Immune Response to Group A Streptococcal C5a Peptidase in Children: Implications for Vaccine Development

Anita Shet, Edward L. Kaplan, Dwight R. Johnson, and P. Patrick Cleary

Departments of Pediatrics, World Health Organization Collaborating Center for Reference and Research on Streptococci, and Microbiology, University of Minnesota Medical School, Minneapolis

The group A streptococcal C5a peptidase (SCPA) is a major surface virulence protein that facilitates the establishment of local infection by group A streptococci (GAS). We measured the human immune response to SCPA, using a standardized indirect enzyme-linked immunosorbent assay. Paired acute and convalescent serum samples from children with GAS-associated pharyngitis were assayed, and a strong immune response to SCPA was demonstrated that was independent of the infecting M type and the age of the patient. Western blot analysis of bacterial extracts revealed that all tested M types expressed SCPA. The immune response to SCPA correlated with the anti-streptolysin O and anti-DNase B responses. These data confirm the immunogenicity of SCPA in humans. Previous knowledge of SCPA's role in virulence, its highly conserved nature, and the results of mouse protection studies make SCPA an ideal vaccine candidate for the prevention of GAS disease.

Group A streptococci (GAS) are major global pathogens with a variety of clinical manifestations. More than 7 million visits to pediatric specialists take place each year in the United States alone for complaints of “sore throat,” and almost one-fourth of these visits are because of streptococcal pharyngitis [1]. Acute rheumatic fever and rheumatic heart disease, which are well recognized nonsuppurative sequelae of infection with this organism, have devastating public health implications worldwide. An apparent resurgence of acute rheumatic fever in the mid-1980s [2, 3], ongoing new cases [4], and the estimated 10,000 cases of invasive streptococcal disease reported annually in the United States [5, 6] confirm that an improved standard of living and the use of antibiotics have not adequately reduced the impact of GAS infections. Thus, there is a need for a safe, efficacious, and cost-effective vaccine against GAS infection.

Virulence is orchestrated by a variety of surface proteins and secreted toxins, of which several have been considered as potential GAS vaccine candidates. For several decades, the antiphagocytic M protein has been a major focus of vaccine development [7]. Different structural components of the M protein have been used as vaccine strategies, and antibodies directed against these components have been shown to be opsonic and protective in animal models [8–10]. However, the potential of different structural components of the M protein to induce a human tissue cross-reactive immune response is a safety concern [11].

Since the original discovery of the GAS C5a peptidase (SCPA), a highly specific endopeptidase that is anchored on the surface of the organism, its role as a major virulence factor has been established [12]. SCPA cleaves the complement-derived chemotaxin C5a at the His⁶⁷-Lys⁶⁸ bond, thus removing the leukocyte-binding site of C5a [13]. As a result, SCPA inhibits chemotaxis, delaying the infiltration of phagocytes, thus retarding the clearance of bacteria from mucosal and subdermal...
surfaces and allowing the organism to establish a site of infection [14, 15]. Animal protection studies that have used SCPA as an immunogen have produced encouraging results. The intranasal immunization of mice with recombinant SCPA induced strong humoral and mucosal immune responses and reduced nasopharyngeal colonization after challenge with several different M types, which suggests cross-protection [16]. It has also been shown that rabbit antibody against SCPA and human serum containing anti-SCPA can neutralize the activity of the peptidase both in vitro [17] and in vivo in mice [18]. A preliminary study in humans showed that healthy, uninfected adults had detectable levels of antibody to SCPA more frequently than did healthy children [19]. To our knowledge, however, there are no available data that have qualitatively or quantitatively characterized the immune response to SCPA in humans.

Because SCPA is a prime vaccine candidate, knowledge of the human immune response to this protein has increased importance. Therefore, we established a sensitive and specific ELISA to reproducibly measure SCPA antibodies in human serum samples. Using this assay, we were able to demonstrate that there is a strong immune response to SCPA. Furthermore, we also found a correlation between the humoral immune response to SCPA with that of anti–streptolysin O (ASO) and anti–DNase B, which are established indicators of GAS infection. These latter analyses allowed us to examine a possible clinical diagnostic role of anti-SCPA as an indicator of previous streptococcal infection.

