Pertussis Pathogenesis—What We Know and What We Don’t Know

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Pertussis is a worldwide public health threat. Borderella pertussis produces multiple virulence factors that have been studied individually, and many have recently been found to have additional biological activities. Nevertheless, how they interact to cause the disease pertussis remains unknown. New animal models, particularly the infection of infant baboons with B. pertussis, are enabling longstanding questions about pertussis pathogenesis to be answered and new ones to be asked. Enhancing our understanding of pathogenesis will enable new approaches to the prevention and control of pertussis.

Keywords. Borderella pertussis; pertussis; pathogenesis; virulence factors; whooping cough.

Pertussis is a classic example of an infectious disease for which multiple virulence factors have been identified and their effects characterized at the molecular and cellular levels [1]. Despite this extensive body of knowledge, woefully little is known about the mechanisms by which these factors act in concert to cause “whooping cough” in humans. In this review, we describe existing concepts of how infection with Bordetella pertussis causes disease. To provide a balanced perspective, we also note deficits in our knowledge. The resultant discussion will hopefully serve as the basis for additional research on this organism and the disease it causes, leading to improved methods for prevention and treatment.

Clinical Pertussis
Examination of pathogenesis for a disease such as pertussis must begin with discussion of its defining features. In fact, it is not easy to answer the question: “What is pertussis?” By definition, it is an illness that results from infection with B. pertussis. It is a cough illness for the majority of those affected, yet infants, who can have serious/fatal infections with this organism, may experience apneic episodes and never cough. B. pertussis causes a localized infection, rarely disseminating from the respiratory tract. Beyond the paroxysmal cough, however, there are systemic manifestations, including: (1) lymphocytosis; (2) dysregulated secretion of insulin; (3) post-tussive vomiting, causing dehydration and malnutrition; (4) alterations in neurologic function (confusion, syncope, seizures, and loss of consciousness); and (5) recurrence of paroxysmal cough, days to weeks after the infection has been cleared. Although the relationship between these additional signs and symptoms and the course of clinical pertussis is unclear, some features appear to be attributable to virulence factors with known activities, as described below.

The mechanisms of microbial pathogenesis and the roles of individual components can be put into a working context by considering the proven or hypothesized contribution of each virulence factor to specific pathogenic processes [2]. These steps in pathogenesis are:

- Exposure/inoculation.
- Tissue tropism/attachment.
- Proliferation and production of virulence factors.
- Evasion/modulation of host defenses.
- Local and systemic cell and tissue dysfunction/damage.
- Chronic infection, death or clearance and resolution of symptoms.

KNOWN OR PRESUMED VIRULENCE FACTORS OF B. PERTUSSIS

Components of B. pertussis that are likely virulence factors include proteins categorized as “toxins” and “adhesins,” as well as other molecules that interact with host cells to alter their function. There are certainly other bacterial products.
that are important for pertussis pathogenesis but heretofore have not been identified or recognized for their relevance. This discussion will focus on those products implicated by data from in vitro and/or in vivo studies. Molecules present in current acellular pertussis vaccines are marked with an asterisk (*), and the rationale for their inclusion in acellular vaccines is noted.

**Pertussis Toxin** (PT)
This toxin was named in response to Dr Margaret Pittman’s paper suggesting that PT was the molecule responsible for pertussis [3]. This hypothesis was not correct, in that administration of PT to animals or humans does not elicit clinical pertussis [1, 4]. PT is important but not essential, and its ADP-ribosylation of hetero-trimeric G proteins affects signal transduction (disrupts function) in many cell types. The resulting biological effects include induction of lymphocytosis, alteration in insulin secretion, and enhancement of sensitivity to histamine and other mediators, in humans and/or animals [1]. Each of these effects contributes to pathophysiology; for example, it appears that the elevated numbers of white blood cells are involved in pulmonary hypertension, a significant cause of pertussis morbidity and mortality [5]. How these diverse effects of PT, apparently via a common molecular mechanism, contribute collectively to clinical pertussis remains unknown.

