Invasive *Haemophilus influenzae* Type b (Hib) Infection in an Adult Patient with a Selective Deficiency of Antibody to the Hib Capsular Polysaccharide

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A 31-year-old woman without any underlying disease contracted severe invasive *Haemophilus influenzae* type b (Hib) infection but developed no antibodies to the Hib capsular polysaccharide. Serum immunoglobulin levels were normal, but she had an isolated deficiency of antibody to Hib. Subsequently, immunization with a tetanus toxoid–conjugated Hib vaccine induced only a minimal response. However, she had a protective level of antibody (>1 μg/mL) after the fifth vaccination.

Invasive *Haemophilus influenzae* type b (Hib) infections occur almost exclusively in young children and are rarely seen in adults [1]. Protective antibodies to the Hib capsular polysaccharide (CP) are normally acquired during childhood and thereafter prevent invasive Hib disease [2]. The low immunogenicity of bacterial polysaccharides in young children can be overcome by coupling the CP to protein carriers, a technique used in current conjugated Hib vaccines [3]. Such conjugated Hib vaccines were in 1993 (the time of investigation of this case) included in the Swedish infant vaccination program.

Case Report

In February 1992, a 31-year-old woman with no history of increased susceptibility to infections contracted an acute high fever (temperature, 40°C), myalgia, throat pain, and difficulty in swallowing. Two children in the same household (6 months and 1.5 years of age, respectively) were also ill with uncomplicated upper respiratory tract infections. On the third day of illness, she had increasing malaise, shivering, and persistent high fever and was admitted to the hospital. At the time of admission, localized supraglottic swelling, but no classic epiglottitis, was observed. Treatment with cefuroxime (1.5 g t.i.d.) was given, and blood cultures later yielded β-lactamase-producing Hib. After 1 day of intensive care, her condition rapidly improved, and a CT on the fifth day showed that moderate parapharyngeal edema remained. After 1 week, she was discharged.

Laboratory studies revealed the following normal values: WBC counts; levels of serum IgG, IgM, IgA, and IgG subclasses; C3, C4, and factor B levels; T lymphocyte profile; lymphocyte subsets; and response to mitogen activation. HIV serology was negative, but she had IgG antibodies to several other viruses.

The levels of all IgG antibodies to Hib CP were studied by using a previously described avidin-biotin–based ELISA [4] with some modifications. Briefly, microplates were precoated with 5 μg of avidin/mL, and after overnight incubation, they were coated with biotinylated Hib CP (concentration, 2 μg/mL). After blocking of nonspecific binding, serum samples in serial dilutions were incubated overnight. Mouse antibody to human IgG conjugated with alkaline phosphatase was added to the plates and incubated; after the reaction with enzyme substrate (o-nitrophenyl-β-D-galactopyranoside) in 1 M diethanolamine buffer (pH, 9.8) was developed, the plates were read spectrophotometrically at 405 nm. The reference for the quantification of antibody levels used was isolated human IgG to Hib CP (U.S. Food and Drug Administration, Bethesda, MD). We considered the concentration >1 μg/mL as a protective level of antibody to Hib CP since such a level achieved after vaccination clearly confers long-lasting protection against invasive Hib disease even in infants [5]. We found only very low (nonprotective) amounts of specific antibody to Hib CP (0.05 μg/mL) in this patient by ELISA.

In April 1994, immunization with a conjugated vaccine (0.5 mL subcutaneously; Act-HIB, Pasteur-Mérieux MSD, Lyon, France) was started; however, in contrast to the one dose that is usually sufficient for normal adults, multiple doses given at 0, 2, 4, 8, and 15 months were required. The antibody response developed very slowly (figure 1), and protective levels of antibodies to Hib CP (>1 μg/mL) were reached only after five vaccinations (3.1 μg/mL). The prevaccination level of antibody to Hib CP in our patient’s healthy sister (who was 2 years younger) was 0.16 μg/mL, but the sister had a good response after a single dose of Act-HIB (10.4 μg/mL).

Serum samples were analyzed by a combination of isoelectric focusing and affinity immunoblotting [6] with use of the following reagents: Hib CP; tetanus toxoid; pneumococcal CPs 6B and 14, meningococcal CPs A and C, or *Escherichia coli* K100 CP; outer membrane proteins of *H. influenzae*; and the monoclonal antibody LuC9, which specifically recognizes anti-Hib CP idiotype 1 (Id-1) [7]. Such clonotypic analysis of anti-
bodies to Hib CP showed that both the patient and her sister responded to vaccination with two different IgG2 clones (figure 1A). Our patient developed the visible clones only after the third dose of vaccine, while her sister responded to the first dose with one IgG2 clone (shown by more strongly stained bands in figure 1A) and one IgG1 clone. No IgG1 clones developed in our patient after all vaccinations.

A few weakly stained bands of IgG2 antibodies to E. coli K100 CP were detected in all serum samples, which did not change during the course of vaccination and did not correspond to the bands of antibodies to Hib CP. We conclude that no cross-reaction between E. coli K100 CP and antibodies to Hib CP was found in either sister’s serum.