MATERIALS AND METHODS

Study population and serum specimens. Paired serum samples previously obtained from 202 children aged 2–12 years residing in 19 states in the United States were used in the study. These children had signs and symptoms of acute pharyngitis and had GAS isolated from their throats at the initial visit. Acute-phase serum samples were obtained at the acute presentation, and convalescent-phase serum samples were obtained 4 weeks later. These serum samples were collected between January 1994 and March 1995 and were kept frozen at −20°C [20]. Of the 1131 archived serum samples available, we selected samples for our study as follows: the first 151 paired serum samples were randomly selected, and the remaining 51 paired serum samples were selected by patient age, to ensure that all age groups were adequately represented. Written informed consent was obtained from patients or their parents and guardians. Human experimentation guidelines of the US Department of Health and Human Services and those of the local institutional review boards at each site of enrollment were followed [20].

Reference serum and quality control serum samples. The reference serum was used to establish a reference curve and was collected from a normal adult convalescing from an episode of documented GAS pharyngitis. Two quality-control serum samples were obtained from 2 individuals with known high and low anti-SCPA titers, respectively, that had been previously determined by a technique similar to that described in the present article [19]. Anti-SCPA IgG concentrations (in micrograms per milliliter) were assigned to the above serum samples on the basis of the standard curve of optical density versus known concentrations of human IgG that were generated as follows: Maxisorb plates (Nunc) were coated overnight at 4°C with purified human IgG (ICN Biomedicals) at known concentrations of 1.56–100 ng/mL. Plates were then processed as described in the section below. Dilutions of the reference and quality control serum samples with optical density values falling in a linear portion of the standard curve were used to calculate specific anti-SCPA antibody concentrations, and the results were expressed as micrograms of anti-SCPA IgG bound per milliliter of serum, as per the modified Zollinger and Boslego technique [21]. By this method, the reference serum was determined to have an anti-SCPA concentration of 35 μg/mL, and the high and low quality-control serum sample concentrations were determined to be 75 and 7 μg/mL, respectively.

ELISA. The antigen used was obtained from Wyeth Vaccines and is a truncated, recombinant SCPA peptide derived from scpA1, with amino acid sequence A89–D1038 and 2 induced mutations at the active sites with the following amino acid replacements: S→A512/D→A130. Polysorb plates (Nunc) were coated with 100 μL of SCPA diluted to 2 μg/mL in 0.05 mol/L bicarbonate buffer (pH 9.6) and incubated overnight at 4°C. Excess or unbound antigen was discarded, and plates were washed 4 times, with PBS that contained 0.1% Tween-20 (pH 7.4), in an automated microplate washer (ELx405; Bio-Tek Instruments). Eight 2-fold dilutions in PBS that contained 0.1% Tween-20 (pH 7.4; starting dilution, 1:200) of reference and test serum samples, as well as the quality-control serum samples, were made directly in the microtiter plate by well-to-well transfer with a multichannel pipetter. Each run included duplicate assays of the reference serum, for the establishment of a standard curve (see below). The test and quality-control serum samples were assayed singly in each run, because preliminary analyses had revealed that well-to-well variation within duplicates was <3%. Acute and convalescent serum samples from the same patient were always run on the same microplate under identical conditions. After incubation for 1 h at 37°C, plates were washed as described above, and 100 μL of a 1:5000 dilution (in PBS and 0.1% Tween) of alkaline phosphatase–conjugated goat anti–human IgG (γ-chain specific; Calbiochem) was added to all wells, followed by incubation for 1 h at 37°C. Plates were washed again, and 100 μL of substrate, p-nitrophenyl phosphate (Sigma) at 0.5 mg/mL in bicarbonate buffer with 10 mmol/L MgCl₂, was added to all wells. After incubation at 37°C for 20 min, the enzyme reaction was stopped.
with 50 µL of 3 mol/L NaOH. The optical density at a wavelength of 405 nm (OD405) was measured with an EL340 BioTek microplate reader (BioTek Instruments).

