Group-Specific Antibody Levels Surrounding Invasive Pneumococcal Illness in Children Infected with Human Immunodeficiency Virus

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Pneumococcal antibody levels surrounding systemic pneumococcal illness (SPI) were measured in children infected with human immunodeficiency virus (HIV). Archived serum samples were collected from 28 HIV-infected children who had 34 cases of SPI, caused by pneumococcal groups 4, 6, 9, 14, 19, and 23. Serum samples collected within 23 weeks before and 13 weeks after the SPI were assayed by ELISA for antipneumococcal polysaccharide (PnP) IgG antibody to 6 representative pneumococcal serotypes. There was a wide range (0.16–30.80 μg/mL) of pre-SPI anti-PnP antibody levels to the presumed infecting serotypes, with a geometric mean level of 0.83 μg/mL (n = 34). Seventy-six percent of the antibody values were <2.0 μg/mL, and 95% were <5.0 μg/mL. Homologous seroresponses (≥4-fold rise in anti-PnP antibody) were detected in only 4 (27%) of 15 paired serum samples. Heterologous, noninfecting group seroresponses were detected frequently (72%) in the paired serum samples from these 4 homologous group seroresponders.

Recognition of the antigenic diversity of pneumococcus is important in any study of this human pathogen. First, there are 46 known pneumococcal groups and at least 90 serotypes within these groups. Protection from invasive pneumococcal disease is thought to be related primarily to serum anticapsular antibody [1, 2]. Within pneumococcal groups, serum antibody cross-reactivity between serotypes can occur [3], but heterologous group serologic cross-reactivity has not been well defined.

Children with human immunodeficiency virus (HIV) infection are known to be at high risk for systemic pneumococcal infection (SPI), particularly bacteremia; attack rates of ~10% per year among young, HIV-infected children have been described [4, 5]. Measurements of serum antibody surrounding SPI in HIV-infected children may be helpful in delineating the reason for this increased susceptibility. An ELISA has been developed to measure human serotype-specific serum IgG levels [6].

This study collected information on group-specific (and when available, serotype-specific) pneumococcal antibody from HIV-infected children who experienced SPI. Archived serum samples that were collected close to the time of the SPI were evaluated so that IgG antibody responses to natural infection could be estimated.

Materials and Methods

Study population. Investigators from 9 different medical centers provided anonymously coded medical records on HIV-infected children who (1) had documented bacteremia with Streptococcus pneumoniae; (2) belonged to an identified group (and, when possible, had an identified serotype) of the pneumococcal isolate that caused the illness; (3) had not received blood products within 3 months prior to collection of the serum samples; and (4) had archived serum samples that had been stored at −70°C and were obtained within 23 weeks prior to (or within 12 weeks prior if the
child was <12 months old) or within 13 weeks after SPI. This study was approved by the University of Maryland’s institutional review board.

**Typing of pneumococcal isolates.** Pneumococcal isolates from Yale University (New Haven, CT), University of Maryland (Baltimore), Children’s Hospital of Philadelphia, St. Jude Children’s Research Hospital (Memphis), and the State University of New York (Stonybrook) were typed in the laboratory of Dr. Robert Austrian (University of Pennsylvania, Philadelphia). Pneumococcal isolates from Children’s Hospital, Denver; Children’s Memorial Hospital, Chicago; and Children’s Hospital, San Diego, were typed in the laboratory of Dr. Edward O. Mason (Baylor College of Medicine, Houston, TX). Pneumococcal isolates from New York University Medical Center were typed on site.

**Serum pneumococcal antibody.** Archived serum samples were packed in dry ice and sent to the Johns Hopkins University Dermatology, Allergy, and Clinical Immunology Reference Laboratory (Baltimore), where they were assayed for representative serotype-specific pneumococcal capsular polysaccharide (PnPs) IgG antibody. Serotype-specific PnPs IgG antibodies were measured by a consensus ELISA with antigens from American Type Culture Collection, after exposure of the serum samples to pneumococcal C-polysaccharide absorbent (Statens Serum Institute, Copenhagen, Denmark, lot X-12-13), which was designed to remove non-type-specific antibody [6]. All serologic assays were performed in a blinded manner, and paired serum samples collected before or after an SPI were run simultaneously. The antibody concentrations were standardized to IgG anti-PnPs assigned antibody values for US Food and Drug Administration reference serum 89SF. The analytical sensitivity of these assays varied by serotype, from 0.05 to 0.08 μg/mL.

Participating centers provided anonymous information, such as the subject’s age at SPI, HIV-related illness severity (by using the 1994 Centers for Disease Control and Prevention [CDC] pediatric criteria [7]), and peripheral blood CD4+ cell percentages obtained within 4 months prior to SPI.

