Risk Factors in the Pathogenesis of Invasive Group A Streptococcal Infections: Role of Protective Humoral Immunity

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An impressive change in the epidemiology and severity of invasive group A streptococcal infections occurred in the 1980s, and the incidence of streptococcal toxic shock syndrome cases continues to rise. The reason for the resurgence of severe invasive cases remains a mystery—has there been a change in the pathogen or in host protective immunity? To address these questions, we have studied 33 patients with invasive infection caused by genotypically indistinguishable M1T1 strains of *Streptococcus pyogenes* who had different disease outcomes. Patients were classified as having severe (n = 21) and nonsevere (n = 12) invasive infections based on the presence or absence of shock and organ failure. Levels of anti-M1 bactericidal antibodies and of anti-streptococcal superantigen neutralizing antibodies in plasma were significantly lower in both groups than in age- and geographically matched healthy controls (P < 0.01). Importantly, the levels of these protective antibodies in plasma samples from severe and nonsevere invasive cases were not different. Together the data suggest that low levels of protective antibodies may contribute to host susceptibility to invasive streptococcal infection but do not modulate disease outcome. Other immunogenetic factors that regulate superantigen responses may influence the severity of systemic manifestations associated with invasive streptococcal infection.

After years of steadily declining morbidity and mortality due to group A streptococcal infections, a resurgence of severe, invasive disease has been ongoing since 1980 (9, 12, 17, 19–21, 24, 25, 31, 32, 49), leading to the recognition of streptococcal toxic shock syndrome (STSS) (52), the most severe form of invasive infection (10, 13, 49). STSS patients suffer from severe acute hypotension, multiorgan failure, and in some cases deep soft tissue destruction (31). The rise in STSS cases is persisting (reviewed in reference 31), and ongoing surveillance studies in Ontario, Canada, revealed a marked increase in the number of reported cases of invasive group A streptococcal infections from 1992 to the present (10, 13). The increased incidence of these infections has been accompanied by a remarkable vigor in virulence and severity, with numerous cases of STSS and necrotizing fasciitis (NF) (4, 7, 23). The reason for this impressive change in the epidemiology and clinical manifestation of group A streptococcal infections remains a mystery—have the bacteria acquired new virulence, or has the host susceptibility to factors produced by reemerging strains of *Streptococcus pyogenes* been compromised due to the lack of protective immunity against these strains?

These possibilities are not mutually exclusive, and there is little doubt that the disease outcome is determined by host-pathogen interplay. Group A streptococci produce a number of virulence factors that can contribute to the pathogenesis of invasive group A streptococcal disease. These include the surface M protein, hyaluronic capsule, proteases, DNases, lipopolysaccharides, streptococcal toxins such as streptolysins O and S, and the streptococcal pyrogenic exotoxins (SpeS) (1, 19, 22, 26, 33, 35, 42, 44, 51). As superantigens, the SpeS can cause activation of large numbers of immune cells to synthesize and release massive amounts of inflammatory cytokines that have been shown to mediate many of the systemic manifestations associated with sepsis, including hypotension and organ failure (reviewed in references 26, 27, and 50). Although it may be hypothesized that the resurgence of invasive group A streptococcal infections is related to production or overproduction of specific virulence factors, studies of clusters and disease outbreaks revealed that the same streptococcal strain can be isolated from STSS cases, nonsevere invasive cases, and asymptomatic contacts, indicating a strong influence of host factors in disease pathogenesis (5, 8, 23, 24, 34, 36, 45, 47).

Patients with invasive group A streptococcal disease, including those infected with indistinguishable M1T1 strains, can be classified as having severe or nonsevere invasive disease based on the presence or absence, respectively, of shock and organ failure. Therefore, even if pathogen virulence products are contributing to the increase in invasive disease, host factors must play a pivotal role in determining the severity of the systemic manifestations.

