated with Group A Streptococci
• Presence of obsessive–compulsive disorder (OCD) and/or tic disorder
• Abrupt onset and episodic course of symptoms
• Association with GAS infections
• May include neurological abnormalities (motor hyperactivity, subtle choreiform movements)
• Autoimmunity to basal ganglia of brain in longstanding cases (similar to Sydenham's chorea)

Swedo (1998, 2004).

marginatum, or subcutaneous nodules. SC may be considered a symptom of ARF (the American Heart Association Jones Criteria includes it as a major manifestation), a form of ARF (because SC occurring alone is sufficient for a diagnosis of ARF), or as an alternative manifestation of poststreptococcal disease.

That ARF is caused by GAS infection was controversial when first suggested over a century ago (Cunningham, 2000). Clinical observations and epidemiologic studies increasingly supported the association of GAS infection with the subsequent development of ARF (Stollerman, 1997). In addition, GAS antigens such as M proteins were shown to cross-react with human heart and connective tissues, and antistreptococcal antibodies were found during episodes of rheumatic fever (Kaplan, 1965). Largely on the basis of these epidemiological and experimental data, GAS infection is accepted as a cause of ARF.

In 1989, Swedo noted a high prevalence of OCD in children seen at the US National Institutes of Mental Heath (NIMH) who had SC (Swedo et al., 1989). This was actually a rediscovery of observations made earlier in the twentieth century (Chapman, Pilkey, and Gibbons, 1958). Interest grew further in 1992 when Swedo presented an abstract at the American Society of Psychiatry annual meeting and later published a paper linking OCD, tics, and GAS (Swedo, Leonard, and Kiessling, 1994). In 1993, a group working at a neurodevelopmental assessment clinic in Rhode Island noted a temporal relationship between a surge in GAS infections and a surge in the number of children presenting to their clinic with new onset of tics. Serum samples were obtained from the children and an evaluation was made to detect serum antibodies directed to human caudate nucleus tissue sections from cadavers. The percentage of children with tics whose sera was strongly positive for antineuronal antibodies (44%) was very similar to that previously found in children with SC (46%) (Kiessling, Marcotte, and Culpepper, 1993). Swedo and others extended these observations over the next several years by drawing attention to the frequent overlap of symptoms among patients with SC, OCD, and TS (Swedo et al., 1989; Allen, Leonard, and Swedo, 1995) and similarities in pathogenesis involving the basal ganglia in the brain (Peterson, 1995; Giedd et al., 1996; Klieger et al., 1997; O'Sullivan et al., 1997). In 1998, Swedo et al. described a

Table 2 PANDAS historical perspective

- 1998: Sue Swedo describes high prevalence of OCD in kids with Sydenham's chorea
- 1992: Swedo presents an abstract at Am Soc Psychiatry meetings linking OCD, tics, and GAS.
- 1993: Outbreak of GAS tonsillitis in Rhode Island associated with 10-fold increase in kids with tics—concept of poststrep tics is born.
- 1994: Swedo links Sydenham's to autoimmune neuropsychiatric disorders (OCD, tics).
- 1997: Swedo describes the first 50 cases of a new syndrome she calls PANDAS.

Swedo et al. (1989), Kiessling (1994), Swedo et al. (1994), Swedo et al. (1998).

series of 50 cases of acute onset OCD and tic disorders immediately preceded by GAS infections; therein, the PANDAS syndrome was born (Swedo et al., 1998) (Table 2).

3 Pathogenesis

Infection with GAS produces neuropsychiatric sequelae through molecular mimicry. Molecular mimicry involves the production of antibodies by the host that are directed against antigenic epitopes of the bacteria that are also shared by the host's tissues. In rheumatic fever, antigenic epitopes present in connective tissue and cardiac myosin cross-react with M proteins located within the bacterial wall of GAS. This cross-reaction is thought to misdirect the host's immune system to attack the host's own myocardium and connective tissues, just as it attacks the invading GAS bacteria. Antigenic similarities have also been identified between GAS proteins and brain tissue, which may account for the cross-reactivity reported between GAS antigens and caudate, putamen, thalamus, and subthalamic nuclei within the brain (Singer et al., 1998; Trifiletti et al., 1998; Trifiletti, 1998; Husby et al., 1976). Thus, similar to the proposed pathogenesis of myocarditis in ARF, streptococcal antigens may mimic human brain tissue antigens. This mimicry may thereby induce the production of cross-reactive auto-antibodies that attack brain tissues in addition to the GAS bacterium.

In support of an auto-antibody pathogenesis, serum IgG from a subset of TS patients has been reported to produce motor and vocal stereotypies when infused into rodents (Hallett et al., 2000). Some patients with OCD and tic symptoms have antibodies directed against human putamen and caudate antigens (Kiessling, Marcotte, and Culpepper, 1994; Church et al., 2003; Church, Dale and Giovannoni, 2004; Morshed et al., 2001; Dale et al., 2005). Antibodies from PANDAS patients have been shown to react with the neuronal cell surface of the basal ganglia and induce calcium–calmodulin-dependent protein kinase II activity in neuronal cells (Kirvan et al., 2006). Depletion of serum IgG abrogated CaM kinase II cell signaling and reactivity of CSF was blocked by streptococcal antigen *N*-acetyl-beta-D-glucosamine (GlcNAc) (Kirvan et al.,

2006). Antibodies against GlcNAc in PANDAS sera were inhibited by lysoganglioside G M1. (Kirvan et al., 2006). Dale et al. (2006) showed that neuronal surface glycolytic enzymes are autoantigen targets in poststreptococcal autoimmune CNS disease. The autoantigens were neuronal glycolytic enzymes—NGE (pyruvate kinase M1, aldolase C, neuronal-specified, and non-neuronal enolase). These are multifunctional proteins that are all expressed intracellularly and on the neuronal cell surface. On the neuronal plasma membrane, NGEs are involved in energy metabolism, cell signaling, and synaptic neurotransmission. In vitro experiments using cultured neurons showed that commercial anti-NGE antibodies induced apoptosis (Dale et al., 2006). GAS also expresses glycolytic enzymes on cell surfaces that have 0–49% identity with human NGE, in support of the notion that molecular mimicry and autoimmune cross-reactivity may be the pathogenic mechanism in poststreptococcal CNS disease.

Our group identified a group of 12 children with a first episode of PANDAS seen in a community-based practice. Prompt antibiotic treatment appeared to result in rapid disappearance of all PANDAS-associated symptoms (Murphy and Pichichero, 2002). GAS produce many toxins and it is well known that bacterial toxins can bind to gangliosides that are abundantly present on neurons. Thus we speculated that sentinel episodes of PANDAS may be toxin mediated whereas long-standing and recurrent cases may be due to autoantibodies.

4 Heritability

Data concerning the genetics of PANDAS come from a family association (Lougee et al., 2000) and a biological marker study (Swedo et al., 1997). The family association study reported that 67% of PANDAS patients had at least one relative with OCD, subclinical OCD, obsessive–compulsive personality disorder, or tic disorder (Lougee et al., 2000). An increased occurrence of B lymphocyte antigen D 8/17 has been reported to occur in patients with PANDAS (Swedo et al., 1997).

5 Neuroimaging

Several case studies have suggested the presence of tic or OCD symptoms associated with radiographic evidence of edema and swelling of the basal ganglia nuclei following a GAS infection (Murphy et al., 1997; Lin et al., 2002; Kienzle et al., 1991; Traill, Pike and Byrne, 1995; Giedd et al., 1995; Peterson et al., 2000), similar to the radiographic findings reported in Sydenham's chorea. Two large, controlled studies showed the presence enlarged basal ganglia nuclei

associated with either PANDAS (Murphy et al., 2000) or with serological evidence of recent infection with GAS (Peterson et al., 2000).

6 Clinical Diagnosis

The typical patient with PANDAS is a child between 5 and 12 years of age who presents with the acute onset of OCD or tics. Acute onset is a critical distinguishing feature of PANDAS. Usually the parent can pinpoint the onset of symptoms to an exact day and hour. The abrupt onset is the key because the typical child with OCD or tic/TS has a gradual escalating onset of symptoms, often not reaching a degree of severity for several years when medical attention is sought. PANDAS patients may have very mild choreiform (very mild piano playing like) hand movements, or they may suddenly become motorically clumsy or experience sudden deterioration in the quality of their handwriting. PANDAS patients often exhibit behavioral symptoms commonly seen in patients with Sydenham's chorea, including emotional lability, depression, irritability, anxiety, motoric hyperactivity, distractibility, or impulsivity. PANDAS patients frequently have comorbidities (Table 3).

Obsessive thoughts and compulsive behaviors in PANDAS patients often meet diagnostic criteria for OCD. The OCD symptoms occurring in PANDAS patients are the same as childhood-onset OCD that is not associated with PANDAS and, similar to childhood-onset OCD, they often co-occur with a tic disorder. A common obsession in our series of sentinel episodes of PANDAS was obsession with urination followed by hand washing (Murphy and Pichichero, 2002). Importantly the urinary frequency was not associated with dysuria, nocturia, or fever and always was associated with a normal urinalysis. The urinary frequency obsession of PANDAS is distinguished from pollakuria by the severity of its intrusiveness to the patient's daily life.

Two recent case control studies have found an association with acute onset of tics and GABHS infections (Cardona and Orefici, 2001; Mell, Davis, and Ownes, 2005). However, tics are common symptoms in the general population, affecting up to 24% of all children (Snider et al., 2002). Tics occur in clusters or

Table 3 PANDAS comorbid symptoms

- Emotional lability (66%)
- Separation anxiety (46%)
- Night-time fears and bedtime rituals (50%)
- Cognitive deficits
  - Deterioration in school performance (60%)
  - Deterioration in math skills (26%)
- Comorbid symptoms always started abruptly, began at the same time as OCD symptoms or tics, began or worsened and were also associated with elevated antistreptococcal antibody titers

Swedo (1998, 2004).

bouts and wax and wane in severity. Motor tics most commonly affect musculature of the face, neck, and shoulders. Common vocal tics include throat clearing, sniffing, grunting, peeping, or popping noises. Chronic tic symptoms on average tend to worsen in children until the age of 11 or 12, before declining gradually through adolescence. Complex tics may be difficult to distinguish from the symptoms of OCD.

7 Laboratory Testing

Reliably distinguishing PANDAS from OCD or tic/TS is difficult because the protean clinical manifestations of the syndrome and these other conditions are identical. Therefore, at this time to meet the working diagnostic criteria an association of symptoms with current or recent GAS infection is essential. Appropriate GAS laboratory tests include rapid antigen detection tests or cultures of the throat, nose, skin, or anus if erythema or other evidence of infection is present.

Although the preferred method of diagnosing GAS infection is with a positive rapid test or culture in patients who have pharyngitis, the recovery of GAS is confounded by the fact that a small proportion of children not actively infected but only colonized will be identified.

An alternative to cultures are serological studies. Serum antibody titers to streptococcal antigens usually rise during convalescence from an acute infection. Antistreptolysin O (ASO) titers rise within 1 week of infection peaking at 2–4 weeks, and persisting for 2–3 months. AntiDNase-B antibody titers rise in 2–3 weeks, peak in 4–6 weeks, and persist for 4–6 months. The precise upper limits for a normal ASO titer depends on the particular laboratory where the assay is run and the age of the child, but typically is around 200 IU/ml and 400 IU/ml for ASO and anti-DNase, respectively. Antibody titers must be evaluated carefully, however, because both ASO and anti-DNase-B titers are frequently elevated in school-aged children (Kaplan, Rothermel and Johnson, 1998).

8 Treatment

PANDAS symptoms by definition may worsen following streptococcal infection. This has naturally led to the use of antibiotics in PANDAS. The data to support penicillin prophylaxis are lacking. A double-blind study of 37 PANDAS patients treated with oral penicillin V failed to demonstrate statistically significant improvement of tic or OCD symptoms associated with the syndrome (Garvey et al., 1999). However, antibiotic prophylaxis in this study did not significantly decrease the frequency of streptococcal infections in the active treatment group, so a benefit could not be anticipated. A subsequent study

using more effective antibiotic prophylaxis with azithromycin did demonstrate an effect on OCD or tic symptoms in a group of PANDAS patients (Snider et al., 2005). There is a single case report suggesting that symptoms in a PANDAS patient with recurrent streptococcal pharyngitis may have improved following tonsillectomy (Orvidas and Slattery, 2001).

Plasma exchange and intravenous immunoglobulin (IVIG) have been tried to remove potentially deleterious circulating auto-antibodies in PANDAS patients (Perlmutter et al., 1999). In that study of 30 carefully selected PANDAS patients, both plasma exchange and IVIG when compared with sham IVIG or untreated patients reduced symptoms 1 month and 1 year after treatment. OCD symptoms seemed to be more responsive than were tics, and plasma exchange appeared to be more effective than IVIG. The results have prompted Swedo and others to suggest that such treatments may only be effective in patients who have documented exacerbations following streptococcal infections, but they are not proven therapies and should only be used in carefully selected cases under a research protocol.

If a clinical history is suggestive of PANDAS, throat culture and serum antibody titers should be obtained at the time of presentation and during the course of therapy, and subsequently at times of acute exacerbation of OCD and tics behaviors and during an episode of pharyngitis. If evidence for a current or recent infection with GAS is found (using throat cultures or antibody titers, respectively), and the patient is symptomatic (i.e., had a pharyngitis, a sinusitis, or an acute exacerbation of tic or OCD symptoms), then an empirical trial of antibiotic therapy should be considered (Murphy et Pichichero, 2002).

9 Perspective on the PANDAS Construct

PANDAS is not yet a validated nosological construct (Table 4). Tic and OCD symptoms putatively associated with PANDAS are in effect distinguished from tic and OCD symptoms not associated with PANDAS only by virtue of an acute onset of symptoms in temporal proximity to GAS infection. Acute onset of tic or OCD symptoms is not uncommon in the absence of GAS infection. The rates of OCD and tic disorders reported in first-degree relatives of PANDAS patients, however, are similar to those observed in family studies of individuals with tics or childhood-onset OCD who presumably do not have PANDAS (Giedd et al., 1996; Rasmussen and Tsuang, 1986; Pauls et al., 1991; Pauls, 2001). Moreover, the percentage of children in the general population who have either a positive GAS throat culture and are silent carriers and/or who have elevated antistreptococcal antibodies (about 20%), depending upon the criteria established for normal values at any given laboratory, at any time in temporal cross-section is substantial. Therefore, the likelihood of a child with tics or OCD also having evidence of recent contact or infection with GAS, simply on the basis of chance alone, is not small (Luo et al., 2004).

