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Bordetella pertussis: new concepts in pathogenesis and treatment

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Abstract

Purpose of review—The purpose of this review is to summarize and discuss recent findings and selected topics of interest in *Bordetella pertussis* virulence and pathogenesis and treatment of pertussis. It is not intended to cover issues on immune responses to *B. pertussis* infection or problems with currently used pertussis vaccines.

Recent findings—Studies on the activities of various *B. pertussis* virulence factors include the immunomodulatory activities of filamentous hemagglutinin, fimbriae, and adenylate cyclase toxin. Recently emerging *B. pertussis* strains show evidence of genetic selection for vaccine escape mutants, with changes in vaccine antigen-expressing genes, some of which may have increased the virulence of this pathogen. Severe and fatal pertussis in young infants continues to be a problem, with several studies highlighting predictors of fatality, including the extreme leukocytosis associated with this infection. Treatments for pertussis are extremely limited, though early antibiotic intervention may be beneficial. Neutralizing pertussis toxin activity may be an effective strategy, as well as targeting two host proteins, pendrin and sphingosine-1-phosphate receptors, as novel potential therapeutic interventions.

Summary—Pertussis is reemerging as a major public health problem and continued basic research is revealing information on bacterial virulence and disease pathogenesis, as well as potential novel strategies for vaccination and targets for therapeutic intervention.

Keywords

Bordetella; pertussis; therapeutics; virulence factors; whooping cough

INTRODUCTION

Pertussis (whooping cough) is caused by acute respiratory infection with the bacterial pathogen *Bordetella pertussis*. Several countries are experiencing significantly increased numbers of pertussis cases in recent years [1], including the United States where the number

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Conflicts of interest

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of reported cases in 2012 was a 50-year high [2]. The reemergence of pertussis is occurring despite widespread vaccination. However, use of effective whole-cell pertussis vaccines has been discontinued in most of the developed world because of concerns about their reactogenicity, and currently used acellular pertussis vaccines provide relatively ineffective and short-lived immunity [3]. Because of this, there is currently much discussion in the pertussis field on the development of new vaccines and vaccination strategies [4–9]. The scope of this review is more basic aspects of *B. pertussis* virulence and disease pathogenesis and treatment.

B. pertussis is transmitted by aerosols and infects the ciliated epithelium of the airways. There is no further dissemination of the infection, but bacterial toxins produced in the respiratory tract contribute to local and systemic disease pathogenesis [10–13]. Typical pertussis is characterized by severe paroxysmal coughing that can persist for weeks after initial onset. However, the specific cause of the severity and longevity of pertussis cough is unknown. Pertussis in young infants can be more serious, with complicated respiratory problems including apnea and pneumonia, as well as marked leukocytosis and pulmonary hypertension [14]. Hospitalization and intensive care treatment is often required and a significant number of pertussis deaths occur in this age group [15,16▪▪]. Despite several decades of research, there are still significant gaps in our understanding of the role and activity of *B. pertussis* virulence factors and of the pathogenesis of pertussis disease, especially the severe disease in young infants. However, the development of new animal models in recent years [17,18] and the possible implementation of human volunteer experiments in the near future provide the opportunity to increase our basic understanding of pertussis and hopefully to develop novel effective vaccines and therapeutics.

ROLE AND ACTIVITY OF *B. PERTUSSIS* VIRULENCE FACTORS

Recent reports have shed new light on the role and activity of several virulence factors in *B. pertussis*, as described in the following subsections.

Filamentous haemagglutinin

Filamentous haemagglutinin (Fha) is an important adherence factor for *B. pertussis* synthesized as a preprotein (FhaB) that is processed to the mature Fha molecule [19]. Fha also appears to act as a suppressor of inflammation in the airways [19,20]. As Fha is a component of acellular pertussis vaccines, this immunomodulatory activity may be a problem for the efficacy of these vaccines. Locht's group found that human monocyte-derived dendritic cells exposed to full-length Fha secreted various cytokines including the immunosuppressive cytokine interleukin (IL)-10, whereas an 80 kDa N-terminal fragment of Fha-induced secretion of the other cytokines but not IL-10 [21▪]. Fragments of Fha may therefore be superior vaccine antigens to the full-length molecule. Another group found that Fha stimulated responses through the pattern recognition receptor Toll-like receptor (TLR)2 but not through TLR4 or TLR5, and that the TLR2 stimulatory region of Fha is within a central fragment C-terminal to the known adherence domains [22]. As this domain is not included in the 80 kDa N-terminal fragment of Fha, this TLR2-stimulatory activity may be responsible for the IL-10 production induced by full-length Fha. However, Sebo's group