Several host factors have been shown to increase the risk of severe invasive streptococcal disease. Differences in confounding factors such as age, underlying disease (10), and ongoing viral infections can be accounted for in multivariate analyses, thereby allowing studies to focus on the role of host immune defense mechanisms in modulating the severity of invasive streptococcal infections. We have reported that host immune responses to the various streptococcal virulence factors can vary (28, 40, 41), and we believe that this interindividual variation can potentially affect the severity of systemic manifestations associated with invasive infections.

The lack of protective immunity to specific virulence factors produced by the bacteria is likely to affect host susceptibility to infection. Previous studies have suggested that low levels of antibodies directed to specific SpeS or to the M protein may
render the host susceptible to invasive infections (21, 48). In fact, several investigators have proposed that low levels of anti-M1 protein in the general populations of the United States, Canada, and Scandinavian countries may have contributed to the remarkable change in the epidemiology of invasive group A streptococcal infections and may be responsible for the impressive rise in the number of STSS cases (14, 21, 48). However, in the majority of these studies, evaluation of the levels of protective antibodies was performed against isolates that were not necessarily recovered from the patients being evaluated, and thus the clinical relevance and immunological specificity of these antibodies could not be ascertained. Furthermore, the role of the antistreptococcal protective antibodies in modulating the severity of invasive streptococcal infections has not been addressed directly.

The goal of this study was to determine if differences in severity of the systemic manifestations of invasive group A streptococcal infections are associated with differences in levels in plasma of antibodies to the M serotype of the infecting isolate and/or antibodies that can neutralize the activity of superantigens produced by these isolates. We report that invasive cases had significantly low levels of protective antibodies compared to age-matched healthy controls; however, the levels were equally valid in severe and nonsevere invasive cases. The data indicate that while the lack of this protective humoral immunity may confer risk of invasive infection, it is not a factor in determining the severity of systemic manifestation associated with these infections. Together the data suggest that other host immunogenetic factors, possibly those regulating cytokine responses to superantigens, may be more important in modulating disease outcome.

MATERIALS AND METHODS

Subjects, case definitions, and clinical material. Patients were identified through ongoing surveillance for all invasive group A streptococcal infections in Ontario, Canada. Group A streptococcal infections were classified according to the scheme proposed by the Working Group on Streptococcal Infections (52). Patients were enrolled from 1994 to 1996, and only those who had invasive infection caused by indistinguishable MIT1 strains (as described below) were included in this study (n = 33). Invasive infection meant that the isolate was obtained from a normally sterile site. Invasive cases could be subdivided into severe and nonsevere, depending on the clinical course of the patient and clinical criteria for STSS. Invasive infection patients (n = 21) were those who had STSS, NF, or NF plus STSS. STSS was defined as invasive infection associated with shock and organ failure early in the course of infection. Patients with nonsevere invasive infections (n = 12) had no signs of hypotension or multiple organ failure; they included patients with bacteremia, cellulitis, and erysipelas.

Plasma samples were collected from each consenting patient before the administration of adjunctive therapy, namely, intravenous administration of pooled immunoglobulin G (IVIG). Plasma samples and clinical isolates were frozen promptly and stored at -80°C until processed. Controls were age-matched healthy individuals who resided in the same geographical area as the study patients.

Characterization of bacterial isolates. Clinical isolates were identified as S. pyogenes by standard methodology (13), and each was designated by patient number. M and T serotyping were performed at the National Reference Center for the Streptococcus, Edmonton, Canada. All MIT1 isolates included here were further typed by pulsed-field gel electrophoresis and by random amplified polymorphic DNA analysis. All had identical DNA banding patterns after digestion with the restriction enzymes BamHI and KpnI and subcloned into the PQE30 vector (Qiagen Inc.), which was used to transform M15 Escherichia coli. The bacteria were grown to an optical density at 600 nm of 0.8, and then expression was induced with 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside) for 5 h. Bacterial cells were harvested by centrifugation at 4,000 x g for 10 min, and the pellet was resuspended in sonication buffer (50 mM Na-phosphate [pH 7.8], 300 mM NaCl) and sonicated on ice (10 s bursts, 20 s, 60 to 70 W). This was followed by dialysis for 15 min at 10,000 x g, and the supernatant was collected and filtered through a 0.2-μm-pore-filter size. The His-tagged rSpeF protein was purified on an Ni-nitrilotriacetic acid column and stepwise eluted with a 10, 20, 30, and 300 mM imidazole. The purified protein was dialyzed in 50 mM sodium phosphate and immediately dialyzed against distilled H2O overnight with several changes. The purity of the rSpeF protein was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the specific expression of the protein was determined by immunoblotting. The rabbit antisera (kindly provided by S. Holms, Umea University, Umea, Sweden) and commercial polyclonal antibodies were generated in our laboratory.

