Bordetella pertussis and Pertussis Vaccines

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Bordetella pertussis is a human-specific pathogen that causes whooping cough. The use of pertussis whole-cell vaccines in infants and toddlers led to decreased circulation of the bacterium in the child population and a marked decrease in the incidence of the disease. However, vaccine does not result in life-long immunity; indeed, the circulation of the bacterium has not been controlled in the adult population. Universal adult booster immunization is now possible using pertussis acellular vaccines, which target—and are thus likely to control—the virulence of this bacterium.

Bordetella pertussis causes whooping cough (“le Microbe de la coqueluche”). Despite extensive molecular studies, whole-genome sequencing, detailed protein characterization, and gene expression studies, much remains unknown about this microbe. Effective pertussis whole-cell (Pw) vaccines have been used to vaccinate infants and toddlers; however, the bacterium is still circulating and killing newborns. The potential control of its circulation now needs to be evaluated.

ISOLATION OF B. PERTUSSIS

In Paris in 1900, Jules Bordet and Octave Gengou identified the causative agent of whooping cough. They identified “a small ovoid Gram-negative bacterium” in an expectorate fluid specimen obtained from a 5-month-old child. However, they only isolated this bacterium 6 years later from the expectorate of Bordet’s own son, Paul. They were able to isolate it successfully thanks to the development of a particular medium [1], “Bordet-Gengou medium” (or “BG medium”), which is now widely used. Particular features of this bacterium include its high lability and the fact that it is very difficult to isolate.

In their first publication in 1906, Bordet and Gengou [2] described the very short timeframe in which the microbe can be successfully isolated during the progression of the disease. They also observed a variable distribution of the microbe in biological samples. In particular, they reported that the isolated microbe had a different appearance in artificial medium to that observed in biological samples. This bacterium glows in BG medium and has a striking appearance, reminiscent of a drop of mercury (Figure 1A). However, soon after its discovery, Bordet and Gengou observed other forms of this bacterium—in particular, the appearance of a white, nonhemolytic form—after several subcultures in vitro (Figure 1B). They found that, in contrast to the hemolytic bacteria freshly isolated from patients, these nonhemolytic bacteria were not agglutinated by infected patient serum [2]. This phenomenon was later analyzed by Leslie and Gardner [3] and has been further described in other more recent reviews [4]. The development of new technologies has led to elucidation of the major regulatory mechanisms of the expression of proteins involved in the virulence of the bacterium. This regulation is based on a 2-component system called the Bvg regulon, which is made up of 2 proteins: the sensor BvgS and the activator BvgA. The genetic regulation of various protein-encoding genes expressed by B. pertussis by BvgAS regulon is well established [4, 5]. In 1910, Bordet and Sleeswick [6] observed that the bacterium displays physiological plasticity, adapting to changes in external conditions, and that such adaptation involves changes at its cell-surface receptors. Their early use of the terms plasticity, adaptation, and responses to environment in their 1910 publication is particularly noteworthy. Indeed, it is because of these features of the bacterium that Pw vaccines are not easily prepared in a reproducible manner.

The BvgAS regulon plays a major role in the adaptation to changes in growth conditions. However, despite the development of many sophisticated and modern molecular techniques, the mechanisms regulating the production of virulence factors and the activity of the Bvg AS regulon in B. pertussis in vivo remain unknown.
THE ORIGIN OF B. PERTUSSIS

Pertussis is a relatively recent human infectious disease. According to Nils Rosen von Rosenstein, the disease first appeared in France in 1414. The first epidemic was described in 1578 by Guillaume de Baillou [7, 8].

B. pertussis belongs to the Bordetella genus, which now includes 9 species. B. pertussis, Bordetella parapertussis, and Bordetella bronchiseptica are the 3 most studied species. The genome of a reference strain for these 3 species was sequenced [9], and their phylogenetic relationship was studied in detail [10]. B. pertussis and B. parapertussis may have originated independently from a B. bronchiseptica ancestor [11]. They evolved through genome reduction with large-scale rearrangements [10]. Insertion sequence (IS) elements played a particularly important part in the reduction of the B. pertussis genome. Furthermore, comparative genomic hybridization and multilocus sequence typing studies [12] have shown that the degree of genetic diversity is greatest in B. bronchiseptica, followed by B. pertussis, with B. parapertussis showing the least genetic diversity. These data suggest that the association of B. parapertussis with humans is more recent than that of B. pertussis.

PATHOGENESIS OF B. PERTUSSIS

B. pertussis is a human-specific pathogen that causes a respiratory disease. It is highly labile and cannot survive outside its host. Thus, its aim is to survive in its host, and accordingly, it has maintained its capacity to transmit very efficiently from one host to another.