**Determination of anti-SCPA concentrations.** The optical density values were analyzed using a public-domain software program (ELISA for Windows, version 1.0) developed by the Centers for Disease Control and Prevention [22]. The reference curve (derived from the reference serum) was established by using the 4-parameter logistic-log function. The total antibody concentration was obtained by averaging the values from all serum dilutions that fell within the working range of the reference curve, which was usually between the OD values of 0.3 and 1.5. To control day-to-day variation, both high- and low-titer quality-control serum samples in 8 serial dilutions were included in each plate; the ELISA determination was accepted only if the variation of the control serum samples was <10%.

**Competitive inhibition assay.** Increasing concentrations of 0.015–2 µg of SCPA and heterologous antigens (tetanus toxoid and bovine serum antigen) were preincubated with 200 µL of a 1:1600 dilution of the reference serum. After incubation for 1 h at 37°C, 100 µL of the aliquots of each antigen-serum mixture was transferred into corresponding wells in SCPA-coated microtiter plates and incubated for 1 h at 37°C. Plates were washed, and anti–human IgG enzyme conjugate and substrate were added as described above. The percentage of inhibition was determined by comparison of the absorbances with and without the inhibiting antigens: [(OD405 nonadsorbed serum samples – OD405 adsorbed serum samples/OD405 nonadsorbed serum samples) × 100] [23].

**Determination of SCPA production: SDS-PAGE and Western blot techniques.** Protein extracts were obtained from GAS throat isolates that were grown for 2–3 h (to log phase) in 50 mL of Todd-Hewitt broth at 37°C. The culture pellet was washed twice with 5 mL of cold TE (50 mmol/L Tris-Cl and 1 mmol/L EDTA) buffer that contained 1 mmol/L phenylmethylsulfonyl fluoride and then was suspended in a solution that contained 1 mL of TE-sucrose buffer (20% sucrose), 100 µL of lysozyme (100 mg/mL), and 50 µL of mutanolysin (5000 U/mL) (Sigma). The mixture was gently rotated at 37°C for 2 h and then centrifuged for 5 min at 1500 g. Electrophoresis and Western blot analyses were done as described elsewhere [14]. The primary antiserum used to detect SCPA protein on Western blot was prepared by the immunization of a rabbit with purified recombinant SCPA protein. Binding was detected by goat anti-rabbit antibody–alkaline phosphatase conjugate (Sigma).

**Statistical methods.** The mean anti-SCPA response in acute- and convalescent-phase serum samples after GAS pharyngitis was calculated, and the means in both groups were compared using the paired Student’s t test. The comparison of the mean anti-SCPA increase between groups of children with and without significant increases in ASO and anti–DNase B was done using the Mann-Whitney U test. Correlations between anti-SCPA and established streptococcal antibody tests (ASO and anti–DNase B) were assessed by single linear regression, and significance was evaluated by Spearman’s rank correlation test. The analyses were done using Instat for Macintosh (version 2.0; GraphPad). P < .05 was considered to be significant. Intra- and interassay variation were measured using coefficients of variation.

**RESULTS**

**Standardization of ELISA for SCPA-specific IgG.** A sensitive, directed, and reproducible ELISA was developed to measure the anti-SCPA concentration in human serum samples. The sensitivity or detection limit of the assay was 0.01 µg/mL. This was determined by obtaining the lowest concentration of antibody from the corresponding optical density signal that was 3 SD above the mean for 20 replicate determinations of the zero calibrator that contained the sample diluent and the zero reference serum [24]. A competitive inhibition assay was done with the reference serum. There was a corresponding reduction in anti-SCPA reactivity by ELISA after preincubation with increasing concentrations of SCPA. With 2 µg of SCPA, 85%–90% inhibition was achieved. The preincubation of serum with increasing concentrations of heterologous antigens, tetanus toxoid and bovine serum antigen, resulted in mean inhibitions of 13% and 15%, respectively, regardless of the antigen concentrations (figure 1). These data suggest that the assay is specific and that the antibodies measured are directed against SCPA. The variation within each run was evaluated by testing, in duplicate, 3 different serum samples with anti-SCPA concentrations of 7, 35, and 75 µg/mL, respectively, on 20 different microtiter plates. The day-to-day variation was assessed by testing the above 3 serum samples on 14 different days. The within-run variation was 8%, and the day-to-day variation was 11%, which indicates acceptable reproducibility.

