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REVIEWS

Pathogenesis and histopathology of pertussis: implications for immunization

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Pertussis is a unique infectious disease in that it can be severe and fatal but occurs without fever and other evidence of an inflammatory illness. The authors with others have studied the histopathology of fatal pertussis and also the unique characteristics of severe pertussis in young infants. Histopathologic observations from approximately 100 years ago, and from recent evaluation, indicate that the histopathologic changes of the upper respiratory tract of patients with fatal pertussis are often relatively normal unless there is a secondary bacterial infection. *Bordetella pertussis* contains many protein antigens and perhaps a polysaccharide capsule which contribute to the infectious process. However, only two of these antigens contribute to clinical illness. These antigens are pertussis toxin and the yet to be identified 'cough toxin'. The authors speculate as to the nature of the 'cough toxin' and discuss the implications of their observations and concepts for the future control of pertussis.

KEYWORDS: histopathology • immunization • pathogenesis • pertussis • vaccine

Background

One of the authors (JDC) has been involved in the study of pertussis and its prevention for 37 years [1–34]. During this period, many misconceptions relating to epidemiology, vaccine effectiveness and immunity have been noted. Most recently, the authors, in conjunction with a number of other investigators, have examined closely the pathology of fatal *Bordetella pertussis* infections in young infants [8,28–30]. These studies have resulted in some findings that are at variance with some present beliefs. These experiences have led to this review and the opinions that are presented here.

History

Pertussis is a unique infectious disease [16,26,35,36]. Compared with other classic epidemic infectious diseases such as measles, polio or smallpox, it lacks an ancient history. Even though it is often a severe disease with considerable morbidity and mortality in young infants and deaths in adults as well, it occurs without significant fever unless there is a coinfection or secondary infection with another agent. It has a nonproductive, paroxysmal

cough, which is unique and does not occur in all other respiratory illnesses. As noted by W. H. Holmes over 70 years ago, 'The victim has actual convulsions of coughing which recur explosively at irregular intervals. Between the spasmodic attacks, breathing is just as free and easy as that of any healthy child' [35].

Throughout the first half of the last century, the causative organism (*B. pertussis*) was isolated and subsequently effective pertussis vaccines were developed [16,26,37]. When these vaccines were universally used in children, the rate of reported pertussis was dramatically reduced [1,16,26]. However, during the last 40 years of the 20th century, vaccine reactions (true reactions and temporally related neurologic events) led to a push to develop 'safer' less reactogenic vaccines [15–17,26]. During the same period, *B. pertussis* was studied extensively in the laboratory and much of the information was derived from studies using organ culture, mice and other non-human animal models [8,16,26]. Acellular pertussis vaccines, which are less reactogenic because of the removal of endotoxin, were developed during the last 25 years of the 20th century and are now used in many countries throughout the world [6,8,16].

Studies during the last 35 years document two different epidemiologies – one relating to reported pertussis and the other relating to *B. pertussis* infection [5]. Reporting of pertussis depends upon the reporting systems in a particular district, state or country. In contrast, studies of *B. pertussis* infection have noted that about 15% of prolonged, afebrile cough illnesses in adolescents and adults are due to *B. pertussis* infection and that infection rates in populations can be as high as 6%. Also, prospective studies indicate a symptomatic clinical illness rate in adolescents and adults of 370–500 per 100,000 [34,38]. In addition, other studies indicate that these adolescent and adult illnesses were also occurring in the pre-vaccine era [2]. In fact, the cyclical nature of reported pertussis outbreaks today is similar to that of the pre-vaccine era [1,16,26,27,39]. These data indicate that immunization reduced reported pertussis but did not affect the overall circulation of *B. pertussis*.

Over the last 30 years, there has been a general increase in the rate of reported pertussis [16]. This increase changed dramatically during the last 10 years [6]. There are a number of possible reasons for the resurgence of reported pertussis. The most important of these is a greater awareness because of the many publications relating to the development of acellular vaccines. Also of importance is the use of new diagnostic tests such as PCR and single serum serologic assay. In addition, it is very clear that acellular vaccines are not as good as whole cell vaccines [7,8,16]. Finally, since acellular vaccines contain a small number of antigens, it is likely that genetic changes in *B. pertussis* also may contribute to vaccine failure.