reported that the cytokine-inducing and TLR2-stimulating activities of Fha preparations are because of contaminating endotoxin [23▪], calling the findings on Fha immunostimulatory properties into doubt. Studying the closely related pathogen *Bordetella bronchiseptica* in a mouse model of respiratory infection, Cotter's group found that the FhaB preprotein appears to play a role in bacterial persistence in the airways [24▪]. Deletion of two C-terminal subdomains of FhaB did not affect production of mature Fha, adherence or suppression of inflammation, but resulted in more rapid clearance of the mutant strains from the airways of infected mice. They postulated that transmembrane signaling from these FhaB subdomains somehow aids in bacterial resistance to the early host immune response, and determining the mechanism of this activity will be an interesting challenge. Clearly further understanding of Fha biology is important for both pathogenesis and vaccine considerations. The IL-10 stimulatory activity is important to understand since it may be the cause of immunosuppression associated with pertussis infection or vaccination, as manifested in a recent report on the attenuation of CNS autoimmunity (a model of multiple sclerosis) by *B. pertussis* infection [25].

Fimbriae

Bordetella pathogens produce fimbriae (Fim) that are thought to be adherence factors despite relatively little supporting evidence. Guevara *et al.* [26] studied adherence of *B. pertussis* to primary and immortalized human bronchial epithelial cells. They found that mutations in the major fimbrial subunits Fim2 and Fim3 and the minor adhesin subunit FimD significantly reduced bacterial adherence to these cells, and that addition of purified fimbrial subunits competitively inhibited bacterial adherence. Cotter's group found that *B. bronchiseptica* Fim mediate bacterial attachment to the airway epithelium as well as suppression of inflammatory airway responses, in concert with Fha [27]. If this is also true for *B. pertussis*, then inclusion of Fim in acellular pertussis vaccines may be beneficial in reducing bacterial colonization of the airways, although fragments that avoid the immunosuppressive property may be optimal.

Adenylate cyclase toxin

Adenylate cyclase toxin (Act) targets phagocytic cells via binding to the $\alpha_M\beta_2$ integrin complement receptor 3 (CR3, also known as CD11b/CD18), entering cells to increase cyclic adenosine mono-phosphate (cAMP) levels via its adenylate cyclase domain and forming cation-selective pores in the cell membrane through its hemolysin/repeats in toxin (RTX) domain [11]. Hewlett's group found that Act inhibits neutrophil apoptosis and the formation of neutrophil extracellular traps by cAMP elevation and inhibition of oxidative burst, contributing to its protective capacity against neutrophils [28▪]. Sebo's group has made a number of recent findings on the binding and activity of this toxin. They found that Act binding to the C-terminal section of CD11b is enhanced by N-glycosylation of several residues in this part of CR3 [29]. Furthermore, Act binds to a segment of the integrin distinct from the typical integrin ligand-binding domain, Act binding does not elicit downstream signaling from CR3, and Act-mediated cAMP elevation inhibits CR3 signaling induced by other ligands [30▪]. They also found that Act-mediated cAMP signaling through protein kinase A activates the tyrosine phosphatase protein Srchomology 2 domain protein tyrosine phosphatase 1, which suppresses TLR4-stimulated inducible nitric oxide synthase gene

expression and production of bactericidal nitric oxide, promoting survival of *B. pertussis* inside macrophages [31▪]. In addition, Act-mediated cAMP signaling promoted dendritic cell chemotaxis while reducing T cell-stimulatory capacity and enhancing immunosuppressive IL-10 production [32▪]. The combination of these immunomodulatory activities mediated by Act renders it a powerful virulence factor promoting *B. pertussis* infection, and these authors argue that it should be included (in inactivated form) as a component of future acellular pertussis vaccines, since neutralizing these activities would be beneficial to the host in preventing infection [33]. Indeed, Maynard's group recently showed that the RTX domain of Act is immunodominant and that antibodies directed to this domain can neutralize Act activity, suggesting that a more stable and easily produced fragment of Act may be a candidate vaccine antigen [34▪].

EMERGENCE OF *B. PERTUSSIS* STRAINS WITH INCREASED VIRULENCE?