**Human immunogenicity testing.** Having standardized the assay, we determined mean anti-SCPA levels in acute- and convalescent-phase serum samples from 202 children. Acute-phase serum samples had been obtained within 1–2 days of the onset of symptoms of acute pharyngitis. The mean anti-SCPA for all serum samples concentrations ranged from undetectable to 29.59 µg/mL (SE, 2.58; 95% confidence interval [CI], 24.89–34.99 µg/mL), which is twice the mean acute-phase value. This difference in mean anti-SCPA values obtained for acute- and convalescent-phase serum samples was statistically significant (P < .0001) (figure 2). The acute-phase serum samples had anti-SCPA concentrations ranging from undetectable to 156.62 µg/mL, whereas the convalescent-phase serum samples concentrations ranged from undetectable to 295.29 µg/mL. At the acute presentation, 22% (44/202) children
had undetectable serum levels of anti-SCPA. Almost 60% (26/44) of these children were aged ≤5 years, although this age group constituted only 36% of the total group.

Taking into consideration the assay variation of 8%–11%, an increase in convalescent antibody concentration that was at least 15% greater than the acute concentration was selected to constitute a "significant" increase in anti-SCPA. Of the 202 children studied, 67% (136) responded with a ≥15% increase in antibody concentrations 4 weeks later. Of the remaining 66 children who did not demonstrate a significant increase in anti-SCPA, it was noted that 70% and 80% also did not demonstrate an increase in ASO and anti–DNase B titers, respectively. Of the children with undetectable acute anti-SCPA titers, 59% (26/44) showed a significant response during the convalescent phase. There were 18 children (9%) who were non-SCPA responders who had undetectable levels 4 weeks after the acute presentation. More than two-thirds of these nonresponders also did not demonstrate an increase in either ASO or anti–DNase B.

When anti-SCPA response was examined with respect to patient age, an increase in mean convalescent antibody occurred in each of the age groups between 2 and 12 years (figure 3). There were 13–23 children in each age group. Thus, the ability to respond to SCPA did not appear to be dependent on age. The magnitude of the immune response was highest in the children aged ≤5 years.

A number of the GAS throat isolates obtained from the children at their acute visit had been previously serotyped in our laboratory using conventional methods (T-agglutination or M/OF serotyping) [25]. The M types were available for samples from 127 of these 202 children included in the present study. When the anti-SCPA response was determined according to M type, it was noted that the mean increase in anti-SCPA appeared to occur irrespective of M type (figure 4). Of the 4 children infected with streptococcal M5 strains, 3 showed a significant increase in anti-SCPA concentration, and 1 had a decrease in anti-SCPA. This lowered the overall mean for this group and therefore inappropriately indicated no change between acute and convalescent anti-SCPA concentrations. The positive immune response suggested that all streptococcal M types are likely to produce SCPA during human infections.

Approximately one-third of the children tested did not demonstrate a significant increase in SCPA antibody. This lack of a specific immune response in a portion of the studied population prompted us to reexamine whether all the infecting streptococcal isolates expressed the SCPA protein. SDS-PAGE and Western blot analyses were done on 18 randomly selected streptococcal throat isolates representing 13 different serotypes, including 1 nontypeable strain (M1, M2, M3, M4, M5, M6, M12, M18, M22, M28, M75, and M77). A protein band of 130 kDa, corresponding to the SCPA protein, was present in all tested bacterial protein extracts, confirming that SCPA was expressed by all tested isolates.