Clinical manifestations of *B. pertussis* infections

Infection with *B. pertussis* can result in asymptomatic infection, mild cough illness and typical illness with paroxysmal cough, whoop and posttussive vomiting [16,18,26]. The manifestations in an individual depend upon many factors including previous infection or immunization and transplacentally acquired antibody in a young infant. Other factors include: sex, characteristics of the organism and dose [1,16,18,26]. Once an individual has had an infection or has been vaccinated, leukocytosis with lymphocytosis does not occur with infection. In contrast, all primary infections in children (who do not have passively acquired antibodies) will be associated with leukocytosis with lymphocytosis at various degrees of magnitude. Serologic studies looking for the presence of IgA antibodies (a marker of past infection and not immunization) indicate that by 10 years of age, approximately 70% have had a previous infection and by age 20, everyone has had previous infection [9,10,18]. Contrary to popular belief, adults may have typical pertussis except that leukocytosis with lymphocytosis does not occur [16,18,26,31,40,41]. Fever is not a clinical manifestation of *B. pertussis* infection unless there is a secondary infection with another infectious agent. However, patients often report that they feel warm and parents state that their child is feverish. In a study of 31 infants ≤ 90 days of age with severe pertussis including 6 deaths, whom the authors' group monitored very closely, 30 remained afebrile

throughout their pediatric intensive care experience [CHERRY JD, UNPUBLISHED DATA].

Pathology/histopathology

In Lapin's book published in 1943 on 'Whooping Cough', there is a 14-page chapter on the pathology of pertussis [5,36]. In this chapter, he reviewed 35 citations relating to postmortem data on young children dying of pertussis. Then, as is the situation today, there are no detailed histopathologic data of patients who have survived mild or typical pertussis.

Approximately 100 years ago, Mallory and Horner [42] described the microscopic findings in three children who died of whooping cough. The first child was a 14-month-old girl who died on the 16th day of cough illness. During the last 3 days of life, she had significant fever suggesting to us that she had a superimposed infection. The second child was a 2 $\frac{3}{4}$ -year-old girl who was convalescing from measles and also had findings suggestive of a secondary bacterial infection; her cough illness duration was approximately 6 weeks and she died on the 2nd hospital day. The third child was a 2 $\frac{3}{4}$ -year-old boy who had died 15 years previously. He had an illness duration of approximately 16 days. This child also developed fever shortly before death. Although the authors describe loss of ciliated cells and reduction of the cilia to 'short stubs', their drawings and pictures display relatively normal-appearing ciliated epithelial cells with large numbers of bacteria between the cilia of the cells lining the trachea, bronchi and bronchioles (FIGURE 1A & 1B).

In 1969, Christie and Baltimore [43] reported the deaths of three neonates all of whom had extreme leukocytosis with lymphocytosis. Postmortem examination in one of these infants revealed 'bilateral pulmonary edema and focal hemorrhages'; there was 'diffuse infiltration by macrophages and moderate focal infiltration with inflammatory cells'. This infant also had 'extensive necrotizing bronchopneumonia and thromboemboli'.

In 2008, we described the histopathologic findings in respiratory tissue specimens from 15 infants ≤ 4 months of age who had *B. pertussis* infections [30]. In this group, six were found to have ≥ 1 infection with a viral or bacterial pathogen. We did not find a difference in histopathologic features in those with co-infections in comparison with those in whom we only demonstrated *B. pertussis* infection. Low-to-moderate temperature elevation (37.5–39.1°C) was noted in 8 of the 10 in whom temperature data were reported. Of the 13 infants whose white blood cell count data were available, all had peak counts $\geq 70 \times 10^9$ cells/l and all had remarkable lymphocytosis. Radiographic evidence of pneumonia was documented in eight infants. Also documented were respiratory failure in 10 infants, shock in 11 infants and pulmonary hypertension in 5 infants.