**Filamentous Hemagglutinin** (FHA)
This large surface protein, one component of a 2-partner secretion pair, can participate in the interaction of *B. pertussis* with host cells and thus is appropriately classified as an adhesion; this activity is the basis on which it is an acellular vaccine component. More recently, FHA was reported to exert immunomodulatory effects in vivo by unknown mechanisms [6].

**Pertactin** (PRN)
There is in vitro evidence that PRN can contribute to bacterium-host cell interaction as an adhesin and it was included in acellular vaccines with the hope of preventing establishment of infection. In recent in vivo studies, PRN was found to have a role in defense against neutrophils (PMN), suggesting immunomodulation with consequences similar to those of adenylate cyclase toxin [7].

**Fimbriae** (FIM)
These surface appendages, similar to those in other bacteria, function as adhesins and comprise several of the agglutinogens, which are the basis for *Bordetella* serotyping. Their inclusion in some acellular vaccines was also predicated on the possibility of preventing infection. Like FHA and PRN, FIM are also immunomodulatory [1].

**Adenylate Cyclase Toxin** (ACT)
This toxin delivers an adenylate cyclase domain into host cells, where it increases cAMP levels, resulting in inhibition of phagocyte function and activation of apoptosis in some cell types. ACT is a critical virulence factor and has protective activity demonstrated in mouse infection [1, 8].

**Tracheal Cytotoxin** (TCT)
This disaccharide-tetrapeptide, derived from peptidoglycan, kills respiratory epithelial cells in vitro by a complex mechanism involving intracellular interleukin 1 and nitric oxide [9]. Its role in clinical disease remains to be determined.

**Lipooligosaccharide** (LOS)
This molecule is a biologically active outer-membrane component, which differs structurally and functionally between *B. pertussis* and *B. bronchiseptica*; because pertussis is generally a nonfebrile illness, the role of LOS in disease is unknown. Minute quantities may be present in acellular vaccines.

**Dermonecrotic Toxin** (DNT)
This protein has weak sequence homology and similar function to that of *Pasteurella multocida* leukotoxin, which acts by deamidating specific signaling proteins. Cellular targets for DNT are unknown, and it has no recognized role in pertussis [1].

### B. Pertussis Virulence Factors and the Elements of Pathogenesis

**Exposure/Inoculation**
As demonstrated in recent studies by Warfel et al, transmission of *B. pertussis* can occur by aerosol without contact between infected and naive hosts [10, 11]. Expression of virulence factors is controlled by the Bvg 2-component system, which can be modulated by specific molecules and conditions in vitro, but the actual in vivo signals are not known [1]. Although the so-called Bvg phase is necessary and sufficient for infection, Bvg intermediate phase and the Bvg phases, in which vir-repressed genes (vrgs) are expressed, have been hypothesized to have a role in transmission between hosts [1], and this issue can now be studied in a relevant model.

**Tissue Tropism/Attachment**
Multiple adhesins (such as FHA, PRN, FIM, and others not yet identified) contribute to the interaction with ciliated cells and other cell types in the respiratory tract [1]. FHA is also involved in interaction of *B. pertussis* with PMN, to the detriment of the organism, and anti-FHA antibodies may interfere with that process [12].

**Proliferation/Production of Virulence Factors**
Following initial interaction with host cells, these processes are presumed to occur within the upper respiratory tract (nasopharynx), then perhaps spread to mid- and lower respiratory tract. This concept has not, however, been addressed in an appropriate model of pertussis.

**Evasion/Modulation of Host Defenses**
PT and ACT are active modulators of host defenses [13]. An increasing number of other virulence factors (FHA, PRN, and FIM), however, have also been
shown to possess immunomodulatory activities in vitro and/or in vivo. In addition, the autotransporter protein BrkA (Bordetella resistance to killing) confers serum resistance on B. pertussis, and TCT could be affecting the basic defense mechanism of ciliary clearance [1].