For initial immunization, 0.2 mg of rSpeA and 0.5 mg of rSpeF were emulsified in Freund's complete adjuvant and injected into male New Zealand White rabbits. Boosting doses of rSpeA (0.1 mg) and rSpeF (0.25 mg) were emulsified in Freund's incomplete adjuvant and administered every 2 weeks, for a total of five injections in those rabbits. The high-titer rabbit sera were used as a positive control. Different dilutions of plasma from patients or controls were added to the ELISA antigen. The sequence was deduced from the emm1.0 allele described by Haanes-Fritz et al. (18). Microtiter plates were coated with 0.2 μg of the SMI peptide per ml in coating buffer (0.1 M carbonate buffer, pH 9.6 to 9.8) at 4°C for 18 h, rinsed with wash buffer (0.05% Tween 20 in phosphate-buffered saline [PBS]), and blocked with 1% bovine serum albumin in PBS for 60 min at 37°C. Fetal bovine serum (PBS), diluted 1:100 in PBS, was used as a negative control, and a rabbit anti-M1 antiserum, provided by J. B. Dale and generated as previously described (29), was serially diluted in PBS and used as a positive control. Different dilutions of plasma from patients or controls were added to duplicate coated wells and incubated for 2 h at room temperature. The plates were rinsed with wash buffer, and goat anti-human or goat anti-rabbit immunoglobulin (Ig) peroxidase conjugate diluted 1:1,000 was added to the appropriate wells. After 1 h of incubation, the plates were rinsed with wash buffer and freshly made peroxidase substrate solution (ABTS, Kirkegaard-Perry, Gaithersburg, Md.) was added. The reaction was monitored at 415 nm, and the OD was used to determine antibody titers from a standard curve generated with serial dilutions of the control antibody. Results are expressed as mean ELISA titers ± standard error of the means (SEM).

Measurement of levels of anti-M1 protein antibodies by ELISA. The presence of anti-M1 protein antibodies in plasma samples from patients was determined by ELISA using the peptide copies M1 (1-26) on a nitrocellulose filter, provided by J. B. Dale, as the ELISA antigen. The sequence was deduced from the emm1.0 allele described by Haanes-Fritz et al. (18). Microtiter plates were coated with 0.2 μg of the SMI peptide per ml in coating buffer (0.1 M carbonate buffer, pH 9.6 to 9.8) at 4°C for 18 h, rinsed with wash buffer (0.05% Tween 20 in phosphate-buffered saline [PBS]), and blocked with 1% bovine serum albumin in PBS for 60 min at 37°C. Fetal bovine serum (PBS), diluted 1:100 in PBS, was used as a negative control, and a rabbit anti-M1 antiserum, provided by J. B. Dale and generated as previously described (29), was serially diluted in PBS and used as a positive control. Different dilutions of plasma from patients or controls were added to duplicate coated wells and incubated for 2 h at room temperature. The plates were rinsed with wash buffer, and goat anti-human or goat anti-rabbit immunoglobulin (Ig)-peroxidase conjugate diluted 1:1,000 was added to the appropriate wells. After 1 h of incubation, the plates were rinsed with wash buffer and freshly made peroxidase substrate solution (ABTS, Kirkegaard-Perry, Gaithersburg, Md.) was added. The reaction was monitored at 415 nm, and the OD was used to determine antibody titers from a standard curve generated with serial dilutions of the control antibody. Results are expressed as mean ELISA titers ± standard error of the means (SEM).