Necrotizing bronchiolitis and intra-alveolar hemorrhage were noted in all 14 infants with available specimens. Fibrinous intra-alveolar edema, macrophage-rich alveolar infiltrates and lymphangiectasia were noted in 13 of the 14 infants with available specimens. Other common findings were neutrophilic bronchopneumonia, diffuse alveolar damage, pleural edema or

hemorrhage, interlobular septal edema and fibrin thrombi. FIGURE 2A–2D shows the following histopathologic characteristics: A. abundant bordetellae in the cilia of respiratory epithelial cells; B. necrotizing bronchiolitis; C. abundant intra-alveolar macrophages; and D. aggregates of mixed leukocytes in a pulmonary arteriole. In FIGURE 3A–3C, immunohistochemical staining of *B. pertussis* bacteria in the airways and airspaces is presented: A. tangles of *B. pertussis* in the cilia of tracheal epithelium; B. abundant intra- and extracellular bacteria in alveolar infiltrates; and C. intracellular *B. pertussis* antigens in the columnar epithelia of a bronchiole.

In 2009, Sawal and colleagues [44] described the histological features in 10 infants ≤ 8 weeks of age who died from *B. pertussis* infection. All had necrotizing bronchitis, bronchiolitis and pneumonia in varying degrees. Four of the 10 had diffuse alveolar damage. Eight of the 10 had severe cortical depletion in the thymus. They also noted lymphocyte depletion in lymph nodes from 6 infants and white pulp depletion in the spleens of 9 of 10 of the infants.

Encephalopathy associated with *B. pertussis* infection is not uncommon in young infants. In relation to this, pathologic changes in the brain had been noted in the past, but no recent data are available [36,45]. Microscopic or gross cerebral hemorrhage has been noted along with cortical atrophy.

The evaluation of the pathologic findings and the microbiologic findings in fatal pneumonias is often problematic because of mixed infections, sequential infections with one or more agents and postmortem changes. On comparing the *B. pertussis* death cases with influenza and pneumococcal death cases in young infants, one fact stands out. Specifically, in *B. pertussis* infections, the ciliated epithelial cells of the trachea and bronchi are generally well-preserved (FIGURES 1A, 1B & 3A), whereas the respiratory epithelium of the large and medium-sized airways is frequently severely damaged in patients with seasonal influenza and pneumococcal infections. Also, specific for *B. pertussis* infections are the aggregates of leukocytes in the arterioles, venules and lymphatic channels (FIGURE 3D).

Biologically active & antigenic components of *B. pertussis*

After a person is exposed to a bacterial pathogen, the pathogenesis of infection and disease depends upon four important steps: attachment, evasion of host defenses, local damage and systemic spread [16,26]. Over the last 100 years, a number of biologically active and antigenic components of *B. pertussis* have been described [8,15,16,26]. The functions of these proteins and their antigenicity have largely been demonstrated in studies using mice and organ cultures as models for human infection. During the 1970s, the knowledge about various *B. pertussis* antigens led to suggestions about specific toxins and attachment proteins (adhesins). Recognized toxins were: pertussis toxin (PT), adenylate cyclase toxin (ACT), dermonecrotic toxin, tracheal cytotoxin and lipopolysaccharide. Recognized adhesins were: filamentous hemagglutinin (FHA), pertactin and fimbriae.

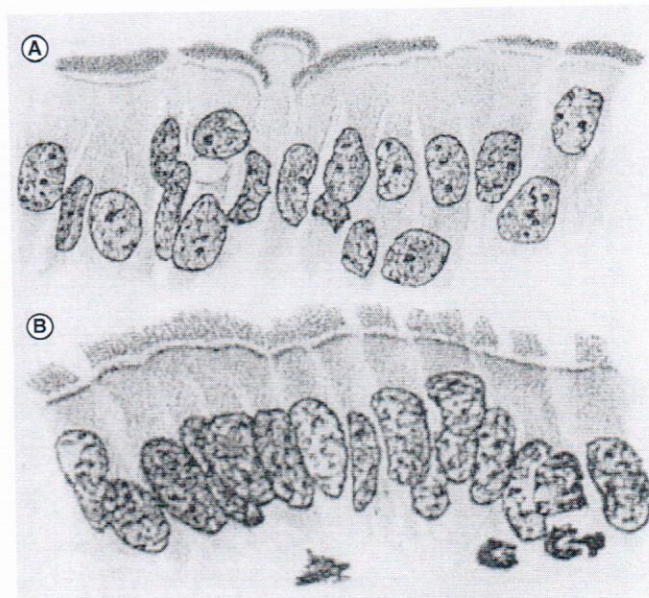


Figure 1. Drawings by Miss Etta R Piotti of ciliated epithelium of a child dying with pertussis. (A) Ciliated epithelium lining the trachea of a child dying in acute stage of whooping cough. Large numbers of minute bacilli present between the cilia, $\times 1000$. (B) Ciliated epithelium lining the bronchus of a child dying in acute stage of whooping cough. Masses of minute bacilli present between the cilia, $\times 1000$. Reproduced with permission from [42].