The study of human-specific pathogens requires collaboration with clinicians and epidemiologists or the development of appropriate animal models. Various models have been proposed or used to study the pathogenicity of B. pertussis, including primates, rats, mice, pigs, and, more recently, piglets. Primates are not used because of the high cost, and the major limitation in mouse and piglet models is the nontransmission of the disease in these animals. Although mice do not have the muscle that allows coughing, they can still be useful in the characterization of virulence factors and analysis of certain aspects of the immune response to infection.

B. pertussis is a sophisticated bacterium that expresses many bacterial factors with immunomodulating functions. It produces several bacterial factors responsible for the symptoms observed during the disease [13]. These include several toxins (e.g., tracheal cytotoxin; pertussis toxin, an A-B toxin; and adenylate cyclase-hemolysin, a Repeats in ToXins [RTX] toxin) that damage ciliated epithelial cells and alveolar macrophages and cause hyperlymphocytosis. The bacterium also produces several adhesins, such as filamentous hemagglutinin, pertactin, and 2 fimbrial proteins (FIM2 and FIM3). B. pertussis possesses all the tools necessary to attach to host cells, to evade host defenses, and to cause damage to the respiratory tract of the host.

GENOMIC VARIATION OF B. PERTUSSIS POPULATION

Soon after the isolation of the bacterium, Pw vaccines were developed using heat-killed bacteria. Given the pediatric nature of the disease, infants and toddlers were intensively vaccinated. The B. pertussis species, being strictly restricted to human hosts, displays a very low level of genetic diversity. Analyses performed over the past 2 decades demonstrate that several types of isolates, which produce various proteins, had been in circulation during the prevaccine era. However, 1 or 2 types predominated, with no significant geographical pattern observed in their distribution. Pw vaccines are generally composed of 1, 2, or 3 different strains, which are selected from these predominant types.

Extensive analyses over the past decade compare clinical isolates circulating during the prevaccine era with those that now
remain in circulation [12, 14]. In areas where Pw vaccination in infants and toddlers was effective, vaccine strains were no longer circulating or circulated at a very low level. In a previous study, my colleagues and I did not find differences between isolates circulating in areas using the same vaccine and the same schedule, with similar coverage [15, 16]. In countries sharing the same vaccine schedules and coverage, but using vaccine strains with different fimbriae, the isolates now circulating in these different regions were similar but, accordingly, produced different fimbriae [17]. In areas with very low vaccine coverage, isolates remaining in circulation are similar to those that were circulating in the pre-vaccine era [18]. Thus, overall, the effective use of Pw vaccines to induce immunity controlled the circulation of some—but not all—isolates. The remaining isolates that are currently in circulation have been shown to be as virulent as the isolates that were circulating in the prevaccine era [18]. However, genomic analyses have demonstrated that they exhibit several differences with the vaccine strains and the prevaccine era isolates. Their genome possesses less genetic material but more IS elements than the isolates circulating during the prevaccine era [19]. The genetic material lost is composed mostly of pseudo-genes but no gene implicated in the survival inside the host or in the virulence of the bacteria. As the number of pseudo-genes decreases, B. pertussis seems to adapt increasingly to surviving in their human hosts. It thus remains possible that B. pertussis will continue to lose genes that are not necessary for human infection. IS elements may be of particular importance due to their potential role in the initiation of gene duplication or deletion events. As described initially by Bordet and Gengou [1], and as observed since by scientists isolating this bacterium from nasopharyngeal samples, the phenotype of freshly isolated bacteria in biological samples differs from that of the bacteria grown in vitro. We previously demonstrated that certain clinical isolates of B. pertussis harbor 2 genes encoding one of this bacterium’s major toxins, the adenylate cyclase-hemolysin. This phenomenon explains the observation of the larger halo of haemolysis produced by these isolates in BG medium than produced by other clinical isolates [20]. However, 1 gene is deleted after 2 or 3 subcultures, with this process being mediated by IS elements. Thus, new B. pertussis clinical isolates need to be collected, and the genome of fresh isolates, rather than of subcultured isolates, needs to be analyzed to address this issue.

**CAN THE VIRULENCE OF B. PERTUSSIS BE CONTROLLED BY VACCINATION?**

Pw vaccination induces a broad immune response against hundreds of bacterial proteins, leading to the control of the circulation of isolates similar to the vaccine strains. However, this vaccination approach did not control the circulation of other isolates and did not control the virulence of the isolates.

