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Serum Immunoglobulin G Antibody Responses to *Bordetella pertussis* Lipooligosaccharide and *B. parapertussis* Lipopolysaccharide in Children with Pertussis and Parapertussis

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Serum immunoglobulin G (IgG) antibodies against the lipooligosaccharide (LOS) of *Bordetella pertussis* and the lipopolysaccharide (LPS) of *Bordetella parapertussis* were measured by enzyme-linked immunosorbent assay in paired sera from 40 children with pertussis and 14 with parapertussis. Wide differences in the individual responses were noted. Both anti-LOS and -LPS IgG levels increased significantly in the children with pertussis, as did anti-LPS but not anti-LOS in those with parapertussis.

Bordetella pertussis and *Bordetella parapertussis* are closely related species and share many virulence factors, including the pertussis toxin gene. The former organism expresses pertussis toxin, while the latter does not (1, 14). The two organisms have different cell wall liposaccharides. The liposaccharide of *B. pertussis* has a lipid A region and a core oligosaccharide most accurately described as a lipooligosaccharide (LOS) (5, 12, 17). *B. parapertussis* has similar lipid A and core regions plus an outer O-specific polysaccharide region composed of poly(→2-α-L-GalA*p*-3→)$_n$, where *n* is about 16 (5, 7). Thus, the liposaccharide of *B. parapertussis* is designated a lipopolysaccharide (LPS).

Characterization of the serum antibody responses against the liposaccharides of these two *Bordetella* species is of interest for two reasons. First, serum antibodies against the liposaccharides of gram-negative organisms can be protective (6). Second, serum antibody responses to *B. pertussis* LOS can be used to diagnose pertussis (8, 9). This is of particular interest in recipients of acellular pertussis vaccines in which LOS is only a trace contaminant.

In the present work, the serum immunoglobulin G (IgG) responses to the LOS of *B. pertussis* and the LPS of *B. parapertussis* in children with the respective diseases and the cross-reactivity between the two liposaccharides were studied.

Children with pertussis. In a double-blind placebo-controlled study of a monovalent pertussis toxoid vaccine, nasopharyngeal samples for culture of *Bordetella* species and paired sera were obtained from study participants and family members with cough persisting for at least 7 days (19). IgG antibodies against *B. pertussis* LOS and *B. parapertussis* LPS were assayed by enzyme-linked immunosorbent assay (ELISA) in paired sera from 40 randomly selected siblings of study participants who fulfilled the following criteria: (i) paroxysmal cough for ≥21 days; (ii) pertussis verified by isolation of the organism (35 children) or by significant antibody increases against both pertussis toxin and filamentous hemagglutinin (5 children); (iii) an acute-phase serum obtained within 14 days after onset of symptoms and a convalescent-phase serum obtained 4 to 6 weeks later; and (iv) not vaccinated against pertussis. The age of the children (18 male, 22 female) ranged between 6 months and 7 years (median, 4 years). The time from onset of symptoms until the first serum was obtained ranged between 5 and 14 days (median, 8 days).

Children with parapertussis. Serum IgG antibodies were assayed in all study participants ($n = 12$) and all siblings ($n = 2$) in the vaccine efficacy trial (19) with laboratory-verified parapertussis for whom paired sera were available. The age of the 14 children (nine male, five female) ranged between 6 months and 6 years (median, 1 year). The diagnosis of parapertussis was verified by isolation of the organism from 10 children. For three children, parapertussis DNA was detected in nasopharyngeal secretion by PCR (13). *B. parapertussis* was isolated from a sibling of one child. In addition, all 14 children had significant increases in antibodies against filamentous hemagglutinin but not against pertussis toxin. The interval between onset of symptoms and the first serum sample ranged between 7 and 16 days (median, 13 days).

Serology. Serum IgG was determined by ELISA. Liposaccharides were prepared from *B. pertussis* (strain Tohama; Culture Collection of Göteborg University no. 15609) and from *B. parapertussis* (American Type Culture Collection strain 15989) by hot phenol-water extraction (21). The crude liposaccharides were treated with RNase and DNase followed by proteinase K (Sigma Chemical Co., St. Louis, Mo.) and then ultracentrifuged. The protein and nucleic acid content of the two preparations was <1%.

Microtiter plates were coated at 23°C with LOS or LPS at 2 μg/ml, diluted in phosphate-buffered saline (PBS) containing 10 mM $MgCl_2$. The plates were washed twice with PBS and blocked with 0.1% bovine serum albumin (BSA) in PBS (0.1% BSA–PBS) for 1 h at 23°C. The plates were then washed three

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TABLE 1. Serum IgG responses to *B. pertussis* LOS and *B. parapertussis* LPS in patients with pertussis and parapertussis