PT is an important toxin [26]. It is an ADP-ribosylating toxin which is synthesized and secreted exclusively by *B. pertussis*. It is an A–B toxin composed of six polypeptides (S1–S5). S1 is the A subunit and S2–5 form the B subunit. The S1 subunit inactivates G-proteins (guanine nucleotide-binding proteins) in eukaryotic (host) cells. The biologic activities of the A subunit in humans include a lymphocytosis promoting effect and pancreatic islet cell activation. PT does not cause the paroxysmal cough in pertussis. We know this because the paroxysmal cough also occurs in *Bordetella parapertussis* infection and this organism does not liberate PT.

Dermonecrotic toxin was described over 100 years ago [26,46]. Its effect in the animal model was that of severe tissue necrosis. More recently, tracheal cytotoxin was described [26,47]. Studies with this toxin in hamster tracheal organ culture, hamster tracheal epithelial cells and human respiratory epithelial cells revealed that it damaged ciliated cells but not nonciliated cells. However, the present and past studies of the authors as well as the data presented by Mallory and Horner, in fatal cases in young infants did not, for the most part, demonstrate damage to the ciliated cells of the large airways (FIGURES 1A, 1B & 3A) [30,42]. The authors and Mallory and Horner demonstrated abundant bacteria attached to the ciliated cells in the trachea and bronchi, but the cells themselves were largely intact.

Presented in TABLE 1 are biologically active and antigenic components of *B. pertussis* [8,15,16,26,46–62]. As observed in TABLE 1,

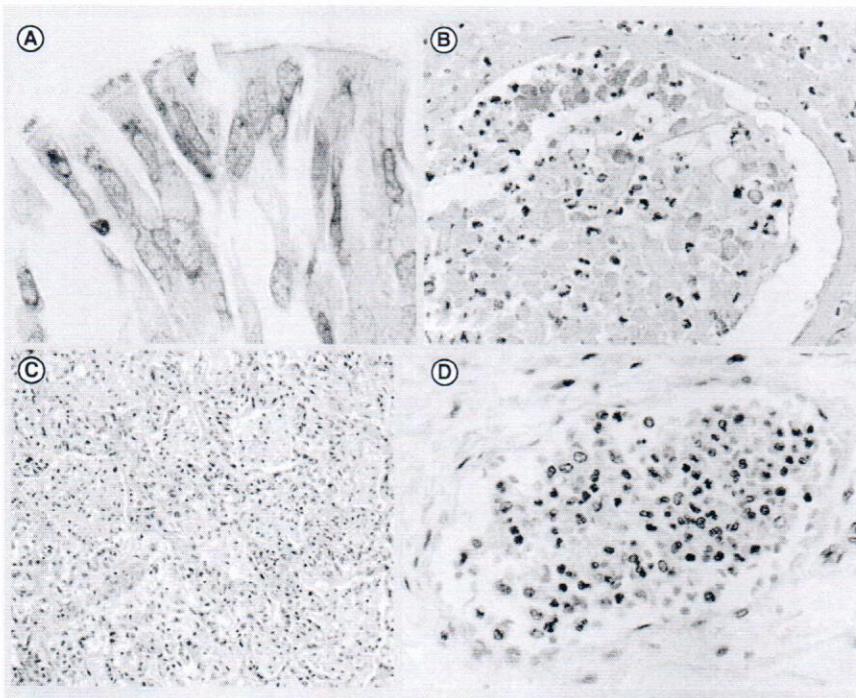


Figure 2. Selected histopathologic finding in the lungs of young infants dying with pertussis. (A) Distribution of *Bordetella pertussis* bacteria within the cilia of respiratory epithelial cells lining the trachea of an infant with fatal pertussis (Giemsa stain, original magnification $\times 250$). (B) Sloughed and necrotic airway epithelium admixed with predominantly neutrophilic infiltrates in the lumen of a bronchiole (hematoxylin and eosin stain, original magnification $\times 100$). (C) Diffuse intra-alveolar collections of macrophages in the lung of an infant with fatal pertussis (hematoxylin and eosin stain, original magnification $\times 25$). (D) Leukocyte aggregate in the lumen of a venule in the lung of an infant with fatal pertussis (hematoxylin and eosin stain, original magnification $\times 50$).

there are a limited number of adhesins and a large number of toxins. In addition, some antigens are listed as both adhesins and toxins. It is also important to separate antigens that participate in infections from those that cause clinical disease. This is examined in the next section.