**Statistical analysis.** Log10-transformed IgG anti-PnPs antibody levels were compared between paired pre-SPI and post-SPI serum samples by using a paired t test. All proportions were compared by using a χ² or Fisher’s exact test when appropriate.

## Results

### Descriptions of SPI

Six pneumococcal groups (4, 6, 9, 14, 19, and 23) caused 34 SPI episodes in 28 children infected with HIV (table 1), between July 1985 and January 1996. The serotypes of the pneumococcal isolates that caused these SPIs, identified when available, also are listed in table 1. Unfortunately, not all the pneumococcal isolates that caused the cases of SPI were serotyped. Because serotypes 6B, 9V, 19F, and 23F were the most common serotypes circulating in the United States during the last several decades, it was assumed that these serotypes were the most appropriate antigens for the ELISA used in this study. Fortunately, pneumococcal groups 4 and 14 have no serotypes.

### Pneumococcal antibody

Pre-SPI serum samples were obtained at a mean of 8.9 weeks (SD, 7.0 weeks) before SPI. Post-SPI serum samples were obtained within a mean of 8.9 weeks (SD, 4.1 weeks) after SPI.

Pre-SPI, presumed-infecting, serotype-specific, anti-PnPs antibody levels varied greatly among the 34 study cases, ranging from 0.16 to 30.79 μg/mL with a geometric mean antibody level of 0.83 μg/mL. The geometric mean antibody levels for each presumed-infecting serotype were as follows: for type 4, 1.17 μg/mL (n = 5); for type 6, 0.58 μg/mL (n = 12); for type 9, 0.23 μg/mL (n = 2); for type 14, 1.74 μg/mL (n = 7); for type 19, 0.36 μg/mL (n = 2); and for type 23, 1.23 μg/mL (n = 6).

Twenty (59%) of the 34 assessable pre-SPI, presumed-infecting, serotype-specific anti-PnPs antibody levels were <1.0 μg/mL, 6 (18%) levels were between 1.0 and 1.99 μg/mL, 6 (18%) levels were between 2.0 and 4.99 μg/mL, and 2 (5%) of the levels were >5.0 μg/mL.

Fifteen paired serum samples were available before and after SPI. Anti-PnPs IgG antibody levels to all 6 representative pneumococcal serotype antigens used in this study were examined, for all paired serum samples, except when the quantity of a serum sample was insufficient (table 2). A seroresponse (defined as a ≥4-fold rise in presumed-infecting, serotype-specific PnPs antibody) was shown in 4 (27%) of 15 of paired serum samples (table 2). Six (40%) of the paired serum samples showed a ≥2-fold rise in presumed-infecting, serotype-specific anti-PnPs antibody.

Notably, in the 4 homologous group seroresponders, a ≥4-fold rise in anti-PnPs antibody response to the other 6 heterologous pneumococcal groups was observed in 13 (72%) of 18 of the assays tested. Heterologous seroresponses (≥4-fold rise) were also shown in 5 (50%) of the 10 assays tested on serum samples from the children who had a ≥2-fold but <4-fold rise in response to the homologous group. Only 1 (2%) of the 42 assays tested on serum samples from children with a ≥2-fold rise in response to the homologous group had a ≥4-fold rise in response to a heterologous-group antigen.

Christmas antiserums were provided by Dr. John J. McGehee (Food and Drug Administration). The antibody concentrations of the antibodies were determined by ELISA at Statens Serum Institute (Copenhagen, Denmark) and the Centers for Disease Control and Prevention (Atlanta, GA).

Serum samples from the 4 homologous-group seroresponders...
and the 2 children with a ≥2-fold but <4-fold rise in response to the homologous groups were retested at Wyeth-Lederle Vaccines and Pediatrics (WLVP; West Henrietta, New York) by using a method similar to that used in the present study, except in 3 key areas. First, the Statens Serum Institute absorbent was not used; instead, a cruder preparation containing cell-wall capsule and capsule-associated proteins was used. Second, medium-affinity, 96-well ELISA plates were used for antigen capture, rather than higher-binding plates. Third, the antigen concentration for coating was not in excess.

As shown in table 2, the data from the WLVP assay showed quantitatively lower antibody levels but still exhibited a large degree of heterologous-group antibody rises. Seroresponses were seen in 9 (45%) of the 20 heterologous groups tested for the 4 initial seroresponders and in 6 (60%) of the 10 heterologous groups tested on the serum samples from the 2 children with a ≥2-fold but <4-fold rise in response to their initial homologous group.