Measurement of levels of anti-M1 opsonic and bactericidal antibodies in plasma. The levels of opsonic and bactericidal antibodies to 21S plaque-forming units (PFU) were determined by a neutrophil-mediated opsonophagocytosis assay by the method of Fischer et al. (15). Neutrophils were isolated from adult venous blood by dextran sedimentation and Ficoll-Hypaque density centrifugation. Bacteria were grown through overnight in Todd-Hewitt broth with 5% normal rabbit serum, and then 50 μl was added to 5 ml of Todd-Hewitt broth and allowed to grow at 37°C with occasional monitoring until the OD at 530 nm
followed by incubation on ice for 15 min. Neutrophils (2 x 10^6 cells) were treated as described above for the opsonic assay except that 10^5 per ml of 1:50-diluted rabbit anti-SpeA, -SpeB, and -SpeF antisera were used as positive controls. Differences in the dilutions of plasma from patients and controls were added to duplicate coated wells and incubated for 2 h at room temperature. The plates were rinsed with wash buffer, and then goat anti-human or goat anti-rabbit IgG were used as secondary antibodies. After addition of peroxidase substrate, the reaction was monitored at 415 nm.
The levels of anti-M1 antibodies in plasma specimens from patients with severe and nonsevere invasive group A streptococcal infections. Inasmuch as the levels of anti-M1 antibodies in plasma were equally low in the patients with the severe and nonsevere invasive infections, it was of interest to determine if their levels of anti-Spe antibodies in plasma were different. As indicated above, all patients were infected with indistinguishable M1T1 strains that harbored the speA, speB, and speF genes. Levels (determined by ELISA) of anti-SpeA, -SpeB, and -SpeF antibodies in plasma specimens of patients with invasive disease were significantly lower than those in controls (P > 0.003) (Table 2). Equally low levels of anti-Spe antibodies were found in plasma specimens of patients with severe and nonsevere invasive infections, with no significant difference between them (P = 0.1), but both groups had levels that were significantly lower than those in controls for all three Spes (Table 2).

Levels of neutralizing anti-streptococcal superantigen antibodies in plasma specimens from patients with severe and nonsevere invasive group A streptococcal infections. Previous studies have indicated that the levels of neutralizing anti-Spe antibodies do not always correlate with the total amount of binding antibodies as determined by ELISA or immunoblotting (37, 38, 42). Further, our studies have suggested that the quality (neutralizing activity) rather than the quantity (binding activity) of anti-streptococcal superantigen antibodies is more clinically relevant (38). Here we compared the levels of neutralizing antibodies in plasma specimens of the patients with the severe and nonsevere invasive M1T1 infections. The ability of plasma from patients or controls to neutralize the mitogenic activity of either the pure Spe proteins or the mixture of superantigens in the partially purified culture supernatants of the patient’s M1T1 isolates was tested. PBMC from a healthy responder were incubated with partially purified supernatant from M1T1 isolates in the presence of either FBS, plasma of the patient from whom the isolate was obtained, or plasma from age-matched healthy controls residing in the same area. The importance of matching the ages of patients and controls is illustrated in Fig. 3A, where it can be seen that the neutralizing activity increases with age.

No significant difference in the levels of neutralizing activity against pure Spe proteins was found between severe and nonsevere invasive cases or between the invasive cases and healthy controls (Fig. 3B). By contrast, significantly lower levels of isolate-specific neutralizing antibodies were found in plasma specimens from patients with severe and nonsevere invasive infections compared to the healthy controls (Fig. 3C) (P < 0.0002). The lack of concordance between the levels of neutralizing antibodies to a pure Spe and the mixture of superantigens produced by the isolate is consistent with the argument.