The association of *B. pertussis* antigens & infection & clinical illness

B. pertussis infection

All antigens listed as adhesins in TABLE 1 have been demonstrated to have roles in the colonization or persistence of infection in the respiratory tract of experimental animals [8,15,16,26,46,63]. In addition, the B subunit of PT, ACT and lipopolysaccharide may also mediate colonization. All autotransporters (pertactin, Vag8, BrkA, BapC, SphB1 and TchA) play a role in blocking the innate immune response. The A subunit of PT and ACT and lipopolysaccharide also have roles in inhibiting the innate immune response, which allows the infection to progress. Fimbriae 2 and 3 are attachment proteins and the B subunit of PT may also function as an adhesin.

Although FHA has been considered an important adhesin for approximately 30 years, this perhaps should be questioned [26]. FHA is secreted, which is not a useful characteristic

of an adhesin except in a decoy role. In addition, in two studies of serologic correlates of protection in children, antibody to FHA did not seem to have a role in protection [11,63].

It has been stated that a *B. pertussis* capsule was identified 80 years ago, but until recently, this had not been confirmed [59]. In 2010, Neo *et al.* [59] presented evidence for an intact *B. pertussis* polysaccharide capsule. This capsule functions as an adhesin and confers protection from complement-mediated killing [49,51].

Using murine models, it has been shown that innate immune mechanisms involve dendritic cells, macrophages, neutrophils, natural killer cells and antimicrobial peptides in the process of controlling infection [63]. In addition, however, in this model, complete bacterial clearance requires cellular immunity mediated by Th1 and Th17 cells. These cellular immune response findings have recently been confirmed in the baboon model [64,65].

Clinical pertussis

In contrast with *B. pertussis* infection in which all the toxins given in TABLE 1 participate, clinical pertussis is a toxin-mediated disease caused by just two toxins: specifically, the A subunit of PT (which inhibits host cell G-proteins) and a second as-yet unidentified toxin that causes the unique cough ('cough toxin'). PT causes leukocytosis with lymphocytosis, pulmonary hypertension, shock and organ failure in young infants [16,26,28–30]. The authors with their other colleagues at the CDC [30] presented a proposed mechanism for the pathogenesis of pulmonary hypertension in infants with fatal pertussis. This suggested that the increased vascular resistance caused by the aggregates of leukocytes in the small pulmonary vessels made the pulmonary hypertension non-responsive to conventional treatment modalities. This concept was considered by others and is the basis for leukodepletion therapy in severe infant pertussis. However, it occurred to the authors and their California pertussis researchers that this hypothesis might be wrong [28]. Specifically, leukocytosis with lymphocytosis might be the marker for the event, but not the cause. It is possible that the inhibition of G-proteins in the heart and/or lungs in the infant may contribute to cardiac failure or respiratory failure and indirectly contribute to the refractory pulmonary hypertension. If this is the case, therapeutic exchange blood transfusion would work not by leukocyte reduction but by removal of PT from the blood and its role in cardiac or pulmonary failure. After primary exposure to PT by