Pneumococcal antibody levels related to preexisting factors. Preexisting factors, such as age at SPI, CDC classification of HIV disease severity, and CD4+ cell counts at the time of SPI, are shown in table 2. Evaluations of these preexisting factors were not associated with any statistically significant differences in seroresponses in this small group of children.

Discussion

This is the first report, to our knowledge, of serum pneumococcal antibody levels before or after an SPI in HIV-infected children. The 6 pneumococcal groups (4, 6, 9, 14, 19, and 23) encountered in this study are the most common groups known to cause invasive pneumococcal disease in the general US population [8, 9]. Although the pneumococcal serotypes were not identified for the entire cohort, those that were (6A, 6B, 9V, 19F, and 23F) are similar to the serotypes that have been identified for the entire cohort, those that were (6A, 6B, 9V, 19F, and 23F) are similar to the serotypes that have been isolated most commonly in the United States in other surveys [8].

Selecting representative serotype antigens used in the ELISA was problematic. However, groups 4 and 14 pneumococci have no subtypes, and children with available serum samples and those who had group 9 infection had serotype 9V identified. There is much cross-reactivity of antibody between serotypes 6A and 6B, so the use of 6B in the assay is satisfactory [3].
Serotype 23F has been overwhelmingly the most common type of this group encountered in the United States [3], so the use of the 23F antigen was appropriate, even though the serotypes were not known for all these isolates. Unfortunately, isolates were not typed in the cases of the children who had group 19 infection. However, serotype 19F represents about two-thirds of the group 19 isolates in the United States, so the choice of 19F as the representative antigen was appropriate but presented the greatest error in terms of interpretation.

Although the number of serum samples available to study was small, it was clear that the range of preexisting, presumed-infecting, serotype-specific anti-PnPs IgG antibody levels was broad (0.16–30.79 μg/mL). However, nearly 76% of the values were <2 μg/mL, and 95% were <5 μg/mL. These findings are similar to those of another study, in which it was estimated that 200–300 ng/mL antibody nitrogen, as measured by radioimmunoassay, was protective in adults; this translates to ~1.3–2 μg/mL as measured by ELISA [1]. Protective antibody levels may be higher in HIV-infected children, compared with what seen in these adults, because of other HIV-related immunodeficiencies. Also, the current ELISA may not measure functional antibody, and other measures of antibody response, such as opsonization, may be more appropriate. The low attack rate of pneumococcal disease and the existence of multiple pneumococcal serotypes make defining human protective antibody levels more challenging.

An advantage of this study was that pre-SPI serum samples generally were collected at a time distant from the SPI. Serum samples collected at the time of SPI may be less than ideal, because anti-PnPs antibody could be bound to the colonizing or invading pneumococcus, which could cause falsely low serum preinfection anti-PnPs antibody measurements.

The relatively poor anti-PnPs antibody response to natural infection observed in this study was not surprising. It is known that HIV-infected children respond poorly to PnPs antigen in the form of the presently licensed polyvalent PnPs vaccine [10]. Conjugation of the PnPs to a protein carrier results in a better serologic response in HIV-infected children [11]; thus, it is hoped that if and when conjugated pneumococcal vaccines are available, the frequency of SPI experienced by HIV-infected children will decrease.

The cross-group heterologous seroresponses observed in this study deserve comment. The ELISA used in this study was developed to measure serotype-specific, anti-PnPs IgG antibody by preadsorbing all sera with reagents that remove nonspecific antibody, particularly C-polysaccharide. However, the findings in this study raise the concern of the lack of serotype specificity of this assay to actual pneumococcal infection.

This type of ELISA has been shown to be serotype specific in studies of infants and their IgG responses to pneumococcal vaccines (WLVP, unpublished data). Perhaps cross-reactive epitopes present on live, invading pneumococcal bacteria are not present on semipurified pneumococcal vaccine antigens. Further studies comparing the specificity of this type of ELISA to antibodies stimulated by vaccination versus actual pneumococcal infection should be performed.

Alternatively, heterologous, cross-group PnPs antibody responses may be explained by a general polyclonal B cell activation that has been recognized to occur in HIV-infected individuals [12]. However, a large portion of the IgG is probably directed against HIV [13], and a broad response to multiple specific bacterial antigens seems unlikely.

In summary, there was a wide range of preinfection antibody levels to the presumed-infecting pneumococcal serotype, thus making an estimate of protective antibody levels difficult in this small sample. Few children responded to their SPI with a seroresponse, which is consistent with what has been reported with PnPs vaccines. The apparent lack of specificity of the ELISAs used in this study would indicate that other types of assays should be considered when evaluating serologic responses to natural pneumococcal infection.

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References