Pertussis acellular (Pa) vaccines were introduced in many developed regions ~10 years ago to replace Pw vaccines. These Pa vaccines consist of 1–5 purified, detoxified toxins and adhesins. They consequently induce immunity against only a few bacterial proteins involved in the virulence of the bacterium. Thus, vaccine-induced immunity is changing, with bacterial virulence becoming the major target. Furthermore, the overall immunity in the human population is increasing, because Pa vaccines are not only used for infants and toddlers but also for adolescents and adults. With the use of Pa vaccines and increased vaccine coverage, successful control B. pertussis virulence thus seems feasible [13, 21]. What mechanisms underlie this potential control of virulence? We recently found that currently circulating isolates have more IS elements in their genome than isolates circulating during the prevaccine era [19]. As explained above, these IS elements are often located at both ends of a structural gene and can lead to gene deletion. Therefore, with an increasing rate of Pa vaccine–induced immunity in the population, we would expect newly collected isolates to lack the vaccine antigens due to deletion or inactivation of their structural genes. Indeed, since 2007, there have been several such isolates reported, some of which do not produce pertussis toxin and others not producing pertactin [22]. The absence of expression of these vaccine antigens is due either to deletion of the structural gene or to an IS element insertion within the structural gene. France may be particularly suited to the collection of such isolates, because its vaccine coverage is one of the highest in the world. Indeed, France introduced a vaccine booster for adolescents in 1998, the “cocoon strategy” vaccination program in 2004 and a vaccine booster, still keeping the “cocoon strategy”, for 26- and 27-year-old persons in 2008 [23–26]. If Pa vaccines are now used for the universal vaccination of adults, the circulation of virulent B. pertussis is likely to decrease, as is the incidence of pertussis, whereas the circulation of isolates that do not produce vaccine antigens is likely to persist.

**THE FUTURE OF B. PERTUSSIS AND OTHER BORDETELLA SPECIES**

With an increasing coverage of Pa vaccine, B. pertussis isolates that do not display the vaccine antigens would be expected to remain in circulation. However, will these isolates be less virulent? This important question is currently being investigated. However, the analysis requires the regular collection of isolates. Freshly collected isolates, however, are becoming increasingly difficult to obtain. Indeed, because of the difficulties in isolating the bacteria, culture is now being replaced by polymerase chain reaction. In addition, infected adults do not tend to visit their general practitioners immediately, during the earliest stages of the disease, preventing isolation of the bacterium in these cases. Furthermore, this tendency might increase if circulating isolates...
are less virulent. Isolates can be more easily collected from newborns and infants. Their immune system is immature, and *B. pertussis* could still be pathogenic, even if not all the virulence factors are produced. Hospital-based surveillance thus probably offers one of the best systems in which to analyze not only the progression of the disease in infants but also in adults in contact with infected infants.

The Pa vaccine–induced immune response targets the virulence of *B. pertussis* and not of *B. parapertussis* [27, 28], another causative agent of the disease. The possibility of *B. parapertussis* taking the place of *B. pertussis* must thus be considered. The epidemiology of *B. parapertussis* is unknown, making it difficult to determine the likelihood of this scenario. *B. parapertussis* seems to circulate at higher levels in some areas of the world than in others [29, 30]; however, it never reaches the levels of *B. pertussis*. Pw vaccines also specifically targeted *B. pertussis*. There was no increase in the circulation of *B. parapertussis* observed following the introduction of the vaccine and resultant decrease in the incidence of the *B. pertussis*–induced disease. Thus, we would not expect an increase in the circulation of *B. parapertussis* after the control of *B. pertussis* by Pa vaccination. However, these infections, and those due to other *Bordetella* species pathogenic in humans, must continue to be monitored.

**CONCLUSIONS**

It is, of course, unlikely that a high Pa vaccine coverage will be achieved worldwide in a short period of time, given the lack of funding and poor understanding of the fact that prevention is important and more economical than treatment. The management of diphtheria provides a useful example. Diphtheria is caused by a bacterium *Corynebacterium diphtheriae*, which produces a toxin. The vaccine is an acellular vaccine containing only detoxified diphtheria toxin. In all areas with long-standing diphtheria toxoid vaccination programs, diphtheria is controlled. Nontoxigenic *C. diphtheriae* isolates, however, now continue to circulate. The appearance of these isolates is attributed to selective pressure induced by the vaccine [31]. Diphtheria in highly vaccinated areas was controlled by immunizing children as well as adults. This was not possible for pertussis, because Pw vaccines were considered too reactogenic for use in adolescents and adults. We predict that, similarly to the use of diphtheria acellular vaccine, the use of Pa vaccine in all age groups will lead to a decreased circulation of virulent *B. pertussis* and, thus, the control of pertussis. Bacteria that do not produce vaccine antigens, however, will continue to circulate. Pertussis experts emphasize that neither infection nor vaccination guarantees life-long protection and emphasize the need to vaccinate adults now that adult vaccine formulations are available [32]. Recommendations for adult vaccination have already been established in North American and some European countries [26, 33, 34]. These countries must continue to monitor the disease and circulation of the causative agents to determine whether the increase in herd immunity, resulting from high Pa vaccine coverage, will be sufficient to control the circulation of *B. pertussis*. Analysis of pertussis epidemiology will remain important from the perspective of both public health and vaccinology.

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