Recent evidence demonstrates that circulating *B. pertussis* strains have undergone significant genetic changes compared to prevaccine era strains and whole-cell vaccine era strains [35▪, 36,37]. The main driving force for this strain evolution is thought to be immune pressure from vaccination, with emergence of 'vaccine escape' mutants [38,39]. A study by Preston's group on strains from a 2012 outbreak in the UK and other strains from additional outbreaks globally found that acellular vaccine antigen-encoding genes [pertussis toxin (*ptx*), pertactin (*prn*), Fha (*fha*), and Fim (*fim*)] are evolving at a significantly higher rate than genes encoding other surface antigens not included in the vaccine [40▪▪]. Interestingly, this was true (at a lower rate) even in the prevaccine and whole-cell vaccine eras, suggesting either that immunity derived from natural infection or whole-cell vaccination was primarily aimed at this small number of antigens or that changes in these antigens were sufficient to increase virulence to overcome immunity. However, the higher rate of vaccine antigen gene evolution was most pronounced in the current acellular vaccine era, suggesting that the rate of evolution of these genes has accelerated in the face of acellular vaccination.

Another example of apparent vaccine escape mutations in *B. pertussis* strains is the loss of expression of the surface protein pertactin (Prn). Naturally occurring Prn-deficient strains have been described just within the last decade, but their frequency has been on the rise and they now predominate in several parts of the world [41–45]. Recent evidence indicates that these strains have been selected for by the use of acellular vaccines [46▪] and that they have a selective advantage over Prn-expressing strains in vaccinated mouse model infections [47,48▪▪]. A study by Lan's group showed that in a mixed infection of a Prn-expressing and a Prn-deficient strain in mouse trachea and lungs, the Prn-deficient strain dramatically outcompeted the Prn-expressing strain in mice vaccinated with acellular vaccine [48▪▪]. Interestingly, the opposite was true in unvaccinated control mice, suggesting that Prn may play a role in bacterial virulence in this model. However, conclusions from these and similar studies are tentative since such small numbers of strains are used (just one strain of each type in the Lan study). Other studies have found no difference in the severity of pertussis disease in human infants infected with either Prn-expressing or Prn-deficient strains [49▪, 50], although it remains possible that compensatory mutations have occurred in Prn-deficient strains to account for the loss of Prn. Very few *B. pertussis* strains deficient in expression of Fha or pertussis toxin (Ptx) have been described [51,52,53▪]. Intriguingly, Fha-

deficient strains showed significantly higher transcription of virulence factor genes than Fha-expressing strains grown *in vitro* (this was not true for Prn-deficient vs. Prn-expressing strains) [52], which could be an effect on the adjacent *bvg* genes that encode the master regulatory system for virulence gene expression. However, the lack of widespread occurrence of Fha and Ptx-deficient strains in the acellular vaccine era suggests that these virulence factors are crucial for *B. pertussis* pathogenicity and/or transmission. Indeed, one of the two reported Ptx-deficient clinical strains showed reduced virulence in a mouse model of infection [51] and the other [53▪] caused no disease in the baboon model of pertussis (Merkel T, personal communication).

Another view is that genetic changes in currently circulating *B. pertussis* strains have not just promoted escape from vaccine-elicited immunity by antigenic loss or variation, but have also increased the virulence of these strains to reduce the effectiveness of acellular vaccines [54]. Mooi and colleagues have identified and analyzed a relatively new group of *B. pertussis* strains characterized by the *ptx* promoter allele *ptxP3*, differing from previously predominant *ptxP1* strains [54,55]. These *ptxP3* strains now predominate in most parts of the world [56–60]. Mooi's group found that *ptxP3* strains produce slightly more Ptx than *ptxP1* strains [61], and concluded that since Ptx is a crucial virulence factor for *B. pertussis* [10,62], this may contribute to greater virulence of these strains. However, a subsequent study showed that the genetic background of *ptxP3* strains, rather than the *ptxP3* allele itself, contributed to increased virulence (in a mouse model) [63]. Interestingly, Mooi's group found that *ptxP3* strains not only produce higher levels of several virulence factors than *ptxP1* strains, but are less sensitive to sulfate-mediated modulation of virulence gene expression through the Bvg regulatory system, probably because of differential expression of sulfate utilization and transport genes [64▪]. It is still unclear whether *ptxP3* strains are really more virulent than *ptxP1* strains, especially since most of these analyses have included very few strains of each type, but one recent study of young children hospitalized with pertussis found a significant association between *ptxP3* strains and severe disease [49▪]. Additional studies similar to this may reveal a true relationship between the *ptxP3* strain genotype and increased virulence, and will spur the development of improved vaccines and therapeutics to account for this increase.

