

Short communication

Characterization of the antibody response to a *Haemophilus influenzae* type b conjugate vaccine in children with recurrent lower respiratory tract infection

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Children with recurrent lower respiratory tract infection (RLRI) may respond poorly to polysaccharide antigens. To examine how such children respond to a polysaccharide coupled to a protein carrier, we immunized 15 children with RLRI aged 8-69 months and 15 carefully age-matched healthy controls once with a *Haemophilus influenzae* type b (Hib) conjugate vaccine. Total IgG subclasses, total antipolysaccharide Hib antibodies, and antipolysaccharide Hib antibodies of IgM, IgG, IgA, and IgG1-4 specificity were determined by ELISA. There were no significant differences between the two groups in any single total IgG subclass, but total IgG measured as the sum of all four subclasses was significantly lower in the children with RLRI than in the controls ($P = 0.036$). Before vaccination, the children with RLRI had significantly less IgG antipolysaccharide Hib antibody than the controls ($P = 0.005$), whereas 1 month later they had significantly more IgM antibody ($P = 0.038$). No other significant differences were found between the groups before or after immunization with respect to antipolysaccharide Hib antibodies. Since naturally occurring IgG antibodies are thought to be acquired partly as a consequence of antigenic stimulation on mucosal surfaces, we hypothesize that the low level of specific IgG found before immunization, as well as the low total IgG in the children with RLRI, may reflect an impaired ability to prime through mucosal surfaces. This is supported by our finding of an increased IgM response to Hib conjugate vaccine in these children, since this isotype predominates in the primary immune response, i.e., in the absence of immunologic memory. In conclusion, children with RLRI can be protected against invasive Hib infection as well as healthy children, but may have an immunodeficiency characterized by defective ability to respond to antigenic stimulation on mucosal surfaces.

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The availability of highly effective polysaccharide protein conjugate vaccines against *Haemophilus influenzae* type b (Hib) has created an opportunity to eradicate invasive Hib infection, which is annually causing thousands of deaths and cases of neurologic disability worldwide. Immunity to Hib disease is closely related to the presence of antipolysaccharide antibodies (18), and the effect of the new conjugate

Hib vaccines is based on their ability to induce such antibodies in infants below the age of 18-24 months (8), in whom most invasive Hib infections occur (6), and in whom protection was not achieved with the earlier uncoupled polysaccharide vaccine (18). The increased immunogenicity of the Hib conjugate vaccines is thought to be due to their acting as T-cell-dependent antigens (22), in contrast to the uncoupled

polysaccharide, which acts as a T-cell-independent antigen (16). However, since most studies on the immunogenicity of Hib vaccines have focused on healthy children, some doubt still exists that protection will be complete in patients with increased susceptibility to infection or hyporesponsive to immunization. Furthermore, although the efficacy of these new vaccines has been proven in large field trials (4, 9, 10, 20), failure to protect such patients may easily be overlooked due to the relative rarity of these conditions, and because exposure to Hib may decrease after initiation of a general vaccination program (24).

Children with recurrent lower respiratory tract infection (RLRI) constitute a major problem in pediatric practice, and, in most cases, even when such patients undergo extensive evaluation, no underlying condition can be identified. An impaired ability to respond to immunization with the uncoupled polysaccharide Hib vaccine has, however, been described in such patients (1, 12, 23), and likewise their IgG2 response to pneumococcal polysaccharide may be decreased (7). To examine how children with RLRI respond to a polysaccharide coupled to a protein carrier, we immunized such children and healthy controls with a polysaccharide protein conjugate Hib vaccine and compared their responses with respect to total antibodies as well as IgM, IgG, IgA, and IgG subclass-specific antibodies. Furthermore, the children were characterized with respect to plasma levels of total IgG subclasses.

Material and methods

Vaccine

Hib conjugate vaccine (PRP-TT) (lot S2190) consisting of purified Hib polysaccharide (PRP) covalently linked to tetanus toxoid was kindly provided by Institut Merieux, Lyon, France.

Patients and controls

Fifteen children with RLRI and 15 carefully age-matched controls (range 8–69/7–80 months, median age 27/26 months; $P=0.97$) were enrolled in the study after informed consent from the parents. The study protocol was approved by the regional ethics committee. The criterion for categorizing a child as having RLRI was at least three cases of radiologically confirmed pneumonia, but most participating children with RLRI had had radiographically proven pneumonia on many more occasions. All children with RLRI received frequent prophylactic antibiotic courses based on monthly bacteriologic examination of sputum obtained by nasopharyngeal suctioning. The sweat test gave normal results in all, and none were leukopenic or granulo-

cypenic. Likewise, all had normal immunoglobulin levels in plasma, and none had congenital malformations. The controls were 10 healthy children and five patients admitted to the department for minor surgery such as herniotomy or orchilysis.

Immunization and serum samples

All participating children were immunized intramuscularly once with PRP-TT. Serum samples were obtained immediately before vaccination and 1 month after and were stored at -80°C until analyzed. We did not interfere with the routine immunization program, which in Denmark is administered by the general practitioner. When this study was conducted, it consisted of pertussis vaccine given at ages 5 weeks, 9 weeks, and 10 months; diphtheria-tetanus-polio vaccine at ages 5, 6, and 15 months; morbilli-mumps-rubella vaccine at 15 months and 12 years; and oral polio vaccine at 2, 3, and 4 years.