Table 4 The PANDAS controversy

Criteria	Unproved Hypothesis	Bona Fide Clinical Entity
1.	Proposed age at onset may be too arbitrary and only reflects age range of original 50 cases	Symptoms were limited to OCD and/or tics to establish a patient cohort for research studies; systematic studies of possible relationship of GAS to other neurologic symptoms have not been done
2.	Clinical features may not be limited to OCD and/or tics; symptom severity required for diagnosis not yet defined	Symptoms were limited to OCD and/or tics to establish a patient cohort for research studies; systematic studies of possible relationship of GAS to other neurologic symptoms have not been done
3.	Whether or not clinical course of abrupt onset or dramatic exacerbation is specific to PANDAS remains to be established	Children with PANDAS have 'an overnight explosion' of COD symptoms; those with non-PANDAS OCD have a slow, gradual symptom onset
4.	Question temporal relationship of symptom onset or exacerbation and GAS infection	Only children with documented GAS infection in conjunction with neuropsychiatric symptoms and with positive throat cultures and/or high anti-strep antibody titers in the PANDAS profile
5.	Patients in original cohort with 'choreiform' movements may have actually been cases of Sydenham's chorea	Choreiform movements in PANDAS children are fine piano-playing movements of fingers, not the writing adventitious movements seen in Sydenham's chorea

Kurlan and Kaplan (2004), Swedo (2004).

The associations reported between elevated antibody titers and PANDAS-like conditions may not represent causal relationships. It is possible, for example, that elevated serum antibodies and either tic or OCD symptoms could be independently associated with some other causal factor. In addition, findings of genetic and neuroimaging studies in PANDAS patients are not clearly different from those reported in studies of OCD and tics that are not associated with PANDAS. The findings thus may not indicate anything specific regarding a poststreptococcal etiology. All infections produce nonspecific host immune and stress responses, such as the release of cytokines and stress hormones. These biochemical mediators of stress and the immune response may be capable of exacerbating tic or OCD symptoms nonspecifically by virtue of the exquisite sensitivity of these disorders to stress (Chappell et al., 1994). In a prospective study of about 814 children our group tried to find a milder form of PANDAS in GAS-infected children (Perrin et al., 2004). We found that any infection (viral or bacterial) can produce an increase in abnormal behavior in children but we could not find mild OCD-like symptoms in the GAS-infected children compared to viral-infected controls.

Evaluating the results of PANDAS-specific treatments can also be difficult. The natural waxing and waning of OCD and tic symptoms complicates the assessment of treatment response in these conditions. This is especially true given that treatment trials tend to be initiated when symptoms are at their worst, so that the natural subsequent improvement in symptoms might tend to be misattributed to the treatment. In addition, adequate blinding of control conditions is difficult with invasive procedures that have been used in studies on the treatment of PANDAS, such as plasmapheresis. Finally, evidence that the magnitude of reduction in antibody burden with these treatments correlates with the degree of clinical improvement is relatively meager (Singer, 1999).

In summary, there are limitations of the PANDAS construct and more extensive investigation is needed before it is accepted as a true clinical entity (Kurlan, 1998). Double-blind, controlled, longitudinal studies assessing the association of GAS infection with symptom exacerbations of tic and OCD symptoms are needed if the associations reported in temporal cross-sectional studies are to be effectively interpreted. Also, more experimental support for a causal basis of the associations reported between immunological disturbances and the neuropsychiatric component of PANDAS are needed.

References

Allen AJ, Leonard HL, Swedo SE. Case study: a new infection-triggered, autoimmune subtype of pediatric OCD and Tourette's syndrome. J Am Acad Child Adolesc Psychiatry 1995;34:307–311.

Cardona F. Orefici G. Group A streptococcal infections and tic disorders in an Italian pediatric population. J Pediatr 2001;138:71–75.

Chapman AH, Pilkey L, Gibbons MJ. A psychosomatic study of eight children with Sydenham's chorea. Pediatrics 1958;21:582–595.

Chappell P, Riddle M, Anderson G, Scahill L, Hardin M, Walker D et al. Enhanced stress responsively of Tourette syndrome patients undergoing lumbar puncture. Biol Psychiatry 1994;36(1):35–43.

Church AJ, Dale RC, Lees AJ, Giovannoni G, Robertson MM. Tourette's syndrome: a cross sectional study to examine the PANDAS hypothesis. J Neurol Neurosurg Psychiatry 2003;74:602–607.

Church AJ, Dale RC, Giovannoni G. Anti-basal ganglia antibodies: a possible diagnostic utility in idiopathic movement disorders? Arch Dis Child 2004;89:611–614.

Cunningham MW. Pathogenesis of group A streptococcal infections. Clin Microbiol Rev 2000;13(3):470–511.

Dale RC, Heyman I, Giovannoni G, Church AJ. Incidence of anti-brain antibodies in children with obsessive-compulsive disorder. Br J Psychiatry 2005;187:314–319.

Dale RC, Candler PM, Church AJ et al. Neuronal surface glycolytic enzymes are autoantigen targets in post-streptococcal autnoimmune CNS disease. J. Neuroimmunol 2006;172:187–197.

Garvey MA, Perlmutter SJ, Allen AJ, Hamburger S, Lougee L, Leonard HL et al. A piolot study of penicillin prophylaxis for neuropsychiatric exacerbatioins triggered by streptococcal infections. Biol Psychiatry 1999;45:1564–1571.

Giedd JN, Rapoport JL, Kruesi MJ, Parker C, Schapiro MB, Allen AJ et al. Sydenham's chorea: magnetic resonance imaging of the basal ganglia. Neurology 1995;45(12):2199–2202.

Giedd JN, Rapoport JL, Leonard HL, Richter D, Swedo SE. Case study: acute basal ganglia enlargement and obsessive-compulsive symptoms in an adolescent boy. J Am Acad Child Adolesc Psychiatry 1996;35(7):913–915.

Giuliano JD, Zimmerman A, Walkup JT, Singer HS. Prevalence of pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection by history in a consecutive series of community referred children evaluated for tics. Ann Neurol. 1998;44(3):556.

Hallett JJ, Harling-Berg CJ, Knopf PM, Stopa EG, Kiessling LS. Anti-striatal antibodies in Tourette syndrome cause neuronal dysfunction. J Neuroimmunol 2000;111(1–2):195–202.

Husby G, van dRI, Zabriskie JB, Abdin ZH, Williams RC, Jr. Antibodies reacting with cytoplasm of subthalamic and caudate nuclei neurons in chorea and acute rheumatic fever. J Exp Med 1976;144(4):1094–1110.

Kaplan MH. Autoantibodies to heart and rheumatic fever: the induction of autoimmunity to heart by streptococcal antigen cross-reactive with heart. Ann N Y Acad Sci 1965;124(2):904–915.

Kaplan EL, Rothermel CD, Johnson DR. Antistreptolysin O and antideoxyribonuclease B titers: normal values for children ages 2 to 12 in the United States. Pediatrics 1998;101(1 Pt 1):86–88.

Kienzle GD, Breger RK, Chun RW, Zupanc ML, Sackett JF. Sydenham chorea: MR manifestations in two cases. AJNR Am J Neuroradiol 1991;12(1):73–76.

Kiessling et al. Speculations on Antineural Antibody-mediated Neuropsychiatric Disorders of Childhood. Pediatr 1994; 93:323–326.

Kiessling LS, Marcotte AC, Culpepper L. Antineuronal antibodies in movement disorders. Pediatrics 1993;92(1):39–43.

Kiessling LS, Marcotte AC, Culpepper L. Antineuronal antibodies: tics and obsessive-compulsive symptoms. J Dev Behav Pediatr 1994;15(6):421–425.

Kirvan CA, Swedo SE, Snider LA, Cunningham MW. Antibody-mediated neuronal cell signaling in behavior and movement disorders. J Neuroimmunol 2006;179:173–179.

Klieger PS, Fett KA, Dimitsopulos T, Karlan R. Asymmetry of basal ganglia perfusion in Tourette's syndrome shown by technetium-99 m-HMPAO SPECT. J Nucl Med 1997;38:188–191.

Kurlan R. Tourette's syndrome and "PANDAS": will the relation bear out? Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection. Neurology 1998;50(6):1530–1534.

Kurlan R, Kaplan EL. The Pediatric Autoimmune Neuropsychiatric Disorders Associated With Streptococcal Infection (PANDAS) Etiology for Tics and Obsessive-Compulsive Symptoms: Hypothesis or Entity? Practical Considerations for the Clinician. Pediatr 2004;113:883–886.

Lin H, Yeh C-B, Peterson BS, Scahill L, Grantz H, Findley DB et al. Assessment of symptom exacerbations in a longitudinal study of children with Tourette syndrome or obsessive-compulsive disorder. J Am Acad Child Adolesc Psychiatry 2002;41.

Lougee L, Perlmutter SJ, Nicolson R, Garvey MA, Swedo SE. Psychiatric disorders in first-degree relatives of children with pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS). J Am Acad Child Adolesc Psychiatry 2000;39(9):1120–1126.

Luo F, Leckman JF, Katsovich L et al. Prospective longitudinal study of children with tic disorders and/or obsessive-compulsive disorder: relationship of symptom exacerbations to newly acquired streptococcal infections. Pediatrics 2004;113(6):e578–e585.

Mell LK, Davis RL, Ownes D. Association between streptococcal infection and obsessive-compulsive disorder, Tourette's syndrome, and tic disorder. Pediatrics 2005;116(1):56–60.

Morshed SA, Parveen S, Leckman JF, Mercadante MT et al. Antibodies against neural, nuclear, cytoskeletal, and streptococcal epitopes in children and adults with Tourette's syndrome, Sydenham's chorea, and autoimmune disorders. Biol Psychiatry 2001;50:566–577.

Murphy TK, Goodman WK, Fudge MW, Williams RC, Jr, Ayoub EM, Dalal M et al. B lymphocyte antigen D8/17: a peripheral marker for childhood-onset obsessive-compulsive disorder and Tourette's syndrome? Am J Psychiatry 1997;154(3):402–407.

Murphy TK, Goodman WK, Ayoub EM, Voeller KK. On defining Sydenham's chorea: where do we draw the line? Biol Psychiatry 2000;47(10):851–857.

Murphy ML, Pichichero ME. Prospective identification and treatment of children with pediatric autoimmune neuropsychiatric disorder associated with group A streptococcal infection (PANDAS). Arch Pediatr Adolesc Med 2002;156:356–361.

Orvidas LJ, Slattery MJ. Pediatric autoimmune neuropsychiatric disorders and streptococcal infections: role of otolaryngologist. Laryngoscope 2001;111(9):1515–1519.

O'Sullivan RL, Rauch SL, Breiter HC et al. Reduced basal ganglia volumes in trichotillomania measured via morphometric magnetic resonance imaging. Biol Psychiatry 1997;42:39–45.

Pauls DL. Update on the genetics of Tourette syndrome. Adv Neurol 2001;85:281–293.

Pauls DL, Raymond CL, Stevenson JM, Leckman JF. A family study of Gilles de la Tourette syndrome. Am J Hum Genet 1991;48(1):154–163.

Perlmutter SJ, Leitman SF, Garvey MA, Hamburger S, Feldman E, Leonard HL et al. Therapeutic plasma exchange and intravenous immunoglobulin for obsessive-compulsive disorder and tic disorders in childhood. Lancet 1999;354(9185):1153–1158.

Perrin EM, Murphy ML, Casey JR, Pichichero ME, Runyan DK, Miller WC, Snider LA, Swedo SE. Does group A b-hemolytic streptococcal infection increase risk for behavioral and neuropsychiatric symptoms in children? Arch Pediatr Adolesc Med 2004;158:848–856.

Peterson BS. Neuroimaging in child and adolescent neuropsychatric disorders. J Am Acad Child Adolesc Psychiatry 1995;34:1560–1576.

Peterson BS, Leckman JF, Tucker D, Scahill L, Staib L, Zhang H et al. Preliminary findings of antistreptococcal antibody titers and basal ganglia volumes in tic, obsessive-compulsive, and attention deficit/hyperactivity disorders. Arch Gen Psychiatry 2000;57(4):364–372.

Rasmussen SA, Tsuang MT. Clinical characteristics and family history in DSM-III obsessive-compulsive disorder. Am J Psychiatry 1986;143(3):317–322.

Singer HS, Giuliano JD, Hansen BH, Hallett JJ, Laurino JP, Benson M et al. Antibodies against human putamen in children with Tourette syndrome. Neurology 1998;50(6):1618–1624.

Singer HS. PANDAS and immunomodulatory therapy. Lancet 1999;354(9185):1137–1138.

Snider LA, Seligman LD, Ketchen BR et al. Tics and problem behaviors in schoolchildren: prevalence, characterization, and associations. Pediatrics 2002;110:331–336.

Snider LA, Lougee L, Slattery M, Grant P, Swedo SE. Antibiotic prophylaxis with azithromycin or penicillin for childhood-onset neuropsychiatric disorders. Biol Psychiatry 2005;57:788–792.

Stollerman GH. Rheumatic fever and streptococcal infection: unraveling the mysteries of a dread disease. Boston: 1997.

Swedo SE. Pediatric Autoimmune Neuropsychiatric Disorders Associated With Streptococcal Infections: Clinical Description of the First 50 Cases. Am J Psychiatry 1998;155:264–271.

Swedo SE. The Pediatric Autoimmune Neuropsychiatric Disorders Associated With Streptococcal Infection (PANDAS) Subgroup: Separating Fact from Fiction. Pediatr 2004; 113:907–911.

Swedo SE, Rapoport JL, Cheslow DL, Leonard HL, Ayoub EM, Hosier DM et al. High prevalence of obsessive-compulsive symptoms in patients with Sydenham's chorea. Am J Psychiatry 1989;146(2):246–249.

Swedo SE, Rapoport JL, Leonard H, Lenane M, Cheslow D. Obsessive-compulsive disorder in children and adolescents: clinical phenomenology of 70 consecutive cases. Arch Gen Psychiatry 1989;46:335–341.

Swedo et al. Syndenharn's Chorea: a Model for Childhood Autoimmune Neuropsychiatric Disorders. JAMA 1994;272:1788–1791.
Swedo SE, Leonard HL, Kiessling LS. Speculations on antineuronal antibody-mediated neuropsychiatric disorders of childhood. Pediatrics 1994;93:323–326.
Swedo SE, Leonard HL, Mittleman BB et al. Identification of children with pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections by a marker associated with rheumatic fever. Am J Psychiatry 1997;154:110–112.
Swedo SE, Leonard HL, Garvey M, Mittleman B, Allen AJ, Perlmutter S et al. Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections: clinical description of the first 50 cases. Am J Psychiatry 1998;155(2):264–271.
Traill Z, Pike M, Byrne J. Sydenham's chorea: a case showing reversible striatal abnormalities on CT and MRI. Dev Med Child Neurol 1995;37(3):270–273.
Trifiletti RR. TS83: Candidate target brain autoantigen in Tourette Syndrome and OCD. Ann. Neurol. 1998;44(3):561.
Trifiletti RR, Altemus M, Packard AM, Bandele AN, Zabriskie JB. Changes in Antineuronal Antibodies following conventional and immunosuppressive therapy for Tourette's syndrome and obsessive-compulsive disorder. Ann. Neurol. 1998;44(3):561.

Fever in the Returned Paediatric Traveller

F. Andrew I. Riordan

1 Introduction

It was unusual for children to travel to the tropics until recently. The speed and ease of air travel means that children can now return from the tropics within the incubation period for most infections. These children can thus present with 'tropical' infections which their local paediatricians may not expect or recognize. It is essential that a detailed travel history is taken in all children presenting with fever.