CRITICAL AND FATAL PERTUSSIS IN INFANTS

An important issue in pertussis is the severe disease in young infants that results in hospitalization and intensive care (critical pertussis) and can progress to a fatal outcome. Leukocytosis, an effect of Ptx activity, is a significant feature of critical pertussis and has been previously associated with poor outcome in infected infants [65,66]. Recent reports have attempted to determine risk factors and predictors of fatal outcome in infants suffering from critical pertussis. A study of pertussis in Swedish infants highlighted the high rate (70%) of hospitalization of pertussis cases among young infants (<3 months old) and the protective effects of vaccination against fatal disease, since all nine deaths occurred in unvaccinated infants [67▪]. In a smaller study of 17 cases of critical pertussis in Tunisia, there was a high rate of fatal outcome (23%) and significant predictors of mortality included leukocytosis, as well as tachycardia, seizures, and shock [68]. A study of US infants suffering from pertussis between 1991 and 2008 noted 258 deaths, all in infants less than 8

months old [69▪]. The study also found that one or more doses of pertussis vaccine significantly protected infants from hospitalization and death. Interestingly, the protective effect was greater for infants receiving the acellular vaccine than the whole-cell vaccine, possibly because the acellular vaccine elicits higher titer antibodies against Ptx than does the whole-cell vaccine. Another recent study compared 53 fatal versus 183 nonfatal hospitalized cases of pertussis in infants less than 4 months old in California between 1998 and 2014 [16▪▪]. Lack of pertussis vaccination, premature birth, low birth weight, younger age at time of cough onset and higher peak leukocytosis were all significantly associated with fatal cases. This study also examined leukocytosis more closely as a predictor of death, finding that a white blood cell count above 70 400/μl was particularly predictive, especially if birth weight was low [16▪▪]. The study also noted a rapid increase in pulse and respiratory rates in these infants and the authors speculated that while leukocytosis may just be a marker of Ptx activity, Ptx inhibition of inhibitory G protein signaling affecting heart and lung function may be the proximate cause of death. Increased understanding of the pathogenesis of critical pertussis disease in young infants, especially the role of Ptx, will inform strategies toward improved and life-saving treatment.

NOVEL POTENTIAL TREATMENTS FOR PERTUSSIS

In an age of increasing pertussis outbreaks, consideration of treatment strategies for individuals suffering from the disease is an important issue [70]. Unfortunately, no proven effective treatment exists for reducing pertussis symptoms. In the latest Cochrane Center systematic review of pertussis treatment trials, the authors found no significant beneficial effect of treatment with diphenhydramine (an antihistamine), dexamethasone (an anti-inflammatory steroid) or salbutamol (a bronchodilator) [71▪]. Macrolide antibiotics are administered to pertussis patients but typically just to prevent further transmission, since antibiotic administration rarely reduces the clinical course of disease in affected individuals [72]. However, recent reports have highlighted the benefit of early antibiotic treatment for young infants with critical pertussis. In the Swedish study, starting antibiotic treatment within the first 6 days after cough onset was associated with shorter duration of coughing than those initiating treatment 2 weeks after cough onset [67▪]. Similarly, in an Australian study of household attack rates, there was an increased risk of transmission from the primary case to contacts when antibiotic treatment was initiated later than 7 days after the onset of symptoms [73▪]. Early antibiotic treatment was also associated with reduced risk of death in young infants suffering from pertussis [16▪▪,69▪]. Antibiotic resistance has not been a major concern for pertussis. However, recent reports from China have highlighted newly emerging *B. pertussis* strains with significantly elevated levels of macrolide resistance [74▪,75▪], a potential concern if these strains spread globally.