ASO and anti–DNase B titers had been previously determined on these serum samples [20]. When comparing these titers with the anti-SCPA response in both acute- and convalescent-phase serum samples, it was found that, among samples that demonstrated a significant increase in ASO and/or anti–DNase B, the mean anti-SCPA increase was 21.54 μg/mL, a value that was 3 times higher than that in samples that did not show an increase in either ASO or anti–DNase B. The anti-SCPA increase among samples that did not demonstrate an increase in ASO or anti–DNase B was 6.98 μg/mL, and the difference between the 2 groups was significant (P < .0001, Mann-Whitney U test) This suggests that anti-SCPA responses
parallel those for ASO and anti–DNase B. The linear correlation between this newly developed anti-SCPA test and established streptococcal antibody tests was examined (figure 5). There was a good linear correlation between acute anti-SCPA and ASO values (Spearman’s rank \( r = 0.68; P < .0001 \)) and between anti-SCPA and anti–DNase B values (Spearman’s rank \( r = 0.65; P < .0001 \)). There was moderate correlation between the tests with convalescent serum samples (anti-SCPA and ASO, \( r = 0.55 \) and \( P < .0001 \); anti-SCPA and anti–DNase B, \( r = 0.36 \) and \( P < .0001 \)). We also observed that, although 136 (67%) of 202 children showed a significant increase in anti-SCPA, only 109 (54%) showed a significant increase in ASO, 91 (45%) in anti–DNase B, and 127 (63%) in either ASO and/or anti–DNase B (figure 6). When the 3 antibodies are compared (ASO, anti–DNase B, or anti-SCPA), the percentage of children showing an increase in any of these titers was 69%, which is not appreciably different from the proportion showing a significant increase in anti-SCPA alone. The above observations suggest a potential use for anti-SCPA determinations in a clinical diagnostic setting.

**DISCUSSION**

Our results demonstrate the application of a solid-phase ELISA using recombinant SCPA to detect naturally occurring serum antibodies to SCPA in children aged 2–12 years after an episode of uncomplicated GAS pharyngitis. They also show that children with GAS pharyngeal infection can produce a strong immune response to SCPA. The assay performance characteristics suggest appropriate sensitivity and specificity. The 90% inhibition of high-titer serum by the truncated recombinant SCPA peptide indicates that specific anti-SCPA antibodies are detected by this assay. The failure to totally block IgG binding with SCPA peptide may reflect some degree of heterogeneity of the antibody specificity and affinity. Residual antibody may have a lower affinity for SCPA and, therefore, under these conditions may still be reactive with plate-bound antigen. Paired acute and convalescent serum samples were run on the same plate adjacent to each other, minimizing interassay and interplate variations. The intra- and interassay variations of 8% and 11%, respectively, show that the assay is reproducible. This standardized ELISA will be used in future studies with human serum samples, to further investigate the immune response to an SCPA vaccine.

The role of SCPA as a major streptococcal virulence factor has been firmly established. SCPA contributes significantly to the organism’s ability to colonize mucosal surfaces. In a study [15] that examined colonization, it was shown that SCPA mutants with intact M protein, but lacking SCPA activity, were cleared significantly more rapidly than wild-type cultures from mouse oral mucosa. This suggested that M protein is not the sole factor in resisting innate defenses and facilitating colonization. The early inflammatory response of the human host greatly influences the ability of the bacteria to colonize mucosal surfaces with pathological consequences. As a primary mediator of chemotaxis, C5a is a crucial factor in the accumulation of neutrophils at the site of infection, which precedes the phagocytosis and clearance of bacteria. This method of host defense is interrupted by the action of SCPA, which enzymatically inhibits the chemotactic activity of C5a by cleaving its neutrophil binding site. Its role in virulence is an important reason for the selection of SCPA as a candidate for a GAS vaccine. New study results have indicated that SCPA also binds to fibronectin and functions as an intracellular invasin [26]. These studies have also shown that anti-SCPA significantly impedes the capacity of GAS to invade epithelial cells.

Rapid changes in prevailing GAS serotypes have been shown to occur within defined communities [27]. This dynamic ep-
Figure 4. Distribution of mean acute- and convalescent-phase anti–group A streptococcal C5a peptidase (SCPA), according to streptococcal M type.

Figure 5. Correlations between anti–group A streptococcal C5a peptidase (SCPA) and anti–streptolysin O (ASO) titers in acute-phase (A) and convalescent-phase (B) serum samples and between anti–DNase B and anti–SCPA titers in acute-phase (C) and convalescent-phase (D) serum samples.