Many paediatricians see returning travellers infrequently, unless they work in an area with a large immigrant population. The distribution of migrants in the UK is uneven with the majority living in London or the South East. However, many South Asians live in cities in the Midlands or North West of England (HPA, 2006). Other regions of the UK are now seeing large increases in the non-UK-born population. Thus, paediatricians in London and the Midlands are most likely to see children with imported infections, but paediatricians working in other parts of the UK will start to see these more often now. For example, a study of children with fever attending a northern British hospital did not record any travel-related infections (Nademi et al., 2001).

Data on imported infections in children are mostly limited to small single centre studies or retrospective single infection cohorts. Many studies are retrospective and likely to be hampered by under notification. Most studies include mainly adults (Doherty et al., 1995; O'Brien et al., 2001). This article will focus on infections seen in children presenting with fever on return from the tropics or subtropics.

2 Children Are at Increased Risk of Imported Infections

Young children are infrequent travellers to the tropics, but those that do travel have an increased risk of infection. Children less than 10 years of age made up a quarter of hospital admissions due to imported infections in studies from

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Scotland, although they only represented 4% of travellers abroad (Cossar et al., 1990). In an area with a high proportion of immigrants (East Birmingham, UK), 1.3% of admissions to a paediatric ward were due to imported infections (Riordan and Tarlow, 1998). Children account for 15–20% of all cases of imported malaria (Ladhani et al., 2007) and 25–33% of cases of imported typhoid and paratyphoid (HPA, 2005).

The risk of a number of imported infections is higher for children compared with adults, including malaria (Phillips-Howard et al., 1988), traveller diarrhoea (Pitzinger et al., 1991), and Hepatitis A (Behrens et al., 1995).

This increased risk of imported infection is due to a number of interconnected factors; young age, reason for travel, destination, and lack of preventative measures.

2.1 Age

Young children have an increased risk of infection due to immunological immaturity. In the tropics the main burden of infection is in children under 5 years. Even when taking into account destination and reason for travel, children less than 15 years have a much greater risk of Hepatitis A than adults (Behrens et al., 1995). Young children less than 2 years have the highest risk of diarrhoea whilst abroad (Pitzinger et al., 1991), they also had the most dysentery as well as prolonged or recurrent episodes of diarrhoea.

2.2 Reason for Travel—Visiting Friends and Relatives

The reasons for travel to tropical areas have changed over the past few decades. This is seen most clearly in children with imported malaria. In the 1970s and 1980s, malaria was mainly seen in immigrants arriving from malaria-endemic areas. More recently, most children with imported malaria live in non-endemic countries, but travel to malaria-endemic areas to visit friends and relatives (Jelinek et al., 2002).

Travellers who visit friends and relatives have an increased risk of infection compared with tourists (Leder et al., 2006). They are less likely to seek pretravel advice, take antimalarial prophylaxis, or bite-prevention measures. They are also more likely to be exposed to infection because they travel to rural areas for longer periods (Leder et al., 2006). This group is also more likely to delay seeking medical help when they return to their country of residence, often because of cultural and language barriers (Brabin and Ganley, 1997).

2.3 Destination

The likely diagnoses seen in travellers returning from the developing world varies with the destination visited (Freedman et al., 2006). Ill adult travellers who return from sub-Saharan Africa, south-central Asia, and Latin America

are more likely to experience fever than any other group (Wilson et al., 2007). Dengue fever is a common cause of fever in travellers from all regions, except Africa. Rickettsial disease was seen most often in travellers from Africa, whilst enteric fever was seen mostly in travellers from South Asia. However, malaria was one of the most common causes of fever among travellers from every region (Freedman et al., 2006).

Children mostly travel to visit friends and relatives in their parents' country of origin. In the UK, 7.5% of the population were born abroad. Of those born in tropical areas, most of them come from the Indian subcontinent or Africa. British children are thus likely to visit these tropical areas and are at risk of malaria and enteric fever.

2.4 Preventive Measures

Children travelling to the tropics often do not take preventative measures. Only 16 of 108 children admitted to hospital with fever after travel to the tropics had received the recommended pretravel vaccinations (West and Riordan, 2003). Malaria prophylaxis was taken by only 3–15% of children with imported malaria (Brabin and Ganley, 1997; Ladhani et al., 2003; Williams et al., 2002).

It is not clear why the uptake of preventative measures is so poor in children. Many are visiting friends and relatives in their parents' home country and this group are less likely to seek pretravel advice or take preventative measures. Some suggest that parents falsely assume that they and their children are protected from tropical diseases, such as malaria, because of previous time spent in endemic areas or because of their ethnic origin (Bradley et al., 1994).

3 Causes of Fever in Children Who Have Travelled to the Tropics or Subtropics

Most studies of fever in the returning traveller have concentrated on adults presenting to tertiary care centres (Doherty et al., 1995; O'Brien et al., 2001). Three studies of children are published (see Table 1). One paediatric study only included children who had returned in the previous 4 weeks (Klein and Millman, 1998). This time period is likely to have missed a considerable number of cases, especially malaria (Brabin and Ganley, 1997). This study was undertaken in London and included children who had mostly visited sub-Saharan African countries. The other two studies were conducted in the West Midlands where the study population had mostly visited the Indian subcontinent (Riordan, 1998; West and Riordan, 2003).

In these studies the commonest tropical infections were malaria, diarrhoea, hepatitis, and typhoid (see Table 1). However, cosmopolitan infections (such as

Table 1 Causes of fever in children admitted to hospital after returning from the tropics

Cause	Klein and Millman (1998) ($n = 31$)	Riordan (1998) ($n = 45$)	West and Riordan (2003) ($n = 153$)
TROPICAL			
Malaria			
Vivax	1	10	19
Falciparum	3	3	3
Diarrhoea			
Travellers	0	6	15
Bacterial	3	3	16
Giardiasis	0	1	5
Cryptosporidium	0	1	2
Hepatitis	1	6	8
Dengue	2	0	0
Typhoid	2	1	5
Pulmonary TB			2
Ricketsial infection			1
COSMOPOLITAN			
Respiratory Infection			
Lower	2	7	18
Upper	1	6	5
Cellulitis/ lymphadenitis	1	2	3
Measles		1	
UTI		1	6
Viral gastroenteritis		4	
Meningococcal disease			2
No diagnosis	14	3	52
OTHER	AML 1	SLE 1	Kawasaki 1

Some children had more than one infection.

respiratory or urinary tract infections) were as common as tropical infections. This differs from adult studies which found higher rates of malaria and lower rates of cosmopolitan illnesses (Doherty et al., 1995; O'Brien et al., 2001).

3.1 Investigations

The largest prospective series of febrile children returning from the tropics identified a high proportion of treatable conditions (46%) (West and Riordan, 2003). The majority of the diagnoses requiring treatment were made using simple investigations (stool culture, blood film for malaria, chest X-ray, and blood culture).

In addition, thrombocytopenia was often present in those with malaria. A platelet count above 190×10^9/L had a negative predictive value of 97% for malaria versus all other causes of fever in the population studied. The authors

suggest that a platelet count above 190×10^9/L is a useful predictor of the absence of malaria in febrile children who have returned from a malaria-endemic area. White cell count was generally unhelpful. Haemoglobin, neutrophil, and eosinophil counts were also not diagnostically specific.

Febrile children who have travelled to the tropics or subtropics in the preceding year should have the following investigations; full blood count, blood film for malarial parasites, stool culture, and chest X-ray. For children who have travelled in the preceding month, a blood culture for enteric fever should also be taken (Shingadia et al., 1996).

4 Common Imported Infections in Children Presenting with Fever

4.1 Malaria

Children account for 15–20% of all cases of imported malaria (Stauffer and Fischer, 2003). Children need to be considered separately from adults because they have different risk factors. Children also have a higher risk of developing severe disease since they are likely to be non-immune to malaria (Ladhani et al., 2007).

4.1.1 Presenting Symptoms

Most children develop symptoms of malaria after they return to their country of residence (Lobel and Kozarsky, 1997). The time interval between being bitten by an infected mosquito and the development of symptoms varies considerably with the malaria species responsible. *Plasmodium falciparum* malaria mostly presents within a month, whereas *P. ovale* and *P. vivax* infections can present up to a year after travel (Brabin and Ganley, 1997).

Presenting symptoms of imported malaria in children vary considerably. This is due to the different populations studied and the various infecting *Plasmodium* species. For example, many refugee children with malaria are asymptomatic, probably because they are partly immune to malaria.

Non-immune children with imported malaria are likely to present with non-specific symptoms (fever, lethargy, malaise), especially gastrointestinal symptoms (nausea, abdominal pain, vomiting, diarrhoea). Children also have hepatomegaly (56% of children vs. 25% of adults), splenomegaly (48% vs. 25%), and jaundice (48% vs. 34%) more often than adults (Shingadia and Shulman, 2000).

The characteristic patterns of fever associated with malaria are seen in less than a quarter of paediatric cases. However, children are more likely to have fever greater than 40 °C than adults and may present with febrile convulsions. Symptoms and signs can be masked in children who have received prophylaxis or partial treatment for malaria.

4.1.2 Diagnosis

Delays in diagnosis are associated with an increased risk of developing severe malaria and death (Bradley et al., 1994; Shingadia and Shulman, 2000).

The diagnosis of malaria is usually made by microscopic examination of thick and thin blood films. These should be requested in any febrile child who has travelled to a malaria-endemic area in the preceding 12 months. Thick blood films are the most sensitive in detecting malaria parasites, whilst thin films are most valuable for confirming the species of parasite. However, the diagnosis may be missed since the initial blood film may be negative in up to 7% of cases (Ansdell et al., 1974). Children with suspected malaria, who have a negative blood film, should have at least two repeat blood films before the diagnosis of malaria can be safely excluded.

Thrombocytopenia is characteristic of malaria and is present in 45–71% of children with imported malaria. However, thrombocytopenia in children with malaria is not associated with bleeding unlike in adults (Ladhani et al., 2003).

4.1.3 Management

A detailed review of the treatment of malaria is beyond the scope of this chapter and is described elsewhere (Lalloo et al., 2007; Maitland et al., 2005). Management varies according to the malaria species responsible and the severity of the disease. Children with uncomplicated malaria can mostly be treated with oral antimalarial drugs.

4.2 Non-Falciparum Malaria

Uncomplicated *P. vivax* and *P. ovale* infections are usually treated with oral chloroquine followed by primaquine to eradicate hepatic hypnozoites (Lalloo et al., 2007).

4.3 Falciparum Malaria

4.3.1 Uncomplicated Falciparum Malaria

Oral quinine, atovaquone-proguanil, and coartem can all be used for the treatment of uncomplicated falciparum malaria in children. National guidelines in the UK suggest that oral quinine is usually well tolerated by children and is an appropriate drug for the treatment of uncomplicated falciparum malaria (Lalloo et al., 2007), although some disagree with this (Maitland et al., 2005).

4.3.2 Severe/Complicated Falciparum Malaria in Children

The main clinical presentations of severe malaria in children are cerebral malaria, severe anaemia, and respiratory distress/acidosis. Management of severe or complicated malaria in children requires provision of intensive care with respiratory and cardiovascular support (Maitland et al., 2005).

Children with anaemia may require blood transfusions, although the haemoglobin level at which transfusion should be given remains uncertain. In patients with malaria, tachypnoea, and increased work of breathing (respiratory distress) usually indicate underlying hypovolaemia, which should be treated with volume resuscitation (Maitland et al., 2005). Hypoglycaemia and electrolyte imbalances should be corrected at the earliest opportunity.

Concurrent bacterial infections are rare in children with severe imported malaria. However, empiric broad-spectrum antibiotics are often given until bacterial co-infection (meningitis or septicaemia) can be safely excluded (Ladhani et al., 2005).

Intravenous quinine is the drug of choice for the treatment of severe malaria in children in the UK (Lalloo et al., 2007). There is evidence from adult studies that injectable artesunate may improve outcome in severe malaria compared with quinine. However, there is limited evidence supporting the superiority of injectable artesunate in children, trials are currently underway in Africa. Until the results of these large multicentre trials in children are available, intravenous quinine remains the drug of choice.

4.4 Diarrhoea

Traveller's diarrhoea is one of the commonest illnesses to affect people who travel to the tropics or subtropics (Consensus Conference, 1985). Young children have the highest risk of getting traveller's diarrhoea, despite their parents taking the greatest precautions about what they eat (Pitzinger et al., 1991). The clinical course in infants may be severe and protracted (Pitzinger et al., 1991). Some infants have been reported to develop severe enteropathy, needing parenteral nutrition, and prolonged hospital admission (Hutchins et al., 1982; Msengi et al., 1988).

Children admitted to hospital with traveller's diarrhoea are older and more likely to have bacterial and protozoal infections than other children admitted with gastroenteritis (Riordan et al., 2000). Traveller's diarrhoea should thus be viewed as a different entity to other forms of gastroenteritis. However, prolonged admission with traveller's diarrhoea was uncommon, except in young infants or children with other infections (Riordan et al., 2000).

The organisms found in the stools of children with traveller's diarrhoea were similar to those found in adults with traveller's diarrhoea (Black, 1990) (Campylobacter, Shigella, Salmonella, Enteropathogenic *E. coli*, Giardia, Cryptosporidia).

4.4.1 Treatment

TD is usually self-limiting; however, antibiotic treatment (depending on sensitivities) may be needed for infants and those with other underlying diseases.

4.5 Hepatitis

Hepatitis A virus infection causes a range of illness from asymptomatic disease (common in children) to hepatitis and rarely liver failure. It is predominantly transmitted by the faecal–oral route and is endemic worldwide.

The incidence of hepatitis A is generally low in the developed world. It is therefore more likely to occur in non-immune travellers who visit highly endemic areas such as the Indian subcontinent (Mutsch et al., 2006). The risk is greatest in children under 15 years travelling to the Indian subcontinent (Behrens et al., 1995; Gungabissoon et al., 2007).

In the UK it is probable that travel associated hepatitis A is under reported. Where information on foreign travel was available, the Indian subcontinent (particularly India and Pakistan) was the most commonly reported region of travel (Morris et al., 2002).

4.5.1 Clinical Features

The incubation period is around 28–30 days. Jaundice occurs in 70–80% of infected adults, but hepatitis A is usually subclinical in children. However, hepatitis A in children can occasionally be severe and require hospitalization. Hepatitis A rarely causes fever and jaundice at the same time. If this occurs, then another diagnosis is likely.

4.5.2 Treatment

Treatment is supportive.

4.5.3 Prevention

Immunization with hepatitis A vaccine is recommended for children aged 1 year and over travelling to moderately or highly endemic areas, such as the Indian subcontinent (DoH, 2006).