Patient group	IgG titer in serum from indicated phase			
	Pertussis LOS		Parapertussis LPS	
	Acute	Convalescent	Acute	Convalescent
Pertussis (n = 40)				
GMT	<50	364	<50	379
Median	<50	374	<50	385
Range	<50–1,045	<50–12,045	<50–684	50–20,240
Parapertussis (n = 14)				
GMT	<50	<50	68	646
Median	<50	<50	<50	1,308
Range	<50–122	<50–73	<50–3,334	<50–4,625

times with 0.05% Tween 20. The acute- and convalescent-phase sera were diluted 1:50 and tested on the same plate in eight threefold dilutions. The plates were incubated overnight, and after washing, alkaline phosphatase-coupled anti-human IgG (Jackson Immuno Research Lab) diluted in 0.1% BSA–PBS was added. The plates were incubated at 23°C for 5 h. After washing, the plates were developed with nitrophenyl phosphate substrate at 1 mg/ml (Sigma) in 1 M Tris-HCl buffer, pH 9.8, with 3 mM $MgCl_2$ and read at 460 nm (Titertek Multiscan; Flow Laboratories). The optimal concentrations of the reagents were tested by checkerboard titrations with each serum tested in duplicate. Wells containing all reagents but with PBS instead of serum were used as controls. A serum with high anti-LOS and anti-LPS IgG titers was used as a positive control. The antibody content was expressed as the reciprocal serum dilution (titer) giving an absorbance of 0.3 above the background. An optical density (OD) of 0.3 above the background was chosen because the linear part of LOS and LPS antibody curves started at an OD of 0.25 above the background. The lower part of the curve was the most constant and showed the least variability between repeated assays. Sera with a titer of <50 were arbitrarily assigned a titer of 25 when geometric mean titers (GMT) were calculated.

Statistical analysis. Comparisons between acute- and convalescent-phase sera were performed with a paired t test after logarithmic and rank transformation of data. All P values are two-tailed.

Serum IgG in patients with pertussis. Table 1 shows that there was approximately an eightfold rise in the GMT of anti-LOS IgG from *B. pertussis* between the acute- and convalescent-phase sera in the children with pertussis ($P < 0.0001$ for logarithmically and rank-transformed data). The range of increases varied widely: 10 had no detectable anti-LOS IgG in their convalescent-phase sera, 5 had titers between 50 and 100, 8 had titers between 100 and 1,000, and the remaining 16 had titers between 1,000 and 12,045.

With the *B. parapertussis* LPS antigen, there was also an approximately eightfold rise in the GMT of anti-LPS IgG in the children with pertussis ($P < 0.0001$ for logarithmically and rank-transformed data). Most children with anti-LOS IgG increases also had increases in anti-LPS IgG, but some exceptions were noted. One child had an increase from 73 to 10,147 against LOS, while anti-LPS IgG was <50 in both sera. Three children had increases from levels of less than 100 to more than 5,000 against LPS, while anti-LOS IgG was below 100 in both acute- and convalescent-phase sera. In these three children, the diagnosis of pertussis was verified by both isolation of the organism and significant increases in pertussis toxin IgG.

Serum IgG in patients with parapertussis. Table 1 shows that IgG antibodies to the LPS of *B. parapertussis* increased significantly (~10-fold) in the children with parapertussis ($P < 0.0001$ for rank-transformed and $P < 0.0003$ for logarithmically transformed data). Only one child with parapertussis had nondetectable anti-LPS IgG in the convalescent-phase serum, and two had titers of 50 to 100.

In contrast to the heterologous anti-LPS IgG response of the patients with pertussis, none of the 14 patients with parapertussis had a rise in anti-LOS IgG.

Comments. This study shows that the IgG response following parapertussis is directed only to the LPS of *B. parapertussis,* while infection with *B. pertussis* elicits antibodies reactive with the liposaccharides of both *Bordetella* species. Our explanation for these different responses is based upon the structures of the two surface liposaccharides. The core region of the LPS of *B. parapertussis* is "covered" by the O-specific polysaccharide region, while antibodies to the shared core region, elicited by *B. pertussis*, are reactive both with the LOS of this organism and with the purified *B. parapertussis* LPS, as shown in vitro (11, 17). An LOS IgG response in patients with pertussis has been documented previously (8, 16). There seems to be no difference in the overall immunogenicity of these two pathogens in patients, as the serum IgG responses to the filamentous hemagglutinin and pertactin were similar in both patient groups (2).

We have no explanation for the finding that three pertussis patients developed *B. parapertussis* anti-LPS but not *B. pertussis* anti-LOS IgG. Possibly, they had a mixed infection with both pathogens.

Although the LOS of *B. pertussis* and the LPS of *B. parapertussis* have structural and biological properties similar to those of other respiratory and enteric gram-negative pathogens, including pyrogenicity (20), fever is not seen in *Bordetella* infections. Accordingly, it is unlikely that these surface liposaccharides exert a major pathogenic role in the coughing of pertussis or parapertussis.

Serum antibodies to the liposaccharides of bordetellae are found in many individuals without a recent history of a severe cough (8, 16). These so-called "natural" antibodies were probably stimulated by cross-reacting antigens. The presence of LOS antibodies in patients who later develop pertussis argues against their protective role against *B. pertussis*. Although they induce an in vitro bacteriolysis of *B. pertussis* in the presence of diluted complement, the failure of anti-LOS IgG to protect against pertussis may be due to the inaccessibility of this comparatively small surface antigen (4, 5, 7, 10, 17).

Similar to the LPS of other gram-negative bacteria and in contrast to the LOS of *B. pertussis*, the O-specific polysaccharide region of the LPS shields *B. parapertussis* against the cidal action of complement alone (3, 14, 15, 18). Accordingly, the LPS region may confer virulence on *B. parapertussis*.

In summary, serum IgG increases against LOS of *B. pertussis* and LPS of *B. parapertussis* are common but not constant findings in patients with pertussis and parapertussis, respec-

tively. Many patients with pertussis had IgG increases against *B. parapertussis* LPS, while the reverse was not seen. These findings may be of importance for diagnosing pertussis in patients who have the disease despite vaccination with acellular pertussis vaccines and for studying parapertussis epidemiology in populations in which pertussis has become eradicated due to mass vaccination with acellular pertussis vaccines.

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