Variation in the Bordetella pertussis Virulence Factors Pertussis Toxin and Pertactin in Vaccine Strains and Clinical Isolates in Finland

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There is evidence that pertussis is reemerging in vaccinated populations. We have proposed, and provided evidence for, one explanation for this phenomenon in The Netherlands: antigenic divergence between vaccine strains and circulating strains. Finland has a pertussis vaccination history very similar to that of The Netherlands, and yet there is no evidence for an increase in the incidence of pertussis to the extent that it was observed in The Netherlands. A comparison of the Bordetella pertussis strains circulating in the two countries may shed light on the differences in pertussis epidemiology. Here we investigated whether temporal changes had occurred in pertussis toxin and pertactin types produced by the Finnish B. pertussis population. We show that strains isolated before 1964 produced the same pertussis toxin and pertactin variants as the vaccine strains. However, these vaccine types were replaced in later years, and in the 1990s most strains were distinct from the vaccine strains with respect to the two proteins. These trends are similar to those found in the Dutch B. pertussis population. An interesting difference between the contemporary Finnish and Dutch B. pertussis populations was found in the frequencies of pertactin variants, possibly explaining the distinct epidemiology of pertussis in the two countries.

There is evidence that despite vaccination, the incidence of pertussis is increasing in a number of countries, such as Australia, Canada, The Netherlands, and the United States (1, 3, 7, 8). We have proposed elsewhere that this may be due to antigenic divergence between vaccine strains and circulating strains (14). Pertussis toxin and pertactin are two important virulence factors of Bordetella pertussis and have been shown elsewhere to confer protective immunity in animals and humans (14). Recently, it has been shown that the presence of antibodies against pertactin, and to a lesser extent pertussis toxin, correlates with clinical protection (5, 13). In view of their important role in protective immunity, it is perhaps not surprising that the S1 subunit of pertussis toxin and pertactin are polymorphic (2, 11). We have shown previously that S1 and pertactin variants identical to those included in the Dutch whole-cell pertussis vaccine were found in 100% of the Dutch strains from the 1950s, the period when the whole-cell pertussis vaccine was introduced in The Netherlands. However, nonvaccine types of S1 and pertactin gradually replaced the vaccine types in later years and were found in ~90% of the Dutch strains from the 1990s (11). These results are consistent with the hypothesis that vaccination has selected for strains that are antigenically distinct from vaccine strains.

Here we report the first results of a similar analysis of B. pertussis strains from Finland. In Finland, pertussis vaccination was started in 1952, and nowadays 98% of children receive four doses of diphtheria-tetanus-pertussis vaccine during their first 2 years of life (9). The Finnish pertussis vaccine (produced at the National Public Health Institute, Helsinki, Finland) has contained a mixture of two B. pertussis strains in equal amounts since 1976. Strain 18530 has been used since 1962. In 1976, a second strain, 1772, was added to the vaccine. Polymorphism in the genes for S1 (s1) and pertactin (prn) was studied with 54 clinical isolates and the 2 vaccine strains by DNA sequencing as described previously (11). The clinical isolates were from 1953 to 1964 (n = 5), 1982 (n = 6), and the 1990s (n = 43) (Table 1). The strains from 1982 were from a single outbreak, whereas strains from the 1990s were isolated from five outbreaks (n = 24) and nonoutbreak conditions (n = 19).

The s1 gene of S1 strains was sequenced completely. In the Dutch study, variation in the S1 subunit was found to occur in three residues, located in two regions previously identified as B- and T-cell epitopes (11, 12). Indeed, one of the polymorphic residues has been implicated in binding to the T-cell receptor (6), underlining the immunological relevance of polymorphism of this residue. Three s1 alleles were found in The Netherlands and are designated s1A, s1B, and s1D (11). The two Finnish vaccine strains contained s1D (strain 18530) and s1B (strain 1772). Strains from 1953 to 1964 all contained s1B (n = 5). However, all strains isolated in 1982 and the 1990s differed from the vaccine strains and harbored the s1A allele (n = 46) (Table 1).

Sequencing of prn was limited to two regions containing repeated sequences (designated region 1 and region 2) (11).

TABLE 1. Frequencies of s1 and prn alleles in different periods in Finland

<table>
<thead>
<tr>
<th>Period</th>
<th>No. of strains with allele (%)</th>
<th>s1</th>
<th>s1B</th>
<th>s1D</th>
<th>prn1</th>
<th>prn2</th>
<th>prn3</th>
<th>prn4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1953–1964</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1982</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1990–1996</td>
<td>43</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>31</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

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Region 1 was sequenced in all strains (n = 54), while region 2 was sequenced in eight randomly selected strains. Variation in prn was found only in region 1, as previously observed with Dutch strains. This region harbors the RGD motif, which is important for the function of pertactin, i.e., binding to host cell receptors (10). Four allelic variants, designated prn1 to prn4, which differ in the number of repeat units were found (Fig. 1). The prn1 to prn3 alleles were also found in The Netherlands (previously the products of prn1 to prn3 were designated P.69A to P.69C [11]), while prn4 is a novel type. Since prn4 was new, it was sequenced completely. The sequence revealed that polymorphism was restricted to region 1. In Finland, the two vaccine strains, and strains isolated in 1953 to 1964 (n = 5), contained prn1, whereas all strains isolated in 1982 (n = 6) contained prn2 (Table 1). In the strains from the 1990s (n = 43), two additional prn alleles were found, prn3 and prn4. The frequencies of alleles prn1 to prn4 in the 1990s were, respectively, 7, 72, 12, and 9% (Table 1).

The results of Dutch and Finnish studies show in principle a similar trend. The Dutch and Finnish vaccine strains contained the same s1 and prn alleles (i.e., s1B, s1D, and prn1). Further, in both countries divergence was observed between vaccine strains and clinical isolates after the introduction of vaccination, and in the 1990s, the s1 and prn alleles present in the vaccine strains were found to be largely replaced by new types. The frequency of the s1A allele in the 1990s in both countries was comparable (90 and 100% in The Netherlands and Finland, respectively). An interesting difference between the Dutch and Finnish B. pertussis populations was observed with respect to the prn frequencies in the 1990s. The prn4 allele was not observed in the Dutch study. Further, the frequencies of prn2 and prn3 differ significantly in the two countries: 36 and 51% in The Netherlands and 72 and 12% in Finland, respectively. In Sweden, frequencies similar to those in Finland were found (10a).

The incidence of pertussis has increased in Australia, Canada, The Netherlands, and the United States (1, 3, 7, 8). The antigenic divergence between vaccine strains and clinical isolates, observed both in The Netherlands and in Finland, is consistent with the notion of vaccine-driven evolution. There is, however, no evidence for major nationwide outbreaks of pertussis in Finland. This may suggest that the Finnish vaccine is potent enough to offset antigenic variation. It is also possible that pertussis vaccines protect less well against strains with particular prn alleles, such as prn3, which predominate in some countries, such as The Netherlands, but are less common in Finland. The lower vaccine efficacy may be a direct effect of prn3 or due to other polymorphic regions in B. pertussis which are linked to prn3. Preliminary experiments with a mouse model indicate that the whole-cell vaccine is more effective against prn1 strains than against prn2 or prn3 strains (10a).

**Nucleotide sequence accession number.** The accession number of the sequence of prn is AJ011015.

REFERENCES


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