4.6 Enteric Fever in Travellers

Enteric fever includes both typhoid fever and paratyphoid fever. These are systemic infections caused by *Salmonella enterica* (including serotypes Typhi and Paratyphi). Humans are the only reservoir of *Salmonella enterica*. The

most common modes of transmission are through faecally contaminated water or food and person-to-person transmission. The risk to travellers varies by geographic region visited, but the greatest risk is with travel to the Indian subcontinent (Ekdahl et al., 2005). Imported enteric fever is seen in all ages but 25–33% of imported cases in the UK occur in children under 15 years (HPA, 2005).

4.6.1 Presentation

Descriptions of the clinical presentation of imported enteric fever are scarce. Children in developing countries with enteric fever have fever, diarrhoea (8–35%, especially young children) and abdominal pain, but rarely have relative bradycardia. Some children have a mild viral-like illness (Bhutta, 2006; Davis et al., 1999; Walia et al., 2006).

4.6.2 Investigation

The most useful investigation is blood culture; 40–60% are positive. Other cultures may also be positive; stool (30%), urine (0–58%), bone marrow (55–67%). White blood cells (WBC) is often low, but may be raised in young children. In severe disease there may be thrombocytopenia and deranged liver function tests. The Widal test has poor sensitivity (47–77%) and specificity (50–92) and is not used in the UK.

4.6.3 Treatment

Resistance to the commonly used antibiotics has increased in endemic countries (ampicillin, cotrimoxazole, and chloramphenicol); multidrug-resistant enteric fever. There is now also increasing resistance to quionolones (Cooke et al., 2007). Multidrug-resistant typhoid was found in 22% of UK isolates, whilst 39% were quinolone resistant (Cooke et al., 2007). Thirteen per cent of isolates were both MDR and likely to show poor response to quinolones. The drug of choice for imported enteric fever in children is ceftriaxone. If the isolate is found to be quinolone sensitive then fluoroquinolones can be given (Connor and Schwartz, 2005).

4.6.4 Prevention

Vaccination against typhoid fever should be considered for travellers to the Indian subcontinent, the Middle East, and Africa. At present vaccination only protects against *S typhi*, but not *S paratyphi*.

4.7 Dengue Fever

Dengue fever is a mosquito-borne viral illness that has become a common cause of fever in the returned traveller (Freedman et al., 2006). Dengue is transmitted by mosquitoes that live in close association with humans and bite in the

daytime. Between 100 and 150 cases of dengue are imported into the UK every year. The majority of cases travelled to South and South East Asia (HPA, 2005). Other popular tourist destinations where travellers are likely to acquire dengue fever are the Caribbean and South and Central America. The risk of infection to travellers is low if they take measures to reduce mosquito bites.

4.7.1 Clinical Features

The incubation period is commonly 4–7 days. Dengue fever begins with a fever for 1–5 days. Most children with dengue fever have a non-specific febrile illness. Adults and older children may have muscle aches ('break bone fever'), back pain, headache, pharyngitis, and arthralgia. A transient generalized macular rash is common early in illness.

Dengue haemorrhagic fever develops following a second infection with a different dengue serotype. It is therefore rare in travellers.

4.7.2 Treatment

There is no specific treatment for dengue.

4.7.3 Prevention

There is no vaccine or chemoprophylaxis for dengue fever, prevention is by reducing mosquito bites.

4.8 Ricketsial Infection

Rickettsia are transmitted to humans via the bite of an infected tick. *Rickettsia* do not occur in the United Kingdom so rickettsial infections diagnosed in the UK are likely to have been acquired abroad. Between 1990 and 2002 there were 66 laboratory reports of rickettsial infections in England and Wales (HPA, 2004). Rickettsial spotted fever was the most common; 28 of 42 cases with recent travel history had visited to sub-Saharan Africa. This reflects the endemic areas for rickettsial infections, especially African tick typhus.

4.8.1 Spotted Fevers

The most common 'spotted fevers' are Rocky Mountain spotted fever and Mediterranean spotted fever (Jensenius et al., 2004). Rocky Mountain spotted fever occurs in Brazil, Canada, Colombia, Mexico, and the south eastern states of the US. The main clinical features are: abrupt onset of fever, severe headaches and muscle pains, and a rash that usually develops after 2–3 days on the soles of the feet, wrists, and forearms. Mediterranean spotted fever occurs in

coastal Mediterranean countries. The clinical features are similar to Rocky Mountain spotted fever but commonly there is an 'eschar' at the site of the tick bite. The eschar may be absent leading to a clinical picture resembling Kawasaki disease (Jenkins et al., 1997).

African tick typhus is prevalent throughout Africa, especially South Africa. It is caused by *R. conorii*. Travellers on safari are at particular risk of exposure in savannah and veldt areas (Jensenius et al., 2004).

4.8.2 Diagnosis

Diagnosis is mostly made by serology.

4.8.3 Treatment

Tetracyclines are the drugs of choice for children aged 9 years and older. Younger children should be treated with a macrolide (Jensenius et al., 2004).

4.8.4 Prevention

Travellers to endemic areas should take measures to avoid tick bites.

4.9 Tuberculosis

Tuberculosis (TB) occasionally presents as fever in the returned traveller, although only three cases were diagnosed in children entering the UK during a 14-month period (Teo et al., 2006). The majority of children with TB in the UK are from families from the Indian subcontinent or Africa (Teo et al., 2006); many of these children will visit friends and relatives in their parents' country of origin. Some of these children were born in countries with high rates of TB and are at much higher risk of developing TB. Most cases of TB in children born abroad occur within 5 years of arrival in the UK (Teo et al., 2006), but few are diagnosed when children enter the UK.

4.9.1 Treatment

Children with active TB should be treated with four antituberculous drugs (rifampcin, isoniazid, pyrazinamide, and ethambutol) (NICE, 2005).

4.10 Cosmopolitan Infection

In the reported studies of fever in returned paediatric travellers cosmopolitan infections (such as respiratory or urinary tract infections) are as common as tropical infections. This differs from adult studies and probably reflects the

increased rates of these infections in children. Twenty per cent of children had both cosmopolitan and tropical infections (Riordan, 1998).

Influenza is reported to be a frequent health problem among travellers to subtropical and tropical countries with an attack rate of 1.2–2.8% (Mutsch et al., 2005). However, attributing fever in a returned traveller to influenza could lead to missing a treatable tropical disease (malaria, typhoid, etc.).

An important imported cosmopolitan infection is measles. Between 1995 and 2001, most measles in the United Kingdom was acquired from an imported infection (Ramsay et al., 2003). A known link to an imported case was reported in 70–86% of cases of measles in Europe in 2001–2002. Fifty-one per cent of the imported cases were from Asia (Muscat et al., 2003).

References

Ansdell VE, Boosey CM, Geddes AM, Morgan HV. (1974). Malaria in Birmingham 1968–1973. Br Med J **2**:206–8

Behrens RH, Collins M, Botto B, Heptonstall J. (1995). Risk for British travellers of acquiring hepatitis A. BMJ **311**:193

Bhutta ZA. (2006). Current concepts in the diagnosis and treatment of typhoid fever. BMJ **333**:78–82

Black RE. (1990). Epidemiology of traveller's diarrhea and relative importance of various pathogens. Rev Infect Dis **12** Suppl 1:S73–9

Brabin BJ, Ganley Y. (1997) Imported malaria in children in the UK. Arch Dis Child **77**:76–81

Bradley D, Warhurst D, Blaze M, Smith V. (1994). Malaria imported into the United Kingdom in 1992 and 1993. Commun Dis Rep CDR Rev **4**:R169–72

Connor BA, Schwartz E. (2005).Typhoid and paratyphoid fever in travellers. Lancet Infect Dis **5**:623–8

Consensus Conference. (1985). Traveller's diarrhea. JAMA **253**:2700–4

Cooke FJ, Day M, Wain J, Ward LR, Threlfall EJ. (2007). Cases of typhoid fever imported into England, Scotland and Wales (2000–2003). Trans R Soc Trop Med Hyg. **101**:398–404

Cossar JH, Reid D, Fallon RJ, Bell EJ, Riding MH, Follett EA, Dow BC, Mitchell S, Grist NR. (1990). A cumulative review of studies on travellers, their experiences of illnesses and the implications of these findings. J Infect **21**:27–42

Davis TM, Makepeace AE, Dallimore EA, Choo KE. (1999). Relative bradycardia is not a feature of enteric fever in children. Clin Infect Dis **28**:582–6

Department of Health. (2006). Hepatitis A. In: Immunisation against infectious disease, 2006. HMSO. http://www.dh.gov.uk/en/Policyandguidance/Healthandsocialcaretopics/Greenbook/DH_4097254

Doherty JF, Grant AD, Bryceson AD. (1995). Fever as the presenting complaint of travellers returning from the tropics. QJM **88**:277–81

Ekdahl K; de Jong B; Andersson Y. (2005). Risk of travel-associated typhoid and paratyphoid fevers in various regions. J Travel Med **12**:197–204

Freedman DO, Weld LH, Kozarsky PE, Fisk T, Robins R, von Sonnenburg F, Keystone JS, Pandey P, Cetron MS; GeoSentinel Surveillance Network. (2006). Spectrum of disease and relation to place of exposure among ill returned travelers. N Engl J Med. **354**:119–30

Gungabissoon U, Andrews N, Crowcroft NS. (2007). Hepatitis A infection in people of south Asian origin in England and Wales: analysis of laboratory reports between 1992 and 2004. Epidemiol Infect. **135**:549–54

Health Protection Agency. (2004). Unusual infections associated with foreign travel – Part 2: Rickettsial infections. CDR Weekly **14**: no 19

Health Protection Agency. (2005). Foreign travel-associated illness, England, Wales, and Northern Ireland – annual report 2005. London: HPA. Available at http://www.hpa.org.uk/publications/PublicationDisplay.asp?PublicationID=2

Health Protection Agency. (2006). Migrant health. Infectious diseases in non-UK born populations in England, Wales and Northern Ireland. A baseline report – 2006 http://www.hpa.org.uk/publications/2006/migrant_health/migrant_health.pdf

Hutchins P, Hindocha P, Phillips A, Walker-Smith J. (1982). Traveller's diarrhoea with a vengeance in children of UK immigrants visiting their parental homeland. Arch Dis Child **52**:208–11

Jelinek T, Schulte C, Behrens R, Grobusch MP, Coulaud JP, Bisoffi Z, Matteelli A, Clerinx J, Corachan M, Puente S, Gjorup I, Harms G, Kollaritsch H, Kotlowski A, Bjorkmann A, Delmont JP, Knobloch J, Nielsen LN, Cuadros J, Hatz C, Beran J, Schmid ML, Schulze M, Lopez-Velez R, Fleischer K, Kapaun A, McWhinney P, Kern P, Atougia J, Fry G, da Cunha S, Boecken G. (2002). Imported falciparum malaria in Europe: sentinel surveillance data from the European network on surveillance of imported infectious diseases. Clin Infect Dis **34**:572–6

Jenkins DR, Rees JC, Pollitt C, Cant A, Craft AW. (1997). Mediterranean spotted fever mimicking Kawasaki disease. BMJ **314**:655–6.

Jensenius M, Fournier PE, Raoult D. (2004). Rickettsioses and the international traveler. Clin Infect Dis **39**:1493–9.

Klein JL, Millman GC. (1998). Prospective, hospital based study of fever in children in the United Kingdom who had recently spent time in the tropics. BMJ **316**:1425–6

Ladhani S, El Bashir H, Patel VS, Shingadia D. (2003). Childhood malaria in East London. Pediatr Infect Dis J **22**:814–9

Ladhani S, Shingadia D, Riordan FA. (2005). Proposed guidelines for severe imported malaria in children need more evidence. BMJ **331**:1025.

Ladhani S, Aibara RJ, Riordan FA, Shingadia D. (2007). Imported malaria in children: a review of clinical studies. Lancet Infect Dis. **7**:349–57

Lalloo DG, Shingadia D, Pasvol G, Chiodini PL, Whitty CJ, Beeching NJ, Hill DR, Warrell DA, Bannister BA for the HPA Advisory Committee on Malaria Prevention in UK Travellers (2007). UK malaria treatment guidelines. J Infect **54**:111–21

Leder K, Tong S, Weld L, Kain KC, Wilder-Smith A, von Sonnenburg F, Black J, Brown GV, Torresi J; GeoSentinel Surveillance Network. (2006). Illness in travellers visiting friends and relatives: a review of the GeoSentinel Surveillance Network. Clin Infect Dis. **43**:1185–93

Lobel HO, Kozarsky PE. (1997). Update on prevention of malaria for travellers. JAMA **278**:1767–71

Maitland K, Nadel S, Pollard AJ, Williams TN, Newton CR, Levin M. (2005). Management of severe malaria in children: proposed guidelines for the United Kingdom. BMJ **331**:337–43

Morris MC, Gay NJ, Hesketh LM, Morgan-Capner P, Miller E. (2002). The changing epidemiological pattern of hepatitis A in England and Wales. Epidemiol Infect **128**:457–63

Msengi AE, Phillips AD, Risdon RA, Walker-Smith JA. (1988). Traveller's diarrhoea among children returning to the United Kingdom from visits abroad. Ann Trop Paed **8**:173–80

Muscat M, Glismann S, Bang S. (2003). Measles in Europe in 2001–2002. Euro Surveill **8**:123–9

Mutsch M, Tavernini M, Marx A, Gregory V, Lin YP, Hay AJ, Tschopp A, Steffen R. (2005). Influenza virus infection in travellers to tropical and subtropical countries. Clin Infect Dis **40**:1282–7

Mutsch M, Spicher VM, Gut C, Steffen R. (2006). Hepatitis A virus infections in travellers, 1988–2004. Clin Infect Dis **42**:490–7

Nademi Z, Clark J, Richards CG, Walshaw D, Cant AJ. (2001). The causes of fever in children attending hospital in the north of England. J Infect **43**:221–5

NICE. (2005). Clinical diagnosis and management of tuberculosis, and measures for its prevention and control. http://guidance.nice.org.uk/CG33/guidance/pdf/English

O'Brien D, Tobin S, Brown GV, Torresi J. (2001). Fever in returned travellers: review of hospital admissions for a 3-year period. Clin Infect Dis; **33**:603–9

Phillips-Howard PA, Bradley DJ, Blaze M, Hurn M. (1988). Malaria in Britain: 1977–1986. Br Med J **296**:245–8

Pitzinger B, Steffen R, Tschopp A. (1991). Incidence and clinical features of traveller's diarrhea in infants and children. Pediatr Infect Dis J **10**:719–23

Ramsay ME, Jin L, White J, Litton P, Cohen B, Brown D. (2003). The elimination of indigenous measles transmission in England and Wales. J Infect Dis **187**:S198–207

Riordan FA. (1998). Children should be investigated for malaria. BMJ **317**:1390

Riordan FA, Tarlow MJ. (1998). Imported infections in east Birmingham children. Postgrad Med J. **74**:36–7

Riordan FAI, Quigley T, West N. (2000). Travel associated diarrhoea in children admitted to hospital. J Infect **40**:A28

Shingadia D, Al-Ansari H, Novelli V. (1996). Investigation and diagnosis of fever in the returning traveller. Curr Paediatr **6**:108–13

Shingadia D, Shulman ST. (2000). Recognition and management of imported malaria in children. Semin Pediatr Infect Dis **11**:172–7