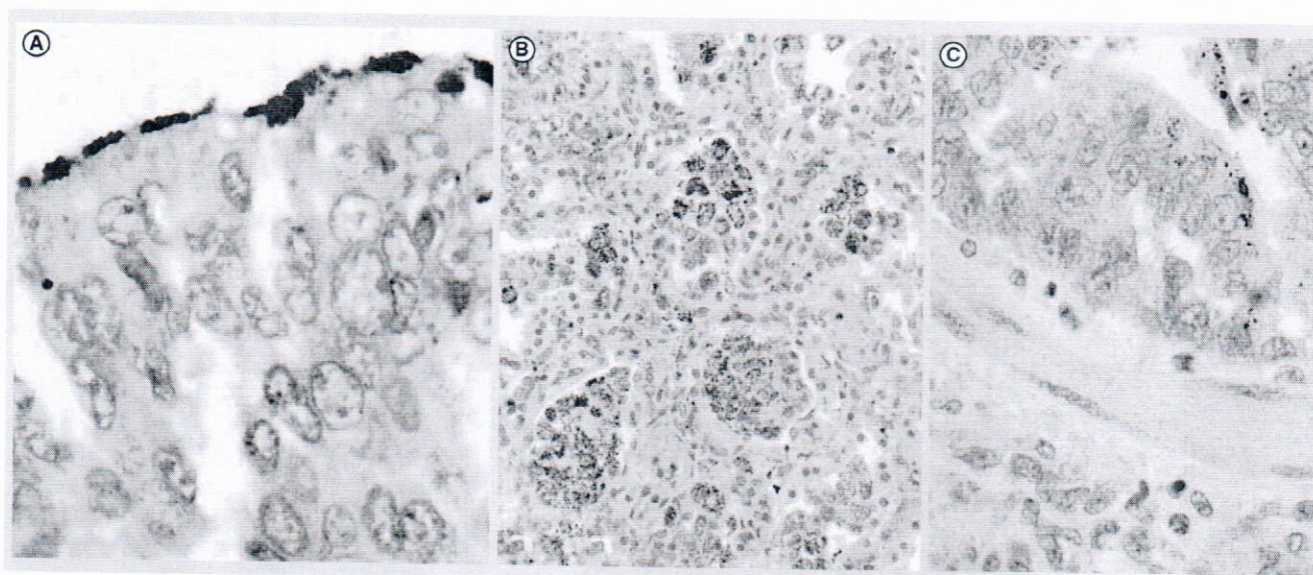


Figure 3. Extracellular and intracellular antigens of *Bordetella pertussis* in the airways and lungs of infants with fatal pertussis. (A) Abundant bacterial antigens and intact bordetellae enmeshed in the cilia of respiratory epithelium. **(B)** Bacterial antigens within alveolar macrophages. **(C)** Intracellular antigens in the respiratory epithelial cells of a bronchiole. Immunoalkaline phosphatase technique with naphthol-fast red and hematoxylin counterstain, original magnifications $\times 158$ (A and C) and $\times 50$ (B).

immunization or natural infection, the clinical manifestations of PT never reoccur with subsequent infections, presumably because of a primed and rapid antibody response to this toxin.

In persons who have previously responded to PT, subsequent manifestations of *B. pertussis* infection are caused solely by the as-yet unidentified 'cough toxin'. Because persons of all ages experience the characteristic cough associated with *B. pertussis* infection, it would seem that the 'cough toxin' is poorly immunogenic or that immunity to it wanes rapidly.

The genome size of *B. pertussis* is approximately 4,086,186 bp. There are approximately 3816 predicted genes and 3438 predicted proteins. Of the predicted proteins, 315 are poorly defined. When the authors first became interested in the concept of 'cough toxin', it seemed that it was likely a protein and one or more of those poorly defined. More recently, they started thinking that the 'cough toxin' could be related to the polysaccharide capsule. There are some bits of evidence that make this hypothesis attractive. Diphtheria and tetanus toxoids and whole cell pertussis vaccine (DTwP) vaccines are made from laboratory-grown strains and it is possible that the final product does not contain a capsule. If this were the case, protection from cough illness by immunization would depend upon antibodies to proteins that present infection; no 'anti-cough toxin' antibody would occur. In addition, polysaccharides (unless linked to a protein) are poorly immunogenic in the first 2 years of life.

Implications of our concepts for immunization

Pertussis in young infants

Pertussis in young infants is a severe illness which can often be fatal. Since this severe illness is due to the effects of PT, all of our presently available vaccines (DTwP, diphtheria and tetanus

toxoids and acellular pertussis vaccine (DTaP) and tetanus and diphtheria toxoids and acellular pertussis vaccine (Tdap)) have the potential to address this problem. Antibody to PT prevents leukocytosis with lymphocytosis and, in general, blocks the inhibition of G-proteins. Presently, however, most severe infant pertussis occurs in the first 4 months of life when a substantial number of them have not been immunized. To address this, the Advisory Committee on Immunization Practices (ACIP) has recommended the immunization of all pregnant women (in the 2nd and 3rd trimesters) with Tdap. Immunization of pregnant women with Tdap provides transplacentally acquired antibody to PT in the young infant, which prevents the adverse effects of this toxin. Unfortunately, this practice has not yet become routine in obstetrical practices. Another approach is to lower the