Epidemiology, as well as the fact that a large proportion of GAS isolates from developing countries are characterized as nontypeable strains [28], strongly suggests the need for a vaccine that will confer a broad, non–type-specific immunity. Previous studies from our laboratory showing that antibodies to recombinant SCPA derived from 1 serotype cross-reacted with >20 different serotypes of GAS have suggested that the SCPA protein is universally present in all GAS strains [16, 18]. The scpA gene has also been found to be widely distributed among several different strains of GAS [29]. The nucleotide sequences of the scp genes from 4 GAS isolates representing 3 serotypes and from group B streptococcus have been predicted to be 95%–98% identical [30] (P.P.C., unpublished data). In our current work, we further confirmed the highly conserved nature of SCPA, by showing that all 13 different M types tested expressed antigenically cross-reactive SCPA on their cell surfaces. Because there are now >130 M or emm types that have been identified [31], we are presently studying additional strains, to further substantiate this finding.

Because of the genetic and structural similarity between SCPA and the C5a peptidase found on group B streptococci (SCPB) [32], a portion of the total measurable antibody concentration may represent anti-SCPB or antibody induced by group B streptococcus. Nevertheless, we believe that this possibility was very unlikely to have influenced our results, because all of the children whose serum samples were used in the present study had documented GAS pharyngitis, a mean significant increase in the convalescent serum samples was noted, and group B streptococcus is a rare pathogen in this age group.

At present, there is no published information regarding the potential immunological cross-reactivity between with SCPA and human tissues; such studies are in progress elsewhere. A case for the safety of SCPA in this regard may be made on the basis of its near identity to SCPB protein [30]. Group B streptococcal infections are not associated with any known human immunological sequelae. Therefore, one may assume that SCPA is very unlikely to induce human tissue cross-reactive antibodies.

The nonsuppurative and invasive consequences of GAS are most likely to occur when the bacteria find a way into the bloodstream and have an opportunity to induce auto antibodies or directly invade soft tissues. In a study confirming the role of SCPA in streptococcal virulence, it was shown that wild-type GAS that express SCPA disseminated to the spleen at 4 h after infection in mice, whereas mutants lacking SCPA activity were more often found in local lymph tissue, which indicated that SCPA-positive bacteria tend to be more invasive [14]. These observations led us to suggest that bacteria neutralized by anti–SCPA antibody are less likely to enter the bloodstream and are more likely to be phagocytized and then cleared.

Earlier experiments using a chemotaxis inhibition assay clearly showed that human serum samples containing high titers of anti–SCPA IgG could successfully inhibit and neutralize SCPA activity [19]. More recently, it has been shown that the
inhibition of SCPA activity is not serotype specific. Rabbits vaccinated with SCPA produced specific antibody that was able to neutralize the SCPA activity associated with several different serotypes of GAS [16]. The strong immune response generated in children infected with GAS in our study could represent non–serotype-specific neutralizing antibodies that could provide protection against the early establishment of infection by the bacteria. On the basis of available data, we postulate that the protection afforded by anti-SCPA is by 2 mechanisms: the neutralization of streptococcal anti-chemotactic activity and the inhibition of the intracellular invasion of epithelial cells.

Although antibody to a highly similar C5a peptidase on group B streptococci has been found to be opsonic and initiate macrophage killing of the bacteria [33], anti-SCPA has not been conclusively shown to be opsonic for GAS (P.P.C., unpublished data). The presence of SCPA neutralizing antibody in the host can facilitate chemotaxis and preserve the C5a-mediated inflammatory response by inactivating the bacterial mechanism that specifically inactivates the host chemotactic signal. Thus, the establishment of infection by the streptococci is prevented, and the probability of streptococcal invasive disease and sequelae is greatly reduced.