Stauffer W, Fischer PR. (2003). Diagnosis and treatment of malaria in children. Clin Infect Dis **37**:1340–8

Teo SSS, Alfaham M, Clark J, Coen P, Hoskyns W, Novelli V, Riordan A, Sharland M, Shingadia D (2006). British paediatric surveillance unit childhood tuberculosis study. Arch Dis Child **91**(Suppl I):A2

Walia M, Gaind R, Paul P, Mehta R, Aggarwal P, Kalaivani M. (2006). Age-related clinical and microbiological characteristics of enteric fever in India. Trans R Soc Trop Med Hyg **100**:942–8

West NS, Riordan FA. (2003). Fever in returned travellers: a prospective review of hospital admissions for a 2 1/2 year period. Arch Dis Child **88**:432–4

Williams JP, Chitre M, Sharland M. (2002). Increasing plasmodium falciparum malaria in Southwest London: a 25-year observational study. Arch Dis Child **86**:428–30

Wilson ME, Weld LH, Boggild A, Keystone JS, Kain KC, von Sonnenburg F, Schwartz E, GeoSentinel Surveillance Network. (2007). Fever in returned travelers: results from the GeoSentinel Surveillance Network. Clin Infect Dis **44**:1560

Molecular Diagnostics of Primary Immunodeficiencies: Benefits and Future Challenges

Mirjam van der Burg, Menno C. van Zelm and Jacques J.M. van Dongen

1 Introduction

Primary immunodeficiencies (PID) are inherited disorders with defects in one or more components of the immune system. According to the latest classification, they can be divided in eight subgroups (See Table 1) (Geha et al. 2007). Although many PID are the result of a single gene mutation, making a molecular diagnosis for a patient can be difficult. Over 135 candidate PID genes have been identified, but the prevalence of most of the genetic defects is low. Furthermore, different genetic defects can result in a similar clinical presentation, whereas patients with different defects in the same gene can present with diverse clinical pictures. Therefore, it is important to take stepwise approach for the diagnosis of PID. First, the patient needs to be evaluated clinically and immunologically using the international multistage diagnostic protocol, designed by the European Society for Immunodeficiencies (ESID) for patient-centered screening for primary immunodeficiency (De Vries 2006). This is followed by flow cytometric immunophenotyping and functional studies (when applicable) of blood, bone marrow, or other specimens to define the immunological defect (Noordzij et al. 2002a, 2002c). These two steps are of great value for guiding the selection of candidate genes for molecular diagnostics (step 3). Finally, genetic counseling and prenatal diagnostics are facilitated with the identification of a genetic defect.

In this chapter, the different types of disease-causing mutations are introduced, their interpretation discussed, and the molecular approach and pitfalls in mutation detection presented.

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Table 1 Eight categories of PID (Geha et al., 2007)

PID categories	Candidate genes
1. Combined T cell and B cell immunodeficiencies	*IL2RG/γ_C,JAK3, IL7RA, CD45, CD3D, CD3E, CD3Z, CD3G, RAG1, RAG2, DCLRE1C/Artemis, XLF, LIG4, ADA, CD40L, CD40, PNP, C2TA, RFXANK/RFXB, RFX5, RFXAP, CD8, ZAP70, ORAI1, TAP1/2, TAPBP, FOXN1, IL2RA, STAT5B*
2. Predominantly antibody deficiencies	*BTK, IGHM, IGLL1/CD179B,CD79A/IGA, CD79B/IGB BLNK, ICOS, CD19, TACI, BAFFR, MSH5, CD40L, CD40, AICDA , UNG, IGK, SH2D1A*
3. Other well-defined immunodeficiency syndromes	*WASP, ATM, MRE11, NBS1, BLM, RMRP, SMARCAL1, STAT3, TYK2, SP110, Dyskerin*
4. Diseases of immune dysregulaton	*LYST, RAB27A, AP3B1, PRF1, MUNC13D, STX11, SH2D1A, XIAP, TNFRSF6/CD95, TNFSF6, CASP10, CASP8, NRAS, AIRE, FOXP3*
5. Congenital defects of phagocyte number, function, or both	*ELA2, GF11, G-CSFR, HAX1, MABPIP, WASP, ITGB2, FUCT1, RAC2, Cal DAG-GEF1, RAC2, ACTB, FPR1, CTSC, C/EBPE, SBDS, CYBB, CYBA, NCF1, NCF2, G6PD, IL12Rβ1, IL12p40, IFN-γR1, IFN-γR2, STAT1,*
6. Defects in innate immunity	*NEMO, IKBA, IRAK4, CXCR4, EVER1, EVER2, UNC93B1,TLR3*
7. Autoinflammatory disorders	*MEFV, TNFRSF1A, MVK, CD2BP1, CARD15, LPIN2*
8. Complement deficiencies	*C1–C9, C4A/CDB, C1NH, CFI, CFH, CFD, Properdin, MBP, MASP2, ITGB2, MCP, CD59, PIGA*

2 Relevance of Molecular Diagnostics

Molecular diagnostics are of great importance to both patient and family. They:

- offer a precise diagnosis;
- form the basis for adequate treatment and estimation of prognosis;
- enable the development of long-term preventive strategies to limit complications and irreversible organ damage; and
- contribute to treatment compliance and permit genetic counseling.

Consequently, fast and precise molecular diagnostics offer substantial added value for patient care. A molecular diagnosis is also required for corrective treatment with gene therapy. Gene therapy for PID has not yet been developed beyond clinical trials and has faced some setbacks (Aiuti et al., 2002; Bordignon et al., 1995; Cavazzana-Calvo et al., 2000; Hacein-Bey-Abina et al., 2002, 2003), but it is still the most promising new corrective treatment.

3 Mutation Spectrum in Human Disease

Different types of disease-causing mutations can be identified. A crude division is made between small lesions (<20 bp) and gross lesions (Fig. 1). Small lesions include single-base-pair substitutions, microdeletions (<20 bp), microinsertions

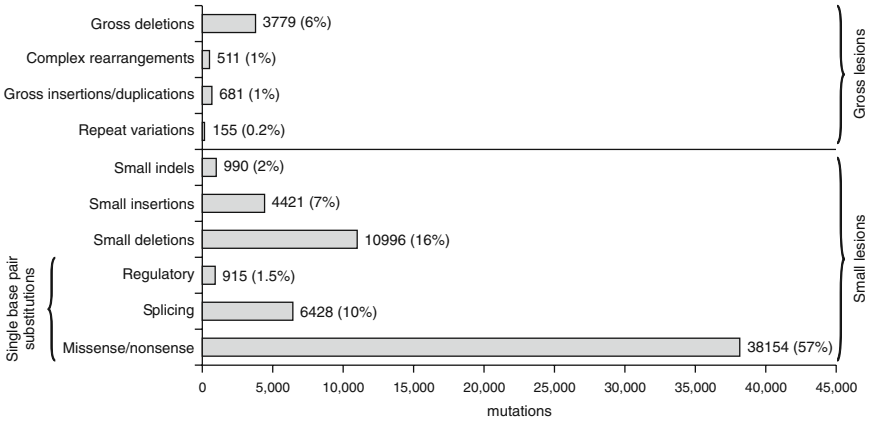

Fig. 1 Spectrum of the different types of human gene mutations recorded in the human genome mutation database (HGMD) as of 3 January 2007, containing 67,030 mutations. Microlesions are the most commonly found gene disruptions underlying human disease, with the vast majority being single-base-pair substitutions. Although not common, a substantial number of gross lesions have also been identified. The majority of these are deletions

(<20 bp), and microindels (<20 bp). Indels are lesions, in which one or more nucleotides were deleted and the same or a different number of new nucleotides were inserted. Recent analysis of the mutations recorded in the Human Gene Mutation Database; HGMD (Stenson et al. 2003) showed that single-base-pair substitutions are most frequently observed: 68% of all mutations (Fig. 1). This is much more than microdeletions (16%), micro-insertions (7%), and micro-indels (1.5%). Gross lesions (>20 bp) include gross deletions (6% of total deleterious mutations), gross insertions (1%), repeat variations (0.2%), or other complex rearrangements (1%) such as inversions.

3.1 Single-Base-Pair Substitutions

Missense and nonsense mutations form 57% of all disease-causing mutations. Missense mutations are single-base-pair substitutions that result in an amino acid change, whereas nonsense mutations directly result in a stop codon. Single-base-pair substitutions in coding regions, which do not result in an amino acid change or stop codon are referred to as silent mutations. This type of mutations can still be disease causing in some cases, when they influence mRNA splicing or stability (Novoyatleva et al. 2006). Single-base-pair substitutions in intronic parts of splice sites (exon–intron border) can also affect mRNA splicing, e.g. by exon skipping or usage of cryptic splice sites. Regulatory mutations (1%) affect promoter or enhancer regions and influence gene expression.

Single-base-pair substitutions are thought to occur nonrandomly throughout the genome (Maki 2002, Rogozin Pavlov 2003). In 2000, Antonarakis and colleagues found a significant excess of transition mutations (purine–purine or pyrimidine–pyrimidine exchange) over transversion mutations (purine–pyrimidine exchange) as compared to random expectation (Antonarakis et al., 2000). In particular, CG dinucleotides have an increased susceptibility to be mutated, a phenomenon observed in a wide range of human genes (Green et al., 1990; Rideout et al., 1990; Youssoufian et al., 1988, 1986). It is thought to be related to methylation of the cytosine in CG dinucleotides (Cooper and Youssoufian, 1988). Deamination of cytosine results in uracil, whereas 5-methylcytosine undergoes deamination to form thymidine. Uracil is recognized and excised by uracil DNA glycosylase. However, thymidine is not specifically excised, as it is one of the four natural bases in DNA. Thus, methylated CG dinucleotides have increased mutation susceptibility into TG or CA.

3.2 Small Deletions and Insertions

Microdeletions and -insertions are associated with short repeats that are usually between 2 and 8 bp (Krawczak and Cooper, 1991). These elements can be direct repeats, palindromes (inverted repeats), and symmetric elements. Most mechanisms proposed to underlie microdeletions are based on the slipped-mispairing hypothesis (Streisinger et al., 1966), which states that during replication one DNA strand can be misaligned via a repeat sequence with the complementary strand (Kunkel, 1990, Kunkel and Soni, 1988). The resulting loop structure can be excised thereby generating the small deletion. This type of deletion can be induced by mutagens (Kimura et al., 1994).

3.3 Gross Lesions

The exact molecular mechanisms by which gross lesions, such as deletions, insertions, or repeat variations arise, are not completely understood, but based on limited sequence data of gross deletion junctions in disease-causing alleles, three mechanisms resulting in gross deletions have been proposed: (1) Mispairing of homologous sequences and unequal crossing over; (2) Non-homologous deletions; and (3) Mispairing between short repeat elements and crossing-over (McNaughton et al., 1998). Nonhomologous gross deletion breakpoints that fall in the latter two categories are frequently located in or near interspersed repeat elements derived from transposable elements (McNaughton et al., 1998). Furthermore, genes with high-transposable element content, such as *IGH* and *DCLRE1C*/*Artemis*, are more frequently disrupted by gross deletions than others. This suggests a role for these elements in mediating gross lesions (Van Zelm et al., 2008).

4 Molecular Analysis of a Candidate Gene

Since the human genome contains >30,000 genes, the PID candidate genes to be sequenced have to be carefully selected based on clinical and immunological findings. Therefore, flow cytometric immunophenotyping and functional assays are important for guiding molecular diagnostics.

Detection of genetic defects can be performed at both the DNA and the RNA level. Mutation analysis at DNA level by sequencing is generally regarded as the gold standard. The first step in mutation analysis is amplification of the candidate gene by a polymerase chain reaction (PCR). PCR primers need to be designed in such a way that the coding exons can be analyzed as well as 30 nucleotides up- and downstream of the exons to ensure identification of splice-site mutations (Fig. 2). Subsequently, PCR products are sequenced and the obtained result is compared to a reference sequence. It is recommended to confirm any identified sequence variant by DNA sequencing of a second (independently obtained) PCR product. In case of a potential splicing mutation, RT-PCR and sequence analysis of RNA transcripts is required (for details see below). Figure 3 illustrates an example of molecular analysis of the *IL2RG* gene in a patient with severe combined immunodeficiency (SCID).

5 Interpretation of Sequence Variants

Interpretation of identified variations in a patient's DNA with respect to the reference sequence needs to be done with great care, because it can either be a polymorphism or a disease-causing mutation. A mutation is a change with a disease-causing effect, whereas a polymorphism is defined as a sequence change occurring in >1% of the population, which does not cause disease. Molecular data on disease-

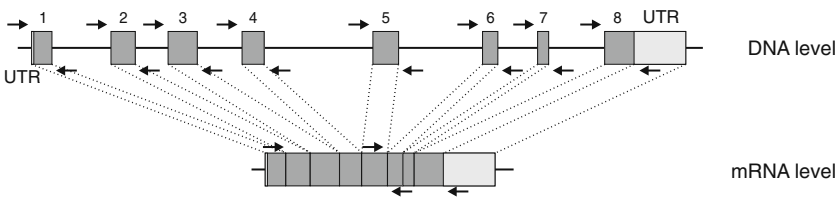

Fig. 2 Strategy for molecular analysis. Coding exons of a candidate gene are amplified by a polymerase chain reaction (PCR). PCR primers need to be designed in such a way that the coding exons can be analyzed as well as 30 nucleotides up- and downstream of the exons to ensure identification of splice-site mutations (*upper part*). PCR products are sequenced and the sequence obtained is compared to a reference. In case of a potential splicing mutation, RT-PCR and sequence analysis of RNA transcripts is required (*lower part*). To ensure analysis of the complete transcript, RT-PCR primer sets should generate overlapping PCR products

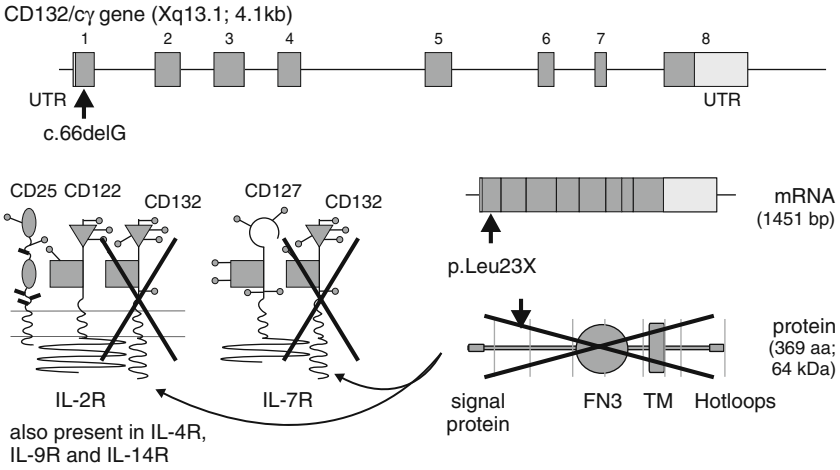

Fig. 3 Molecular analysis of the *IL2RG (CD132)* gene in a patient with X-linked severe combined immunodeficiency (SCID). Molecular analysis of the IL2RG gene revealed a deletion of a G nucleotide in exon 1 at position 66 (c.66delG). This deletion results in the replacement of leucine at amino acid position 23 in a stop codon (p.Leu23X). The common gamma chain (IL2Rγ) is part of the IL-2, IL-4, IL-7, IL-9, and IL-14 receptor; therefore, a mutation in the IL2RG gene affects expression of all these receptors

causing mutations are widely available thanks to inclusion in databases, either genome-wide (Online Mendelian Inheritance in Man; OMIM (Hamosh et al., 2005), and Human Gene Mutation Database; HGMD (Stenson et al., 2003)), or gene-specific (e.g., immunodeficiency mutation databases; IDbases (Piirila et al., 2006)). Information about polymorphisms is also available on several websites (e.g., http://genewindow.nci.nih.gov and www.ncbi.nlm.nih.gov).