Table 1. Biologically active and antigenic components of *Bordetella pertussis* [12,25,32,33,45–61],

Adhesins	Toxins
Fimbriae	Pertussis toxin
B subunit of PT	Adenylate cyclase toxin
Filamentous hemagglutinin	Dermonecrotic toxin
Autotransporters [†]	Tracheal cytotoxin
Polysaccharide capsule	Type III secretion system
	Lipopolysaccharide
	Autotransporters [†]
	Polysaccharide capsule
	'Cough toxin'

[†]Autotransporters: pertactin, Vag8, BrkA, BapC, SphB1 and TcFA.

age of primary DTaP immunization. It should be noted that in the USA, in the period between 1954 and 1974, when pertussis was brought under control, the three-dose primary DTwP series was completed between 3 and 5 months of age [6].

B. pertussis cough illness in all age groups

The prevention of *B. pertussis* cough illness in all age groups depends upon the discovery of the 'cough toxin'. From our histopathologic data, it is reasonable to assume that the 'cough toxin' produces its effect without damage in the respiratory tract. Also, since *B. pertussis* cough illness occurs throughout life, it would appear that it is not very immunogenic or that an immune response to it is short lived. Assuming that this is the case, it would appear that after the 'cough toxin' is identified, a monocomponent vaccine to it could be developed.

We have suggested a monocomponent vaccine because the 'cough toxin' apparently is not or is poorly immunogenic with infection or immunization. If the toxin was the polysaccharide, it could be linked to a carrier protein in a manner similar to present polysaccharide vaccines. If the toxin was a protein, then different adjuncts would need to be investigated. Alternatively, an improved less reactogenic DTwP vaccine could be developed from bacterial cultures grown under different conditions and inactivated by new methods.

Discussion

Pertussis is a fascinating illness that is epidemic in many areas throughout the world. Pertussis 'resurgence' is due to many factors unrelated to this review. However, it is worthwhile to look at some of the factors which we have discussed that have contributed to this process. First, many of the major characteristics of *B. pertussis* infection have been overlooked or ignored for approximately 100 years. Specifically, pertussis is an often severe and fatal disease in young infants, but it is different from all other infectious diseases. It occurs generally without fever, and except for its characteristic pneumonia in young infants, there is no bacteremia or systemic manifestations such as meningitis, arthritis or osteomyelitis.

Second, clinical pertussis is a toxin-mediated infection and disease. A number of toxins and adhesins play a role in infection (TABLE 1). These toxins adversely affect the innate immune response (particularly all autotransporters, ACT and PT) and facilitate attachment to the ciliated epithelial cells (fimbriae 2 and 3 and the B subunit of PT). Disease is due to just two toxins one of which (PT) is well known and the other (the 'cough toxin') awaits discovery. The clinical manifestations of PT only occur in primary infections. Once infection has occurred or vaccination has been performed, manifestations of PT never occur again. In contrast, persons of all ages have paroxysmal cough during *B. pertussis* infections indicating that antibody to this toxin does not occur or that immunity to it wanes rapidly and recall of antibody to it is not rapid enough to prevent the cough illness.

The paroxysmal cough in pertussis is distinctive and can be differentiated from other illnesses with persistent coughing,

such as those caused by *Mycoplasma pneumoniae* and adenoviruses, because all air is expelled before a breath occurs, it is nonproductive and the patient is afebrile. The cough continues long after the infection on the ciliated cells has cleared. Of most interest is that adolescents and adults retain memory for the specific paroxysmal cough. Following the original prolonged paroxysmal cough illness, patients will recover only to have the same paroxysmal cough reoccur with the acquisition of a respiratory viral infection. It has been suggested that the pertussis cough is similar to that induced by bradykinin [66]. Although the clinical manifestations of the 'cough toxin' are quite well known, nothing is known about the toxin itself. We recently identified intact *B. pertussis* bacteria within ciliated epithelial cells in infants with fatal *B. pertussis* infection as well as *B. pertussis* lipooligosaccharide in normal ciliated cells of the trachea 68 days after illness onset [30]. Perhaps this retained antigen has a relationship to the memory for the cough that is retained after an illness.