### TABLE 1. Levels of anti-M1 serotype bactericidal antibodies in plasma

<table>
<thead>
<tr>
<th>Study group (n)</th>
<th>% Bactericidal activity (mean ± SEM)</th>
<th>p-value</th>
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<tr>
<td>Healthy individuals (20)</td>
<td>84 ± 5</td>
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</tr>
<tr>
<td>All invasive cases (33)</td>
<td>68 ± 5</td>
<td>0.0074</td>
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<tr>
<td>Severe invasive cases (21)</td>
<td>72 ± 5</td>
<td>0.047</td>
</tr>
<tr>
<td>Nonsevere invasive cases (12)</td>
<td>63 ± 5</td>
<td>0.0066</td>
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* A bactericidal assay was conducted to assess the bactericidal anti-M1 antibodies in plasma specimens from either patients or controls. The assay was performed as described in Materials and Methods, the mean percentage of bactericidal activity was calculated, and the results were compared by using the Student t-test (one tailed).

* Versus results for healthy controls.

* The difference between results for severe and nonsevere cases was not significant (P = 0.14).

<table>
<thead>
<tr>
<th>ELISA antigen</th>
<th>Healthy individuals (n = 20)</th>
<th>All invasive cases (n = 33)</th>
<th>Severe invasive cases (n = 21)</th>
<th>Nonsevere invasive cases (n = 12)</th>
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<tr>
<td>SpeA</td>
<td>114 ± 14</td>
<td>72 ± 6 (P &lt; 0.003&lt;sup&gt;a&lt;/sup&gt;)</td>
<td>67 ± 8 (P &lt; 0.002&lt;sup&gt;b&lt;/sup&gt;)</td>
<td>80 ± 10 (P &lt; 0.025&lt;sup&gt;c&lt;/sup&gt;; P = 0.15&lt;sup&gt;d&lt;/sup&gt;)</td>
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<tr>
<td>SpeB</td>
<td>60 ± 6</td>
<td>28 ± 6 (P &lt; 0.002&lt;sup&gt;a&lt;/sup&gt;)</td>
<td>23 ± 7 (P &lt; 0.001&lt;sup&gt;c&lt;/sup&gt;)</td>
<td>37 ± 8 (P &lt; 0.004&lt;sup&gt;c&lt;/sup&gt;; P = 0.111&lt;sup&gt;d&lt;/sup&gt;)</td>
</tr>
<tr>
<td>SpeF</td>
<td>153 ± 16</td>
<td>90 ± 10 (P &lt; 0.001&lt;sup&gt;c&lt;/sup&gt;)</td>
<td>83 ± 13 (P &lt; 0.0008&lt;sup&gt;c&lt;/sup&gt;)</td>
<td>103 ± 15 (P &lt; 0.016&lt;sup&gt;c&lt;/sup&gt;; P = 0.15&lt;sup&gt;d&lt;/sup&gt;)</td>
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* Versus results for age-matched healthy individuals.

* Versus results for severe invasive cases.
that most of the clinical group A streptococcal isolates produce a mixture of known and novel superantigens. This underscores the importance of evaluating the plasma neutralizing activity of the patient against the mixture of superantigens produced by their respective isolate. For example, 4 of 21 patients with severe invasive infections had high levels of SpeA-neutralizing antibodies (>80% inhibition of SpeA mitogenicity) but low levels against the mixture of superantigens in the isolate supernatant (<25% inhibition of supernatant mitogenicity) (data not shown). Importantly, there was no difference in the levels of neutralizing antibodies against the mixture of superantigens produced by the M1T1 isolates between the severe and nonsevere M1T1 cases (22 ± 6 versus 16 ± 7; P > 0.29), and both were lower than those of the healthy controls (Fig. 3C).

DISCUSSION

The recent resurgence of invasive group A streptococcal infections has puzzled the scientific community for the past decade. Although, there is no clear explanation for this change in epidemiology of streptococcal infections, it is becoming clear that both pathogen and host factors should be considered when attempting to elucidate the pathogenesis of these infections. The strongest indication for the central role of host factors in invasive group A streptococcal infections is derived from the fact that the same bacterial strain can be isolated from individuals who differ considerably in the spectrum of clinical symptoms, ranging from being asymptomatic to having STSS or NF (5, 8, 24, 34, 36, 46). In fact, the cohort of patients studied here were infected with genetically indistinguishable (by pulsed-field gel electrophoresis and random amplified polymorphic DNA analysis) M1T1 strains yet had very different disease outcomes and could be subclassified as having severe or nonsevere invasive infections (5a).