For correct interpretation and accurate documentation, a consistent gene mutation nomenclature is essential. Den Dunnen et al. proposed such a nomenclature, which is now broadly used by the medical genetics community and recommended by the human genome variation society (HGVS) (Den Dunnen and Antonarakis, 2000).

In addition, the mode of inheritance (X-linked, autosomal-dominant, or autosomal-recessive) should be taken into account for correct interpretation. Information on a patient's family history and consanguinity can be indicative for expecting one homozygous mutation or two heterozygous mutations (compound heterozygosity) in autosomal-recessive disorders. Some PID candidate genes can have both an autosomal-dominant and an autosomal-recessive mode of inheritance, e.g. the *FAS* (*TNFRSF6*/*CD95*) gene, which encodes a trimeric protein complex. When a *FAS* mutation results in complete absence of protein, the mutation is only disease causing when both alleles are affected (autosomal recessive). However, when the mutation gives rise to a nonfunctional but stable protein, a heterozygous mutation is already disease causing (autosomal

dominant), because, among all the trimers formed, only those composed of three correct proteins are functional (Van der Burg et al., 2000).

6 Pitfalls and Limitations

Besides the clear advantages, molecular testing also has several clear limitations. The techniques are relatively costly and laborious. Furthermore, it is not always clear whether identified sequence variants are disease causing. Conversely, a negative result can be at odds with clinical and immunological findings. In both situations, additional assays may be useful on occasion.

6.1 Disease-Causing Mutation or Polymorphism?

When a sequence variant is identified that has not been reported in one of the databases, it can be difficult to determine whether it concerns a real disease-causing mutation or a previously undescribed polymorphism. In some cases, it will be relatively easy, e.g. for sequence variants resulting in a stop codon leading to a predicted truncated protein or for amino acid substitutions in a functional domain or an evolutionarily conserved residue. However, single-point mutations that alter one amino acid could also be harmless polymorphisms. In such situations, analysis of protein expression or function might be required to support the genetic diagnosis. When an antibody is available for the candidate gene product, flow cytometric immunophenotyping, or Western blot analysis are the easiest methods for studying protein expression and stability. However, these methods will not provide information on whether the protein is functional. This can only be studied by addressing specific processes such as apoptosis, cellular activation, signal transduction, or granulocyte function.

The effect of potential splicing mutations can also be difficult to estimate. RNA splicing prediction programs are helpful, but analysis of transcripts by RT-PCR and sequencing is the best way to determine the effect of these sequence variants (Noordzij et al., 2002b). Unfortunately, this is not always possible, because of lack of the relevant cell type expressing these transcripts.

6.2 Reduction of False Negative Results

Several types of gene disruptions are missed by sequencing of coding exons and splice sites at genomic DNA level. Therefore, additional assays are required to reduce false negative results.

First, analysis of protein expression by flow cytometric immunophenotyping or Western blot analysis, if not yet performed during an earlier phase of the

diagnostic process, can predict a defect in the candidate gene and is therefore an important screening method.

Second, analysis of mRNA by RT-PCR and sequencing enables detection of RNA splicing defects outside the sequenced region. Mutations in intronic regions that create a new splice site can result in inclusion of a piece of intronic DNA into spliced transcripts making these nonfunctional (Van der Burg et al., manuscript in preparation). Real-time quantitative PCR (RQ-PCR) of mRNA provides information on whether the candidate gene is normally expressed. Reduced expression levels are suggestive of a regulatory defect or RNA stability defect. To explore this further, promoter, enhancer, or untranslated regions can be sequenced.

Finally, assays to detect partial or monoallelic genomic deletions can be performed. The presence of a deletion is hard to confirm with a positive result, i.e. sequencing of the breakpoint region (Van Zelm et al., 2008). Furthermore, the detection of partial gene duplications or inversions is even more difficult, because the genetic material is still present in the genome, and consequently, PCR amplification and sequencing will not demonstrate abnormalities. These gross lesions do result in loss of normal transcripts, which can be identified by (quantitative) analysis of mRNA. Finally, monoallelic deletions can be detected with multiplex ligation-dependent probe amplification (MLPA) (Sellner and Taylor, 2004) or DNA FISH with gene-specific probes (Kanegane et al., 2007).

Although very informative, the methods mentioned above are time consuming, technically demanding, and not always possible to perform due to lack of patient's cell material or required reagents. Therefore, these assays are not routinely performed and need only to be considered when a negative result strongly contradicts clinical and immunological findings.

7 Future Perspectives

During recent years, it has been shown that children with primary immunodeficiencies do not always present with a full constellation of clinical features, but can also present with partial phenotypes. These mild forms of clinical presentation are caused by hypomorphic mutations in known PID candidate genes (Moshous et al., 2003). Moreover, hypomorphic mutations also appear to occur in genes which cause embryonic lethality in cases with a complete defect (van der Burg et al., 2006). Therefore, awareness of the possibility of hypomorphic mutations is likely to become increasingly important in molecular diagnostics of PID.

Another challenge concerns the large group of patients with common variable immunodeficiency (CVID). In CVID patients, molecular diagnostics proves to be extremely difficult; until now genetic defects have been identified in $<10\%$ of patients (Grimbacher et al., 2003; Salzer et al., 2005; Van Zelm et al., 2006). The difficulties result from (1) the heterogeneity of the disease and

(2) the fact that in many cases complex genetic traits rather than monogenetic defects underlie the disease. As stated before, clinical and immunological characterization of the patient will help to guide molecular diagnostics. However, this could still generate multiple candidate genes. Consequently, a different approach using new molecular techniques is needed for large-scale screening and characterization of the genetic basis of disease in CVID.

In conclusion, molecular diagnostics are of great value in good clinical care and are gaining a prominent position in the management of individual patients. Many PID candidate genes have already been identified, but it can be anticipated that the number will increase further over the next decade. Precise clinical and immunological characterization of patients suffering from unknown PID in combination with new approaches and molecular techniques are needed to identify further candidate genes and disease-causing mutations.

References

Aiuti, A., Slavin, S., Aker, M., Ficara, F., Deola, S., Mortellaro, A., Morecki, S., Andolfi, G., Tabucchi, A., Carlucci, F., Marinello, E., Cattaneo, F., Vai, S., Servida, P., Miniero, R., Roncarolo, M. G., Bordignon, C. 2002. Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. Science 296:2410–2413

Antonarakis, S. E., Krawczak, M., Cooper, D. N. 2000. Disease-causing mutations in the human genome. Eur. J. Pediatr. 159 Suppl 3:S173–178

Bordignon, C., Notarangelo, L. D., Nobili, N., Ferrari, G., Casorati, G., Panina, P., Mazzolari, E., Maggioni, D., Rossi, C., Servida, P., Ugazio, A. G., Mavilio, F. 1995. Gene therapy in peripheral blood lymphocytes and bone marrow for ADA- immunodeficient patients. Science 270:470–475

Cavazzana-Calvo, M., Hacein-Bey, S., de Saint Basile, G., Gross, F., Yvon, E., Nusbaum, P., Selz, F., Hue, C., Certain, S., Casanova, J. L., Bousso, P., Deist, F. L., Fischer, A. 2000. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. Science 288:669–672

Cooper, D. N., Youssoufian, H. 1988. The CpG dinucleotide and human genetic disease. Hum. Genet. 78:151–155

De Vries, E. 2006. Patient-centred screening for primary immunodeficiency: a multi-stage diagnostic protocol designed for non-immunologists. Clin. Exp. Immunol. 145:204–214

Den Dunnen, J. T., Antonarakis, S. E. 2000. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. Hum. Mutat. 15:7–12

Geha, R. S., Notarangelo, L. D., Casanova, J. L., Chapel, H., Conley, M. E., Fischer, A., Hammarstrom, L., Nonoyama, S., Ochs, H. D., Puck, J. M., Roifman, C., Seger, R., Wedgwood, J. 2007. Primary immunodeficiency diseases: an update from the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee. J. Allergy. Clin. Immunol. 120:776–794

Green, P. M., Montandon, A. J., Bentley, D. R., Ljung, R., Nilsson, I. M., Giannelli, F. 1990. The incidence and distribution of CpG—TpG transitions in the coagulation factor IX gene. A fresh look at CpG mutational hotspots. Nucleic. Acids. Res. 18:3227–3231

Grimbacher, B., Hutloff, A., Schlesier, M., Glocker, E., Warnatz, K., Drager, R., Eibel, H., Fischer, B., Schaffer, A. A., Mages, H. W., Kroczek, R. A., Peter, H. H. 2003. Homozygous loss of ICOS is associated with adult-onset common variable immunodeficiency. Nat. Immunol. 4:261–268

Hacein-Bey-Abina, S., Le Deist, F., Carlier, F., Bouneaud, C., Hue, C., De Villartay, J. P., Thrasher, A. J., Wulffraat, N., Sorensen, R., Dupuis-Girod, S., Fischer, A., Davies, E. G., Kuis, W., Leiva, L., Cavazzana-Calvo, M. 2002. Sustained correction of X-linked severe combined immunodeficiency by *ex vivo* gene therapy. N. Engl. J. Med. 346:1185–1193

Hacein-Bey-Abina, S., Von Kalle, C., Schmidt, M., McCormack, M. P., Wulffraat, N., Leboulch, P., Lim, A., Osborne, C. S., Pawliuk, R., Morillon, E., Sorensen, R., Forster, A., Fraser, P., Cohen, J. I., de Saint Basile, G., Alexander, I., Wintergerst, U., Frebourg, T., Aurias, A., Stoppa-Lyonnet, D., Romana, S., Radford-Weiss, I., Gross, F., Valensi, F., Delabesse, E., Macintyre, E., Sigaux, F., Soulier, J., Leiva, L. E., Wissler, M., Prinz, C., Rabbitts, T. H., Le Deist, F., Fischer, A., Cavazzana-Calvo, M. 2003. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science 302:415–419

Hamosh, A., Scott, A. F., Amberger, J. S., Bocchini, C. A., McKusick, V. A. 2005. Online Mendelian Inheritance in Man (OMIM), a knowledgebase of human genes and genetic disorders. Nucleic. Acids. Res. 33:D514–517

Kanegane, H., Agematsu, K., Futatani, T., Sira, M. M., Suga, K., Sekiguchi, T., van Zelm, M. C., Miyawaki, T. 2007. Novel mutations in a Japanese patient with CD19 deficiency. Genes. Immun. 8:663–670

Kimura, H., Iyehara-Ogawa, H., Kato, T. 1994. Slippage–misalignment: to what extent does it contribute to mammalian cell mutagenesis? Mutagenesis 9:395–400

Krawczak, M., Cooper, D. N. 1991. Gene deletions causing human genetic disease: mechanisms of mutagenesis and the role of the local DNA sequence environment. Hum. Genet. 86:425–441

Kunkel, T. A. 1990. Misalignment-mediated DNA synthesis errors. Biochemistry 29:8003–8011

Kunkel, T. A., Soni, A. 1988. Mutagenesis by transient misalignment. J. Biol. Chem. 263:14784–14789

Maki, H. 2002. Origins of spontaneous mutations: specificity and directionality of base-substitution, frameshift, and sequence-substitution mutageneses. Annu. Rev. Genet. 36:279–303

McNaughton, J. C., Cockburn, D. J., Hughes, G., Jones, W. A., Laing, N. G., Ray, P. N., Stockwell, P. A., Petersen, G. B. 1998. Is gene deletion in eukaryotes sequence-dependent? A study of nine deletion junctions and nineteen other deletion breakpoints in intron 7 of the human dystrophin gene. Gene 222:41–51

Moshous, D., Pannetier, C., Chasseval Rd, R., Deist Fl, F., Cavazzana-Calvo, M., Romana, S., Macintyre, E., Canioni, D., Brousse, N., Fischer, A., Casanova, J. L., Villartay, J. P. 2003. Partial T and B lymphocyte immunodeficiency and predisposition to lymphoma in patients with hypomorphic mutations in Artemis. J. Clin. Invest. 111:381–387.