The present acellular pertussis vaccines are not as protective as well-made whole cell vaccines. At present, attempts are underway to make better acellular vaccines, but there are numerous regulatory and other impediments to achieving this goal such as cost and lack of an unvaccinated control population. Therefore, at present, we should concentrate on better use of the vaccines that we have. The severe manifestations of infant pertussis caused by PT can be prevented by maternal immunization and early immunization of young infants. Research should be directed towards the discovery of the 'cough toxin', which causes disease throughout life, so that vaccination and other control strategies can be developed.

The lack of significant fever in *B. pertussis* infections of all degrees of severity is particularly intriguing and enigmatic. It is possible that this represents another effect of PT and the inhibition of G-proteins, or perhaps the 'cough toxin' has a direct suppressive effect upon the expected inflammatory response. It is known that alveolar macrophages have both pro- and anti-inflammatory roles in the alveoli [67]. Perhaps one or more *B. pertussis* antigens stimulate the anti-inflammatory effects of the abundant numbers of macrophages seen in the lungs of infants with fatal pertussis.

Expert commentary

Pertussis is a unique infectious disease in that it can be severe and fatal, but unless a secondary bacterial or viral infection is present, it occurs without fever and other evidence of an inflammatory illness. *B. pertussis* has been extensively studied by microbiologists, but many of the major characteristics of human infection have been overlooked or ignored for approximately 100 years. The study of *B. pertussis* in animal model systems (particularly the mouse) has led to the discovery of many antigens that play a role in the infections process. These antigens have been described as toxins or adhesins. In infection, they all adversely affect the innate immune response and/or facilitate attachment to ciliated respiratory epithelial cells. In contrast with infection, clinical disease is due to just two toxins or toxic

processes. The first of these toxins is PT and its manifestations in human infections are quite well understood. Importantly, once a person has seen PT (either by infection or immunization), he/she has rapid recall of antibody to this antigen and demonstrates none of its clinical manifestations. In contrast with PT and its recognized clinical findings, the cause of the distinctive cough in pertussis is unknown. The authors describe their thoughts on the 'cough toxin'. Present research on pertussis should move away from the mouse model and should be directed at the cause of cough in human *Bordetella* infections.

Five-year view

The baboon model would appear to offer considerable promise for the study of clinical manifestations of pertussis. Many aspects of *B. pertussis* infection in the baboon (paroxysmal cough, leukocytosis with lymphocytosis) are similar to those seen in primary infections in children. However, at the present time, there are no histopathologic data from the baboon which can be compared to that seen in human infants. Assuming that the human

and baboon pathology is similar, the cause of the cough can be studied in the baboon. We would expect this to be useful in regard to our present suggestions and could, of course, uncover other significant factors in the cause of the cough.

The findings and conclusions in this review are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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American Society for Microbiology 113th General Meeting, May 20, 2013

Pediatric Academic Societies Annual Meeting, May 4, 2013

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No writing assistance was utilized in the production of this manuscript.

Key issues

- Pertussis is truly a unique infectious disease. It can be severe and fatal, but unless a secondary bacterial or viral infection is present, it occurs without fever and other evidence of an inflammatory illness.
- The cyclic nature of reported pertussis is similar today to what it was in the pre-vaccine era; this indicates that immunization reduced reported pertussis, but did not affect the overall circulation of *Bordetella pertussis*.
- *B. pertussis* infection can result in asymptomatic infection, mild cough illness and typical illness with paroxysmal cough, whoop and posttussive vomiting.
- Once an individual has had *B. pertussis* infection or has been vaccinated, leukocytosis with lymphocytosis does not occur with a subsequent infection.
- In contrast with studies in mice, the ciliated epithelial cells of the trachea and bronchi are generally well preserved in fatal human *B. pertussis* infections.
- Pertussis toxin in primary human infections causes leukocytosis with lymphocytosis and this is related to deaths in young infants.
- Dermonecrotic toxin and tracheal cytotoxin cause severe damage to the ciliated cells of the respiratory tract in mice, but apparently do not in fatal human infections.
- Many *B. pertussis* antigens participate in infection by blocking the innate immune response and/or blocking attachment to ciliated respiratory epithelial cells.
- In contrast with infection, only pertussis toxin and as-yet-unidentified 'cough toxin' are associated with clinical manifestations.
- Further research should be directed at finding the cause of cough in pertussis.

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