Laboratory methods

Total IgG subclasses, total anti-PRP antibodies, and isotype-specific anti-PRP IgM, IgG, IgA, and IgG1–4 antibodies were measured by ELISA at the Laboratory of Medical Microbiology, Panum-institutet, Copenhagen; the Bacteriological Department, Statens Seruminstitut, Copenhagen; and the Laboratory of Medical Immunology, Rigshospitalet, Copenhagen, Denmark, as previously described (11, 15, 19). In the assay used for detection of total IgG subclasses, the sum of these correlated significantly with total IgG ($r=0.50$; $P<0.001$), and the assay used for measurement of total anti-PRP antibody has been shown to correlate well with the radioimmunoassay in use at the National Institute of Health, Helsinki, Finland ($r=0.93$; $P<0.001$). For total IgG subclasses, results were expressed in gram per liter; for total anti-PRP antibody, in microgram per milliliter serum; and for anti-PRP antibodies of isotype specificity, in arbitrary units.

Statistical methods

Since prevaccination values even after log transformation were not normally distributed, the Mann-Whitney U-test was chosen for comparison of the groups. For nominal data, Fisher's exact test was used. Calculations were done with the computer program Systat, and $P<0.05$ (two-sided) was considered to be significant.

Results

There were no significant differences between any single total IgG subclass between the two groups

(IgG1: $P = 0.097$, IgG2: $P = 0.21$, IgG3: $P = 0.24$, IgG4: $P = 0.52$), but the sum of all four subclasses was significantly lower in the children with RLRI than in the controls (median 2.25 versus 5.01 gram per liter; $P = 0.036$). Before vaccination, the children with RLRI had significantly less IgG anti-PRP antibody than the healthy controls ($P = 0.005$), whereas no significant differences were found between the prevaccination levels of total, IgM, IgA, or IgG subclass antibodies in the two groups. However, there was a trend for the children with RLRI to have less IgG1 anti-PRP antibody than the controls ($P = 0.06$ for IgG1; $P > 0.15$ for others). After vaccination, the children with RLRI had significantly more IgM anti-PRP antibody than the controls ($P = 0.038$), but there were no significant differences in the total, IgG, IgA, or IgG subclass antibody responses between the two groups ($P > 0.15$ for all). Eleven of 15 children with RLRI and eight of 15 controls ($P = 0.45$) had a pre-immunization anti-PRP antibody concentration below the estimated protective level ($0.15 \mu\text{g ml}^{-1}$ (13)), whereas postimmunization levels in all except one child with RLRI exceeded this level. The level associated with long-term protection ($1 \mu\text{g ml}^{-1}$ (2, 13)) was reached by all participating children except two controls.

Discussion

Our finding of a significantly decreased level of total IgG expressed as the sum of the four subclasses in the children with RLRI as compared to the controls was surprising since, to our knowledge, general IgG hypogammaglobulinemia, in contrast to isolated IgG subclass deficiency (3), has not been reported before in these patients. One explanation for this finding may be that the children with RLRI in this study were highly selected; i.e., they had had radiologically proven pneumonia on several occasions and were not suffering only from recurrent bronchitis. Unfortunately, due to an insufficient amount of serum, we were not able to measure total IgG by an alternative method, but since the sum of the four subclasses correlated significantly with total IgG in the assay used for detection of total IgG subclasses, we consider our finding to be valid. Before immunization with PRP-TT, specific anti-PRP IgG was also lower in the children with RLRI than in the controls. Since synthesis of IgG antibodies is thought to occur partly as a consequence of antigenic stimulation of mucosal surfaces, these findings may lead to speculation that children with RLRI have an impaired ability to be primed through their mucosal surfaces, not only by PRP or cross-reacting polysaccharides such as *Escherichia coli* K100 (21), but also by other bacterial and viral antigens, leading to increased susceptibility to infection. Outer membrane protein P6

is a highly conserved antigen among both capsulated and noncapsulated strains of *H. influenzae* (17). Because *H. influenzae* is very often harbored in the upper respiratory tract in children (14), most, if not all, children will experience frequent mucosal exposure to this antigen. Therefore, a recent report on another group of infection-prone children, i.e., those with recurrent otitis media, showing that such children have impaired development of IgG antibodies to P6 (25), also supports this hypothesis. Finally, it agrees well with our finding of an increased IgM response to Hib conjugate vaccine in the children with RLRI since IgM is the predominant isotype in the absence of immunologic memory.

The Hib conjugate vaccine used in this study has now been incorporated in the routine immunization program in many countries. Although immunization starts in infancy, many children with RLRI in the age group studied here have been vaccinated, since, at the beginning of a general vaccination program against Hib, it has been recommended that all children up to the age of 5 years receive the vaccine. Because the response in the children with RLRI with respect to both total antibody as well as IgG, IgA, and IgG subclass-specific antibodies was comparable to that of the controls, these children have probably achieved protection against invasive Hib disease to the same extent as healthy children. However, a reduction in the number of RLRI after immunization with Hib conjugate vaccine cannot be expected, since Hib is only an infrequent cause of pneumonia in children (5).

We conclude that children with severe RLRI may have an immunodeficiency characterized by an impaired ability to respond to antigenic stimulation on mucosal surfaces. However, the Hib conjugate vaccine used in this study is able to elicit an antibody response in these patients, and this response is comparable to that of healthy children.

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