Noordzij, J. G., De Bruin-Versteeg, S., Comans-Bitter, W. M., Hartwig, N. G., Hendriks, R. W., De Groot, R., Van Dongen, J. J. M. 2002a. Composition of precursor B-cell compartment in bone marrow from patients with X-linked agammaglobulinemia compared with healthy children. Pediatr. Res. 51:159–168

Noordzij, J. G., De Bruin-Versteeg, S., Hartwig, N. G., Weemaes, C. M., Gerritsen, E. J. A., Bernatowska, E., Van Lierde, S., De Groot, R., Van Dongen, J. J. M. 2002b. XLA patients with *BTK* splice-site mutations produce low levels of wild-type *BTK* transcripts. J. Clin. Immunol. 22:306–318

Noordzij, J. G., De Bruin-Versteeg, S., Verkaik, N. S., Vossen, J. M. J. J., De Groot, R., Bernatowska, E., Langerak, A. W., Van Gent, D. C., Van Dongen, J. J. M. 2002c. The immunophenotypic and immunogenotypic B-cell differentiation arrest in bone marrow of RAG deficient SCID patients corresponds to residual recombination activities of mutated RAG proteins. Blood 100:2145–2152

Novoyatleva, T., Tang, Y., Rafalska, I., Stamm, S. 2006. Pre-mRNA missplicing as a cause of human disease. Prog. Mol. Subcell. Biol. 44:27–46

Piirila, H., Valiaho, J., Vihinen, M. 2006. Immunodeficiency mutation databases (IDbases). Hum. Mutat. 27:1200–1208

Rideout, W. M., 3rd, Coetzee, G. A., Olumi, A. F., Jones, P. A. 1990. 5-Methylcytosine as an endogenous mutagen in the human LDL receptor and p53 genes. Science 249:1288–1290

Rogozin, I. B., Pavlov, Y. I. 2003. Theoretical analysis of mutation hotspots and their DNA sequence context specificity. Mutat. Res. 544:65–85

Salzer, U., Chapel, H. M., Webster, A. D., Pan-Hammarstrom, Q., Schmitt-Graeff, A., Schlesier, M., Peter, H. H., Rockstroh, J. K., Schneider, P., Schaffer, A. A., Hammarstrom, L., Grimbacher, B. 2005. Mutations in TNFRSF13B encoding TACI are associated with common variable immunodeficiency in humans. Nat. Genet. 37:820–828

Sellner, L. N., Taylor, G. R. 2004. MLPA and MAPH: new techniques for detection of gene deletions. Hum. Mutat. 23:413–419

Stenson, P. D., Ball, E. V., Mort, M., Phillips, A. D., Shiel, J. A., Thomas, N. S., Abeysinghe, S., Krawczak, M., Cooper, D. N. 2003. Human Gene Mutation Database (HGMD): 2003 update. Hum. Mutat. 21:577–581

Streisinger, G., Okada, Y., Emrich, J., Newton, J., Tsugita, A., Terzaghi, E., Inouye, M. 1966. Frameshift mutations and the genetic code. This paper is dedicated to Professor Theodosius Dobzhansky on the occasion of his 66th birthday. Cold Spring Harb. Symp. Quant. Biol. 31:77–84

Van der Burg, M., De Groot, R., Comans-Bitter, W. M., Den Hollander, J. C., Hooijkaas, H., Neijens, H. J., Berger, R. M. F., Oranje, A. P., Langerak, A. W., Van Dongen, J. J. M. 2000. Autoimmune lymphoproliferative syndrome (ALPS) in a child from consanguineous parents: a dominant or recessive disease? Pediatr. Res. 47:336–343

Van der Burg, M., van Veelen, L. R., Verkaik, N. S., Wiegant, W. W., Hartwig, N. G., Barendregt, B. H., Brugmans, L., Raams, A., Jaspers, N. G., Zdzienicka, M. Z., van Dongen, J. J. M., van Gent, D. C. 2006. A new type of radiosensitive T-B-NK + severe combined immunodeficiency caused by a LIG4 mutation. J. Clin. Invest. 116:137–145

Van Zelm, M. C., Geertsema, C., Nieuwenhuis, N., de Ridder, D., Conley, M. E., Schiff, C., Tezcan, I., Bernatowska, E., Hartwig, N. G., Sanders, E. A., Litzman, J., Kondratenko, I., van Dongen, J. J. M., van der Burg, M. 2008. Gross deletions involving IGHM, BTK, or Artemis: a model for genomic lesions mediated by transposable elements. Am. J. Hum. Genet. 82:320–332

Van Zelm, M. C., Reisli, I., van der Burg, M., Castano, D., van Noesel, C. J., van Tol, M. J., Woellner, C., Grimbacher, B., Patino, P. J., van Dongen, J. J. M., Franco, J. L. 2006. An antibody-deficiency syndrome due to mutations in the CD19 gene. N. Engl. J. Med. 354:1901–1912

Youssoufian, H., Antonarakis, S. E., Bell, W., Griffin, A. M., Kazazian, H. H., Jr. 1988. Nonsense and missense mutations in hemophilia A: estimate of the relative mutation rate at CG dinucleotides. Am. J. Hum. Genet. 42:718–725

Youssoufian, H., Kazazian, H. H., Jr., Phillips, D. G., Aronis, S., Tsiftis, G., Brown, V. A., Antonarakis, S. E. 1986. Recurrent mutations in haemophilia A give evidence for CpG mutation hotspots. Nature 324:380–382

Human Genetic Resistance to Malaria

Thomas N. Williams

1 Introduction

Recent years have seen a major global drive towards the goal of identifying the genes associated with diseases of the developed world such as the cancers, heart disease, obesity, and stroke. Such research is largely predicated on the assumption that identifying such genes will yield fresh insights into the biology of these conditions that will, in time, result in novel approaches to their prevention and treatment. Genetic effects are likely to explain a considerable proportion of the risk of death from malaria (Mackinnon et al., 2005) and the resistance traits such as the sickle cell trait have often been used as prime examples to justify the gene discovery industry. Nevertheless, to date, the translation from studies of malaria–gene association to products that have improved the lot of those exposed to this common infection has proved profoundly disappointing. This chapter is by no means meant as a comprehensive review of malaria or human genetics which have been the subject of a number of excellent recent articles (Kwiatkowski, 2005; Arese, 2006; Kwiatkowski and Luoni, 2006). Instead, it discusses some of the reasons why progress has not been as rapid as we might have liked and concludes by explaining how this process is set to accelerate in the very near future.

2 Malaria

2.1 Health Impact of Malaria on Global Health and Development

A precise estimate of the global impact of malaria is impossible; morbidity and mortality can only be quantified accurately where appropriate diagnostic

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facilities exist, and the disease is most common in the poorest parts of the world where this rule simply does not apply. Nevertheless, recent estimates suggest that in 2002, 2.2 billion people were exposed to the threat of *P. falciparum* malaria leading to 515 million episodes worldwide (Snow et al., 2005). At least 70% of these events occurred in sub-Saharan Africa, placing an intolerable burden on the population, economic development, and national health infrastructures.

3 Malaria Biology

The biology of malaria is complex at many levels. It is caused by parasites of the genus *Plasmodium*, which includes 172 known species that infect reptiles, birds, and mammals, of which four species can infect man. The most virulent of these, *Plasmodium falciparum*, is probably the most recent of the human parasites, having been acquired via a host switch from birds perhaps as recently as 5,000–10,000 years ago (Boyd, 1949; Livingstone, 1958; Hoeprich, 1989).

Transmitted by female mosquitoes of the species *Anopheles*, which require blood to provide energy for their reproduction, approximately 80 of the 360 *Anopheline* species described to date are capable of transmitting the disease. Malaria-infected mosquitoes incidentally inject sporozoites as they feed and these motile forms access the circulation either directly or via the lymphatic system (Fig. 1). Once inside the human host, sporozoites target hepatocytes via a series of highly specific host–parasite interactions. The major protein expressed by sporozoites, circumsporozoite (CS) protein, binds specifically to the hepatocyte surface abutting the space of Disse (Cerami et al., 1992). Once inside the hepatocyte, sporozoites undergo a clinically silent period of incubation during which they multiply asexually, culminating in the release of many thousands of merozoites from each infected hepatocyte. These motile merozoites then invade red cells through a series of complex cellular interactions which, in *P. falciparum*, involves the parasite protein erythrocyte-binding antigen-175 (EBA-175) (Camus and Hadley, 1985) and sialic acid residues on the erythrocyte surface (Sim et al., 1994). Erythrocyte glycophorin A appears to be an important ligand in this regard, although *P. falciparum* merozoite binding can also be mediated by alternative receptor–ligand interactions (Mitchell et al., 1986; Dolan, Miller and Wellems, 1990). Within the red cell the parasite grows and matures, deriving nutrients both from the red cell contents (mainly haemoglobin) and from host serum. As the parasite grows it passes through a series of discreet morphological stages culminating in nuclear and cytoplasmic division and the production of an erythrocytic schizont. Each round of intraerythrocytic multiplication ends in red cell rupture and the release of daughter merozoites. These rapidly reinvade other red cells leading to a fresh asexual erythrocytic cycle and, at least initially, roughly logarithmic expansion of the parasite biomass.

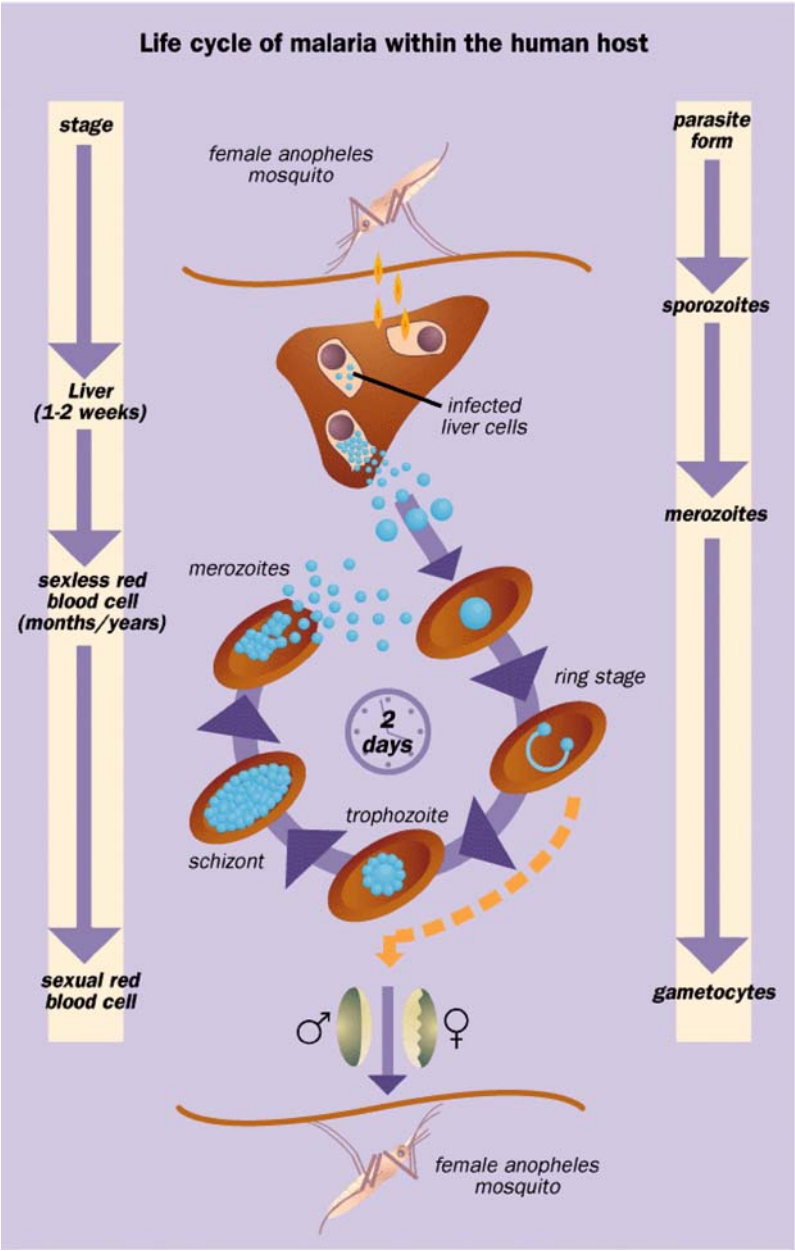

Fig. 1 The life cycle of malaria in the human host

4 Clinical Features

Both the presentation and clinical course of malaria are highly heterogeneous. In many malaria-endemic countries, malaria parasites can be detected in the blood in a high proportion of individuals at any one time yet the vast majority will be completely asymptomatic. Children in many parts of Africa suffer between one and two episodes of clinical malaria each year in their first few years of life. In general, these will be self-limiting or amenable to treatment with simple antimalarial drugs. Such mild clinical episodes usually begin with a prodromal illness lasting 2–3 days and progress into a phase that is dominated by paroxysms of fever accompanied by a range of non-specific additional features that may include sweats, headache, shivers and rigors, vomiting, confusions, and delirium. Typically, each year between 5% and 10% of these children will suffer a more severe episode requiring hospital admission. Only 10% of these cases will be characterized as 'severe' and associated with appreciable mortality.

5 Severe Malaria

While traditionally, severe malaria has been categorized into a number of distinct syndromes on the basis of clinical features (WHO, 1990), in practice, there appears to be considerable overlap between these syndromes (Marsh et al., 1995). Severe malaria probably results from a range of pathophysiological processes that may include haemolysis, cytokine, and nitrous oxide release and organ-specific vascular occlusion caused by sequestration (whereby parasite-infected red cells adhere to vascular endothelium) (Mackintosh, Beeson, and Marsh, 2004) and rosetting (the adherence of uninfected erythrocytes to trophozoite-infected red cells) (Chen, Schlichtherle, and Wahlgren, 2000). These processes can manifest in a range of severity features that may include convulsions and coma, respiratory distress, hypoglycaemia and shock, the precise clinical presentation presumably being determined by the balance of pathological events (Marsh et al., 1996; Maitland and Marsh, 2004).

6 Defining Malaria for the Purpose of Genetic Association Studies

The heterogeneity in the clinical presentation of malaria outlined above is at the heart of the first roadblock to the conduct and interpretation of studies that are aimed at discovering genes that might relate to malaria susceptibility. Given the biology of malaria as we currently understand it, genes could potentially relate to protection through a number of general mechanisms. For example, it is conceivable that there could be genes that reduce host attractiveness to mosquitoes or otherwise interfere with the process of parasite

inoculation; others might interfere with sporozoite invasion into the hepatocyte or their further development therein, while yet others might prevent the invasion of merozoites into red blood cells, accelerate the immune removal of parasites, or reduce the pathological consequences of the disease. It is quite conceivable that at a clinical level, while genes that result in such individual effects should all reduce the likelihood of death from malaria, they might have less predictable effects on intermediate forms of the disease such as asymptomatic parasite carriage or the incidence of mild clinical disease. Yet, these are the phenotypes that are most often considered in studies aimed at identifying malaria-protective genes.

To illustrate some of the difficulties that can be involved in the conduct and interpretation of such studies, there follow examples from two well-known conditions that have now been proven without doubt to be protective against malaria.

7 The Haemoglobinopathies: Sickle Cell Trait and α-Thalassaemia

Sickle haemoglobin is the most celebrated example of all the malaria-protective traits that have been described to date. Haemoglobin S (HbS) is a variant form of haemoglobin composed of two normal α-globin and two abnormal β-globin molecules (βs) ($\alpha_2\beta s_2$). Production of abnormal βs globin results from a point mutation of the β globin gene, such that the codon determining the amino acid at position β^6 is changed from GAG (coding for glutamic acid) to GTG (coding for valine). Homozygotes for the βs mutation suffer from sickle cell disease, a debilitating condition associated with premature death in most developing countries. Nevertheless, on the basis of surveys conducted during the 1940s and 1950s it became obvious that the carrier state (sickle cell trait; HbAS) was extremely common in much of sub-Saharan Africa and that its population frequencies mirrored almost exactly the historic map of malaria incidence (Livingstone, 1989). Epidemiological studies have since confirmed that sickle cell trait is 90% protective against severe and complicated malaria, being equally protective against all the subtypes that have so far been described (Willcox, Bjorkman, and Brohult, 1983; Hill et al., 1991; Williams et al., 2005a; May et al., 2007). Moreover, the trait is more than 60% protective against clinical malaria leading to hospital admission (Williams et al., 2005a; May et al., 2007; Marsh, 1992) and 50% protective against uncomplicated mild clinical attacks (Williams et al., 2005a). Even when children with HbAS do suffer clinical bouts, the parasite densities that are measurable in their circulation are typically 50–90% lower than those measured in normal children. All this is consistent with the conclusion that parasite-infected HbAS red blood cells are more rapidly removed from circulation than infected normal cells most probably by immunological mechanisms (Ayi et al., 2004). Nevertheless, it is striking that on the basis of studies conducted to date there is little evidence that

HbAS protects against infection per se, most studies showing no effect at the level of symptomless parasitaemia (Williams et al., 2005a). This suggests that gene discovery programmes that are based on identifying genes that protect against symptomless parasitaemia would almost certainly fail to identify HbS, the gene with the strongest protective effect against malaria of all those described so far. Here lies the first and most important lesson in malaria gene discovery, a lesson that is equally apposite in any gene discovery programme—that choosing the right clinical phenotype against which to measure the effects of specific genes in protection or predisposition to particular diseases is absolutely critical to the interpretation of such studies. In the case of malaria, the phenotypes that are likely to be most useful in uncovering previously unidentified genes are carefully characterised episodes of severe disease, categorized on the basis of well-defined clinical features.