Treatment of newborns with critical pertussis is a greater concern. In serious cases, extracorporeal membrane oxygenation is performed, sometimes with added leukodepletion because of the extreme leukocytosis associated with pertussis [70]. A recent case report highlighted the effectiveness of this combination therapy in saving the life of a 17-day-old infant hospitalized with pertussis [76]. On the other hand, the California study found that extracorporeal membrane oxygenation, as well as exchange blood transfusion, intubation, and nitric oxide treatment, were more frequently associated with fatal cases of pertussis in

young infants [16■■]. However, this may be because these treatments are only initiated when the disease becomes life-threatening, and that earlier intervention may have been beneficial. Treatment of infants with antipertussis immunoglobulin (containing high titers of anti-Ptx antibodies) has shown some indication of benefit in the past [77]. A new study using humanized forms of Ptx-neutralizing murine monoclonal antibodies found that these antibodies reduced leukocytosis and decreased bacterial colonization in mouse and baboon models of *B. pertussis* infection [78■■], highlighting the potential for this method of treatment directed specifically at Ptx. There was also some indication of cough reduction by this treatment in the infected baboons, although this was not statistically significant.

Research in our lab has revealed two novel potential treatments for pertussis [70]. In one study of Ptx-associated changes in mouse lung gene expression during *B. pertussis* infection, we found that the gene encoding pendrin, an epithelial anion exchanger, was highly upregulated [79■]. Furthermore, pendrin knockout mice exhibited very low levels of lung inflammatory pathology despite higher bacterial loads during *B. pertussis* infection, indicating a role for pendrin in this disorder. We hypothesize that pendrin export of bicarbonate raises pH to optimal levels for inflammatory mediator activity, thus promoting inflammatory pathology. Infected mice treated with the carbonic anhydrase inhibitor acetazolamide (to reduce bicarbonate levels exported by pendrin) exhibited significantly reduced levels of lung inflammatory pathology [79■]. Acetazolamide is a clinically used drug for treatment of a variety of ailments and has been shown to reduce cough responses in human volunteers challenged with low-chloride-ion solutions [80]. Therefore, this drug represents a potential novel treatment for individuals suffering from pertussis cough, and this idea can be tested in the baboon model of pertussis.

In another study, we found that lung cytokine expression and inflammatory pathology in *B. pertussis*-infected mice was dramatically reduced by early intranasal administration of a single dose of the sphingosine-1-phosphate (S1P) receptor ligand 2-amino-4-(4-heptyloxyphenyl)-2-methylbutanol (AAL-R), with little effect on bacterial loads [81■]. More recently, we have found that the same effect is achieved by treatment nearer peak bacterial loads, and that early treatment significantly reduces lethality in *B. pertussis*-infected infant mice (Skerry C *et al.*, unpublished data). The mechanism of this drug effect is unclear and does not appear to be inhibited by Ptx, but likely involves downregulation of a key component involved in stimulating the inflammatory response to the bacterial infection. Importantly, these findings indicate potential therapeutic use of this treatment, especially for young infants with critical pertussis. In addition, similar S1P receptor agonist drugs have been shown to reduce the cytokine storm and lung pathology associated with influenza virus infection in mice [82], and one of these drugs, FTY720 (fingolimod), is in clinical use for treatment of relapsing multiple sclerosis [83]. Therefore, development of these drugs for potential treatment of pertussis should be relatively streamlined, and they represent another promising novel pertussis therapy.

CONCLUSION

Pertussis is reemerging as a serious public health problem in many parts of the world despite widespread vaccine use. This fact highlights our relatively poor understanding of the basics

of *B. pertussis* virulence and infection, the host immune responses, and the pathogenesis of pertussis disease. The problem is especially acute for young infants for whom the disease can be fatal. New studies on the basic biology of virulence factor activities and on the genetics and evolution of *B. pertussis* strains are revealing potentially important information for vaccine considerations. A handful of studies also point to potential novel therapeutic strategies for treatment of pertussis, including a pair of host targets revealed by basic studies in animal models. Continued basic research will be necessary to increase our understanding of pertussis and to develop effective new vaccines and therapeutics.

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KEY POINTS

- New studies highlight the activities of the *B. pertussis* virulence factors Fha, Fim, and Act, especially their immunomodulatory effects.
- Circulating *B. pertussis* strains are evolving to overcome vaccine-elicited immunity and possibly to increase overall virulence.
- Fatality from pertussis remains an issue in young infants, especially in those who are unvaccinated, have a low birth weight, and have high levels of leukocytosis.
- Treatment options for pertussis are extremely limited, but early antibiotic intervention can be beneficial.
- Potential novel therapeutics include antibodies specific for Ptx, as well as drugs aimed at the host targets pendrin and S1P receptors, that reduce lung inflammatory disorder in animal models of pertussis.