Local Cell and Tissue Dysfunction and Damage
ACT (which acts locally) and PT (which acts locally and systemically) disrupt endogenous signaling pathways by producing cAMP (ACT) or catalyzing covalent modification of key molecules in host signaling pathways (PT). These events occur in virtually all cells tested in vitro, so the scope of their effects and contributions to clinical disease are complex and inadequately explored. Probably the most striking disturbance in normal physiology during pertussis is the characteristic paroxysmal cough. Little is known about the mechanism for this cough, but recent work suggests possible heightened sensitivity to inflammatory mediators, perhaps mediated by PT [14]. Alternatively, Cherry [15] has proposed that there may be a previously undiscovered “cough toxin,” which is directly responsible for this phenomenon. The role of TCT and other molecules in tissue damage and impairment of repair in vivo has not been determined.

Chronic Infection or Death vs Clearance and Resolution of Symptoms
In pertussis, “dissemination,” if it occurs, is from upper to lower respiratory, tract and there is rarely systemic spread. Symptoms may last for many months, even when the causal organism has been eliminated by host immune response or antibiotics. There is, however, no evidence for chronic infection with long-term carriage of B. pertussis, as there is for B. bronchiseptica in animals and humans. Furthermore, B. pertussis does not survive well outside of the human host. Despite it being a serious respiratory illness that can last for months, the overall mortality rate is low.

Although the ability of PMN to kill B. pertussis in vitro has been demonstrated and the biological activities of several virulence factors appear to be directed at neutralizing PMN functions, it remains unclear how this bacterium is cleared in immune or nonimmune hosts. In the absence of pneumonia, this infection is noninflammatory and does not cause fever, in contrast to other infections for which PMN are the first line of defense [15].

Questions About Pertussis and Targets for Additional Research
As illustrated by the discussion of B. pertussis virulence factors and pathogenesis, many questions about B. pertussis and whooping cough remain unanswered. Some are attributable to the previous lack of an animal infection model that is comparable and relevant to disease in humans. The infection of infant baboons with B. pertussis, on the other hand, has enormous potential to provide novel perspectives on pathophysiology and immunity in pertussis [10, 11]. In fact, the availability of this model has stimulated discussion of questions that were not previously considered, in part because they were unanswerable with existing approaches. Examples of these issues are:

- Where are the bacteria? With the exception of fatal cases of pertussis (postmortem specimens), the only site from which samples are collected for culture or PCR is the nasopharynx. As a result, it is not known whether organisms need to be present below the larynx for development of classical (nonpneumonic) “whooping cough.” How can contemporary imaging technologies be used to address these issues?
- Is there a “cough toxin,” which is yet to be identified? [15] If so, what are the target cells and tissues, and what is its mechanism of action?
- What can be done to improve the duration of protection elicited by acellular vaccines? Are there additional molecules/antigens that should be considered for inclusion, and what is the nature and significance of host response to these antigens during infection?
- What are the mechanisms for immune-modulatory activities of “adhesins,” such as FHA, PRN, and FIM?
- What are the host responses (innate and acquired) that promote clearance of B. pertussis and protection against subsequent challenge? Several virulence factors appear to have important activities against PMN, yet the in vivo role of PMN in bacterial clearance is not known.

SUMMARY AND CONCLUSIONS
Clinical pertussis resulting from infection with B. pertussis is a contemporary medical and public health problem. Although many so-called virulence factors have been identified and studied, the mechanisms by which these (and yet-to-be-recognized products) result in pertussis remain primarily conjectural, based on in vitro studies and use of bacterial mutants in rodent models. There are exciting recent developments in pertussis research, such as availability of phenotypes and genotypes from multiple B. pertussis strains and experimental infection of infant baboons that results in clinical illness strikingly similar to that in humans. This new information is enabling the old paradigms, which were the basis for concepts of pertussis pathogenesis and for the design of acellular vaccines, to be challenged. Collectively, these data and those yet to be obtained hold enormous potential for revealing the unknowns about pathogenesis and will contribute to the development of more effective vaccines.

Notes
See also Supplement to the Journal (April 1, 2014. Volume 209, Supplement 1) Prevention and Control of Pertussis. To earn journal-based continuing medical education (CME) credit for this article, visit http://nhd.org/pertussis-cme (available after 4/15/14).

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References