8 The Thalassaemias

Unlike sickle cell trait, which is a condition characterized by the production of an abnormal form of haemoglobin, the thalassaemia syndromes are caused by the reduced production of normal haemoglobin. They fall into two main groups, the α- and β thalassaemias, characterized by underproduction of α- and β-globin, respectively (Weatherall and Clegg, 2002). As for HbS, the initial evidence supporting malaria protection by both these forms of thalassaemia was derived from population genetic studies. Surveys conducted throughout the world have shown that, as a group, the thalassaemias are the commonest single gene disorders of humans (summarized by Weatherall and Clegg, 2002). Overall, gene frequencies of >0.1 are the norm in tropical populations, whereas frequencies are somewhat lower in the subtropics and the conditions are rare in temperate zones. In fact, the only tropical populations in which the thalassaemias have not been found at polymorphic frequencies are in the New World, where malaria has only been introduced during the last few 100 years (Dunn, 1965). Isolated examples of extreme frequencies have been described in particular ethnic groups, particularly in certain tribes in India and Nepal (Brittenham et al., 1980; Kulozik et al., 1988; Labie et al., 1989; Modiano et al., 1991). In one of these tribal groups, the Tharu people of Nepal, the α thalassaemia gene frequency reaches 0.78 and there is some evidence to suggest that these people are also more resistant to malaria than their non-Tharu neighbours (Terrenato et al., 1988).

While the global distribution of α thalassaemia provides reasonable evidence for malaria protection, it is the extreme diversity of the molecular origins of this condition that lends the most compelling support for genetic selection as an explanation for its extraordinary distribution. The α thalassaemias have arisen throughout the world, through a wide variety of rare

independent genetic events. However, in some populations they have risen to polymorphic frequencies while in others they have not, suggesting differential selection according to location. While a number of diseases follow a tropical distribution, the probability that malaria was responsible for this selection was further supported by microepidemiological data from the Pacific. In a classic study conducted in the island archipelagos of Oceania, Flint and colleagues (Flint et al., 1986) made four important observations. First, they found that the population frequency of the α gene was consistently low in areas that are known historically to have been non-malarious. Second, they found that α thalassaemia was present in all malaria-exposed populations at gene frequencies that were proportional to the historic incidence of malaria: a trend was found in the α thalassaemia haplotype frequencies both from north to south and with increasing altitude, each of which were associated with similar trends in the historical incidence of malaria. Third, even within this relatively small area, the genetic deletions responsible were numerous and regionally specific. Finally, the population frequencies of other 'neutral' genetic markers including the γ-globin haplotypes -γ and -γγγ and the haptoglobin polymorphism Hp^1, for which there is no evidence of malaria protection, showed no such correlation. Although these observations provided compelling circumstantial evidence for malaria protection by α thalassaemia, in general, results from clinico–epidemiological studies that have been conducted with the aim of proving this protection have proved somewhat confusing. For example, while it is now proven that α thalassemia is associated with protection against both severe (May et al., 2007; Allen et al., 1997; Mockenhaupt et al., 2004; Williams et al., 2005b; Wambua et al., 2006) and fatal (Williams et al., 2005b) malaria on the basis of both cohort and case-control data, this protection does not appear to be uniform against all subtypes of severe disease, being confined to the patients whose clinical picture is complicated by anaemia (Williams et al., 2005b; Wambua et al., 2006; May et al., 2007). Moreover, studies investigating the protective effect of α thalassaemia at the level of uncomplicated malaria have yielded inconsistent findings. Those conducted in the Pacific have tended to show an increased, rather than a reduced incidence of uncomplicated malaria in such children (Oppenheimer et al., 1987; Williams et al., 1996), while one study conducted in Africa identified a small but insignificant effect in the direction of protection. No study conducted to date has found any effect of α thalassaemia at the level of parasite density. The mechanism by which α thalassaemia protects against malaria remains controversial. Whatever the answer, these observations have taught us the lesson that even this genetic variant, one that is presumed to have risen to its current massive frequencies in much of the tropical world through selection driven by malaria, might well be missed in many gene-discovery studies without carefully focusing on the malaria phenotype against which it is protective.

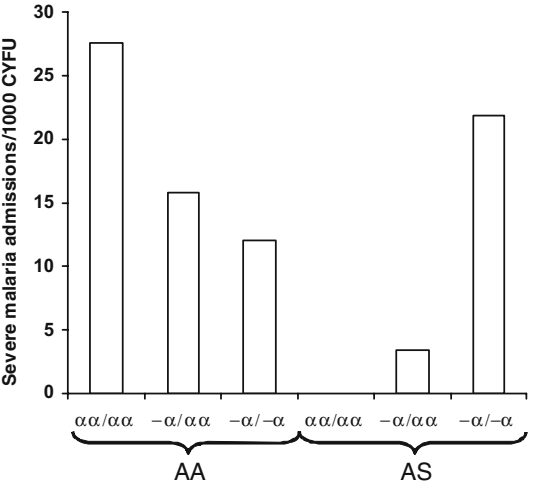

Fig. 2 Epistasis between the genes for α thalassaemia and HbS: The incidence of severe *P. falciparum* malaria in children of different genotype. αα/αα: normal at the α globin locus; −α/αα: heterozygous α^+ thalassaemia; −α/−α: homozygous; AA: normal at the β-globin locus; AS: sickle cell trait. (CYFU – child years of follow-up)

9 Genetic Epistasis

Two studies have recently confirmed what has long been suspected: that when designing and interpreting disease-association studies, it cannot necessarily be assumed that all genes will act independently of one another. In both Kenya (Wambua et al., 2006; Williams et al., 2005c) (Fig. 2) and Ghana (May et al., 2007), it was found that while independently HbAS is strongly protective against all forms of clinical malaria and is associated with reduced parasite densities during the remaining clinical attacks and α thalassaemia is protective against severe malaria anaemia, the protective effects of both genes are lost when both are inherited together. This 'epistatic' interaction, whereby in any individual subject the fitness effect of an allele at one genetic locus is dependent on the genotype with which it is co-inherited at a second, unrelated locus, has a number of important scientific implications. On the one hand, if it occurs commonly, it could make malaria-protective associations more difficult to detect and could complicate the search for mechanisms. On the other, epistatic interactions may provide a powerful tool for dissecting the mechanisms by which specific genes result in protection against malaria.

10 Summary and Conclusions

This brief chapter highlights the need for caution when designing and interpreting studies aimed at seeking new genes that may be associated with malaria protection, or investigating the potential mechanisms for protection in promising candidates. Judging genetic effects on the basis of the wrong clinical phenotype and missing true protective genes because their protective effects are

masked by unpredictable epistatic effects are major potential pitfalls. These issues are by no means unique to malaria: in recent years, the importance of larger sample sizes and careful phenotypic definitions have become appreciated increasingly, particularly for genome-wide studies of complex diseases (Cordell and Clayton, 2005; Burton, Tobin and Hopper, 2005). Until recently, research in the field of malaria genetics has not enjoyed the sort of funding afforded to similar work investigating diseases of importance to the developed world. However, in the last few years, coupled with advances in genetic diagnostics that have led to massive automation and falling costs per gene explored, momentum has grown towards more generous funding that brings with it the opportunity for much larger, multisite cohesive studies. The stage is set for a giant leap forward in the coming years.

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References

Allen, S.J. et al. Alpha(+)thalassemia protects children against disease caused by other infections as well as malaria. *Proc Natl Acad Sci U S A* **94**, 14736–41 (1997).

Arese, P. How genetics and biology helped humanity to survive falciparum malaria. *Parassitologia* **48**, 553–9 (2006).

Ayi, K., Turrini, F., Piga, A. and Arese, P. Enhanced phagocytosis of ring-parasitized mutant erythrocytes. A common mechanism that may explain protection against falciparum malaria in sickle trait and beta-thalassemia-trait. *Blood* **104**, 3364–71 (2004).

Boyd, M.F. A comparative study of all aspects of this group of diseases from a global standpoint. in *Malariology*, Vol. 1 (ed. Boyd, M.F.) 3–25 (Saunders, Philadelphia, 1949).

Brittenham, G. et al. Alpha globin gene number: population and restriction endonuclease studies. *Blood* **55**, 706–9 (1980).

Burton, P.R., Tobin, M.D. and Hopper, J.L. Key concepts in genetic epidemiology. *Lancet* **366**, 941–51 (2005).

Camus, D. and Hadley, T.J. A plasmodium falciparum antigen that binds to host erythrocytes and merozoites. . *Science* **230**, 553–6 (1985).

Cerami, C. et al. The basolateral domain of the hepatocyte plasma membrane bears receptors for the circumsporozoite protein of Plasmodium falciparum sporozoites. *Cell* **70**, 1021–33 (1992).

Chen, Q., Schlichtherle, M. and Wahlgren, M. Molecular aspects of severe malaria. *Clin Microbiol Rev* **13**, 439–50 (2000).

Cordell, H.J. and Clayton, D.G. Genetic association studies. *Lancet* **366**, 1121–31 (2005).

Dolan, S.A., Miller, L.H. and Wellems, T.E. Evidence for a switching mechanism in the invasion of erythrocytes by Plasmodium falciparum. *J Clin Invest* **86**, 618–24 (1990).

Dunn, F.L. On the antiquity of malaria in the Western hemisphere. *Hum Biol* **37**, 385–93 (1965).

Flint, J. et al. High frequencies of alpha-thalassaemia are the result of natural selection by malaria. *Nature* **321**, 744–50 (1986).

Hill, A.V. et al. Common West African HLA antigens are associated with protection from severe malaria. *Nature* **352**, 595–600 (1991).

Hoeprich, P.D. Host-parasite relationships and the pathogenesis of infectious disease. in *Infectious diseases*, Vol. 1 (ed. Hoeprich, P.D.J.M.C.) 41–53 (Lippencott, Philadelphia, 1989).

Kulozik, A.E., Kar, B.C., Serjeant, G.R., Serjeant, B.E. and Weatherall, D.J. The molecular basis of alpha thalassemia in India. Its interaction with the sickle cell gene. *Blood* **71**, 467–72 (1988).

Kwiatkowski, D.P. How malaria has affected the human genome and what human genetics can teach us about malaria. *Am J Hum Genet* **77**, 171–92 (2005).

Kwiatkowski, D.P. and Luoni, G. Host genetic factors in resistance and susceptibility to malaria. *Parassitologia* **48**, 450–67 (2006).

Labie, D. et al. Haplotypes in tribal Indians bearing the sickle gene: evidence for the unicentric origin of the beta S mutation and the unicentric origin of the tribal populations of India. *Hum Biol* **61**, 479–91 (1989).

Livingstone, F.B. Anthropological implications of sickle cell gene distribution in West Africa. *Am J Anthropol* **60**, 533–62 (1958).

Livingstone, F.B. Simulation of the diffusion of the beta-globin variants in the Old World. *Hum Biol* **61**, 297–309 (1989).

Mackinnon, M.J., Mwangi, T.W., Snow, R.W., Marsh, K. and Williams, T.N. Heritability of malaria in Africa. *Plos Med* **2**, e340 (2005).

Mackintosh, C.L., Beeson, J.G. and Marsh, K. Clinical features and pathogenesis of severe malaria. *Trends Parasitol* **20**, 597–603 (2004).

Maitland, K. and Marsh, K. Pathophysiology of severe malaria in children. *Acta Trop* **90**, 131–40 (2004).

Marsh, K. Malaria – a neglected disease? *Parasitology* **104**, S53–69 (1992).

Marsh, K. et al. Indicators of life-threatening malaria in African children. *N Engl J Med* **332**, 1399–1404 (1995).

Marsh, K., English, M., Crawley, J. and Peshu, N. The pathogenesis of severe malaria in African children. *Ann Trop Med Parasitol* **90**, 395–402 (1996).

May, J. et al. Hemoglobin variants and disease manifestations in severe falciparum malaria. *Jama* **297**, 2220–6 (2007).

Mitchell, G.H., Hadley, T.J., McGinniss, M.H., Klotz, F.W. and Miller, L.H. Invasion of erythrocytes by Plasmodium falciparum malaria parasites: evidence for receptor heterogeneity and two receptors. *Blood* **67**, 1519–21 (1986).

Mockenhaupt, F.P. et al. α^+ thalassemia protects African children from severe malaria. *Blood* **104**, 2003–2006 (2004).

Modiano, G. et al. Protection against malaria morbidity: near-fixation of the alpha- thalassemia gene in a Nepalese population. *Am J Hum Genet* **48**, 390–7 (1991).

Oppenheimer, S.J. et al. The interaction of alpha thalassaemia with malaria. *Trans R Soc Trop Med Hyg* **81**, 322–6 (1987).

Sim, B.K., Chitnis, C.E., Wasniowska, K., Hadley, T.J. and Miller, L.H. Receptor and ligand domains for invasion of erythrocytes by Plasmodium falciparum. *Science* **264**, 1941–4 (1994).

Snow, R.W., Guerra, C.A., Noor, A.M., Myint, H.Y. and Hay, S.I. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature* **434**, 214–7 (2005).

Terrenato, L. et al. Decreased malaria morbidity in the Tharu people compared to sympatric populations in Nepal. *Ann Trop Med Parasitol* **82**, 1–11 (1988).

Wambua, S. et al. The effect of α^+-thalassaemia on the incidence of malaria and other diseases in children living on the coast of Kenya. *PLoS Med* 3, 5, e158 (2006).

Weatherall, D.J. and Clegg, J.B. *The thalassaemia syndromes*, (Blackwell Scientific Publications, Oxford, 2002).

Willcox, M., Bjorkman, A. and Brohult, J. Falciparum malaria and beta-thalassaemia trait in northern Liberia. *Ann Trop Med Parasitol* **77**, 335–47 (1983).

Williams, T.N. et al. High incidence of malaria in alpha-thalassaemic children. *Nature* **383**, 522–5 (1996).

Williams, T.N. et al. Sickle cell trait and the risk of *Plasmodium falciparum* malaria and other childhood diseases. *J Infect Dis* **192**, 178–86 (2005a).

Williams, T.N. et al. Both heterozygous and homozygous alpha + thalassemias protect against severe and fatal *Plasmodium falciparum* malaria on the coast of Kenya. *Blood* **106**, 368–71 (2005b).

Williams, T.N. et al. Negative epistasis between the malaria-protective effects of α^+-thalassemia and the sickle cell trait. *Nat Genet* **37**, 1253–7 (2005c).

World_Health_Organization. Severe and complicated malaria. World Health Organization, Division of Control of Tropical Diseases. *Trans R Soc Trop Med Hyg* **84 Suppl 2**, 1–65 (1990).

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