Protective immunity to GAS infection is exceedingly complex and poorly understood. A key question that remains unanswered is why more children, compared with adults, get recurrent streptococcal infections, despite the demonstration of the ability to mount a good immune response. Several possibilities exist: the immune response in children may be transient or poorly functional or it may not represent the right class of antibody or T cells that constitutes long-term protection. There may be an as-yet-unknown developmental process that limits the appropriate immune response in young children. Then again, protection may require multiple episodes of streptococcal infection to produce a response of sufficient magnitude. In the group of pediatric age subjects studied, the preexisting antibody concentration at the time of initial clinical presentation of GAS pharyngitis was, on the whole, considerably lower than the convalescent-phase antibody concentration measured 4 weeks after the acute presentation. More than two-thirds of the children responded with a $\geq 15\%$ increase in anti-SCPA concentration, which indicates that SCPA is expressed and immunogenic during infection. Our results, however, do not directly address the issue of protection. One may postulate that several encounters with the streptococcal antigen (repeated infection with GAS or exposure to SCPA protein administered as a vaccine) are required to produce adequate protective antibody titers. However, the threshold of protective antibody level is still unknown. Studies that characterize the immune response to SCPA—including antibody class and subclass and antibody function such as neutralization and opsonization in serum samples from adults and normal healthy children, as well as from patients with rheumatic fever and poststreptococcal glomerulonephritis—are required to answer this question.

The immune response to both cellular and secreted antigens of GAS has been extensively studied, especially for its diagnostic value when considering poststreptococcal nonsuppurative sequelae like acute rheumatic fever and acute glomerulonephritis, where the presence of the organism at the time of clinical presentation is not always reliable. Currently established commercially available streptococcal antibody tests measure the immune response to the streptococcal extracellular antigens streptolysin O (ASO test) and deoxyribonuclease B (anti–DNase B test) [34].

Having demonstrated that there is a brisk response to streptococcal SCPA in this group of subjects from whom GAS were isolated from their upper respiratory tracts, it was important to compare the antibody responses to ASO and anti–DNase B. A good correlation was seen in acute serum samples between anti-SCPA and these 2 established streptococcal antibody tests. The parallel immune response that we observed when comparing antibody to SCPA with ASO and anti–DNase B suggests a diagnostic potential for anti-SCPA for confirming prior GAS infection. As seen in figure 6, the anti-SCPA response alone identifies almost all of the subjects defined by the 2 reference-standard streptococcal antibodies together. Furthermore, adding a third antibody to the ASO and anti–DNase B assays did not appreciably increase the percentage of children correctly identified as having a true GAS infection. Although these preliminary data must be confirmed in other age groups and in other populations, they suggest that the anti-SCPA response may be a useful adjunct in identifying prior GAS infection.

Of note is the fact that more than three-fourths of serum samples that did not show a significant increase in anti-SCPA concentration also did not demonstrate a significant increase.
in either ASO or anti–DNase B. This may reflect the intensity of infection or genetic differences in the host’s capacity to respond to streptococci in general or to specific streptococcal antigens in particular. It is also possible that these children may have been streptococcal throat carriers and that their symptoms of pharyngitis may have been attributable to viral causes.

Complete information relating to the kinetics of the anti-SCPA immune response and its decline is not available from the present study. SCPA antibody kinetics may also vary with the site of infection and with invasive streptococcal disease. It is possible that higher anti-SCPA values may be seen in patients with acute rheumatic fever and poststreptococcal glomerulonephritis, as is the pattern seen with ASO and anti–DNase B.

In conclusion, we have shown that SCPA is highly immunogenic in humans who have been recently infected with GAS in their upper respiratory tract, and the magnitude of this immune response is independent of the infecting serotype. This vigorous immune response to SCPA was present in all tested age groups of children and occurred irrespective of M type. We have also shown that all tested M types express SCPA, which supports the hypothesis that SCPA is highly conserved in GAS. These data, in conjunction with previous knowledge of the role of SCPA in virulence and the ability of neutralizing antibody to prevent bacterial colonization, make SCPA a prime vaccine candidate for the prevention of GAS disease. Our data also suggest that anti-SCPA may be useful as a diagnostic tool in the clinical microbiology laboratory. Further studies involving larger numbers of children and adults, as well as data regarding the immune kinetics of the antibody response, will be necessary to define its diagnostic potential as an aid to confirming previous streptococcal infection.

Acknowledgments

We thank Stephen Olmsted, Steve Hildreth, and Yury Matsuka, from Wyeth Vaccines for their advice in standardizing the ELISA assay and for supplying the group A streptococcal C5a peptidase protein.

References