A well-defined clone of IgG antibody expressing Id-1 was found in our patient before the first vaccination. It did not change after all the vaccine doses were administered (figure 1B). A few weakly stained bands of IgG antibody expressing Id-1 and two abundantly developing clones were detected in the sister’s serum before and after vaccination, respectively (figure 1B). While the pattern of serum samples from the sister showed the expression of Id-1 by antibodies to Hib CP, the antibodies expressing Id-1 in our patient’s serum were not related to antibodies to Hib CP (figure 1C).

We estimated the response of our patient to other polysaccharide vaccines (Pneumovacc N and Meningovacc A+C, Pasteur-Mérieux MSD) by using the combination of isoelectric focusing and affinity immunoblotting. IgG1 and IgG2 antibodies to meningococcal CPs A and C appeared as very weakly stained bands before vaccination but developed into intensely stained clones of IgG2 antibodies to both CP A and CP C after vaccination.

Antibodies to pneumococcus were studied in samples preabsorbed with C polysaccharide. The results showed the presence of clones of IgG1 and IgG2 antibodies to CP 6B, which became more intensively stained after vaccination. No antibody to CP 14 was found before immunization, but after immunization, numerous clones of IgG2, but none of IgG1, antibody appeared. At the same time, we noticed the presence of IgG2 antibodies to C polysaccharide both before vaccination with Pneumovacc N and more abundantly with numerous clones after vaccination. While we did not quantify the antibody levels, the isoelectric focusing and affinity immunoblotting patterns clearly showed the development of the antibody response to these polysaccharides.

The antibody response to the carrier protein (tetanus toxoid) was quantified after each vaccination. Both sisters showed protective levels of antibody to tetanus toxoid before Act-HIB vaccination. However, these levels rose significantly after the first dose of vaccine and thereafter remained at a constant high level (data not shown in figure 1), thus indicating an unimpaired response to this protein antigen. Clones of IgG1 and IgG2 antibodies to outer membrane proteins of H. influenzae were found in both sisters as well.

**Discussion**

Invasive Hib infections are very uncommon in adults without predisposing underlying disease. An annual incidence of 0.22 case per 100,000 population has been reported [1]. Protective antibodies to Hib CP may develop during childhood because of natural exposure to cross-reacting antigens like E. coli K100 CP [2]. In one study [8], after invasive Hib infection, high levels of antibody to Hib CP were induced in most adults, findings in clear contrast to the low amounts of antibodies found in our patient.

The conjugated Hib vaccines were developed primarily for infants but also are very efficient for vaccinating healthy non-immune adults [3]. Even in immunocompromised persons, e.g., patients without spleens or bone marrow recipients, these vaccines are good immunogens [9, 10]. In our patient, however, the conjugated Hib vaccine did not generate protective amounts of antibodies to Hib CP immediately. Repeated immunizations were required before the patient, in contrast to her sister, slowly responded.

We can conclude that this was a case of a selective deficiency of antibody to Hib CP that could be overcome by repeated
immunizations with a protein-polysaccharide conjugate rendering the polysaccharide T cell dependent. The selective character of this immunodeficiency was confirmed by the ability of the patient to respond to vaccination with other bacterial polysaccharides (pneumococcal and meningococcal CPS) as well as to protein antigens (tetanus toxoid and outer membrane proteins of *H. influenzae*).

The monoclonal antibody LuC9 specifically recognizes Hib Id-1, which was originally discovered to be present on theVkII-A2 chain of human antibody to Hib CP. This idiotype was found to be prevalent in the antibodies to Hib CP in adults [7]. While the pattern of serum samples from the sister showed a clear expression of Id-1 by antibodies to Hib CP after vaccination, the occurrence of antibody expressing Id-1 that was not related to either antibody to Hib or vaccination was found in our patient’s serum. Similar results were recently found in sera from healthy Chinese people in Hong Kong [6]. Reason and Lucas [11] recently showed that Hib Id-1 is not confined to the A2 region of antibodies specific to Hib CP but can be expressed by antibodies using either the A2 or A18 region (a very close homologue to the A2 region in the human κ chain), irrespective of antigenic specificity. We suggest that expression of Hib Id-1 by antibodies that are not specific to Hib CP in our patient may represent a product of the A18 region and that those antibodies are a result of natural exposure to some environmental antigen(s) of unknown specificity. The possibility of an inhibitory effect of a cross-reacting idiotype present before vaccination (or infection) on the antigen-specific response may be considered.

To our knowledge, this is the first case of a selective immunodeficiency of antibody to Hib CP that has been successfully treated with a prolonged vaccination schedule. It illustrates the importance of specific immunologic investigation for adults with invasive Hib infections. In similar cases of selective deficiency of antibody to polysaccharide, an individual vaccination scheme far beyond those usually recommended may need to be applied.

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**References**