Inasmuch as invasive group A streptococcal disease can be caused by a number of distinct serotypes that produce distinct superantigens (6, 10, 23, 24), and since it has been shown that the response of an individual to different serotypes can be very different (40), it follows that host specific immune responses to the infecting strain are more clinically relevant than those to an unrelated serotype. In addition, previous studies (11) have shown that protective immunity to S. pyogenes may distinguish between clones of the same serotype. Accordingly, to understand the contribution of host factors to disease, it was important to conduct our studies with patients who were infected with the same streptococcal strain in order to normalize, as much as possible, for variations in disease severity that could be simply attributed to differences in the virulence and spectrum of superantigens produced by distinct serotypes or even subclones of the same serotype. To this end, we studied patients with severe and nonsevere infections who were all infected with the same M1T1 strain. How can infection with the

FIG. 3. Low levels of anti-streptococcal superantigen neutralizing antibodies in patients with severe and nonsevere invasive group A streptococcal infections. Neutralizing antibodies against the mixture of superantigens produced by the M1T1 isolates in plasma specimens of patients infected with indistinguishable M1T1 strains were evaluated. PBMC (10^6 cells/ml) from a healthy donor were stimulated either with phytohemagglutinin (1 µg/ml) or with the partially purified culture supernatant from M1T1 isolates (diluted 1:100 in RPMI) in the presence of 5% FBS or 4% FBS plus 1% plasma. (A) Neutralizing activity in plasma specimens from healthy individuals (n = 20) of different ages. (B) Neutralizing activity in plasma specimens from healthy individuals (n = 20) of different ages. (B) Neutralizing activity in plasma specimens from patients with severe (n = 21) and nonsevere (n = 12) infections and healthy controls (n = 20) against pure superantigens rSpeA and SpeB. Proliferation was assessed after 3 days of culture, and the mean counts per minute ([3H]thymidine uptake) ± SEM for triplicate cultures was calculated. Neutralizing activity is expressed as percent inhibition of mitogenic activity. (C) Results for patients with severe (n = 21) and nonsevere (n = 12) invasive disease or for age-matched healthy individuals who reside in the same area. Each patient plasma was tested for neutralizing activity against the isolate from that patient, or, in the case of the age-matched healthy individuals, supernatants from six representative isolates (three from severe cases and three from nonsevere cases) were used for stimulation. Proliferation was assessed after 3 days of culture, and the mean counts per minute ([3H]thymidine uptake) ± SEM for triplicate cultures was calculated. Neutralizing activity is expressed as percent inhibition of mitogenic activity.
same organism cause starkly different symptoms in different people?

One of the main objectives of our studies over the past few years is to identify host factors that modulate the severity of invasive streptococcal infections. A variety of host factors can potentially affect disease outcome. These include age, underlying disease, or a preceding viral infection (9, 10, 43, 48); the presence of protective humoral immunity specific to the infecting isolate; and immunogenetic factors that regulate immune responses to streptococcal virulence factors, such as the Sps and other superantigens produced by these isolates. It is well established that the presence of M type-specific antibodies can protect the host from infection, as these antibodies opsonize the bacteria and enhance their elimination by phagocytic cells (3, 16). Our data seem to support this notion, as we have found that patients with invasive disease have significantly lower levels of binding, opsonic, and bactericidal anti-M1 antibodies compared to age- and geographically matched healthy controls. The low levels of anti-M1 antibodies were not due to nonspecific effects of sepsis, since levels of antibodies to other streptococcal components were comparable to those in controls (Fig. 3B). However, we have shown that there was no correlation between low levels of anti-M1 antibodies and disease severity: both the patients with the severe and nonsevere invasive infections had significantly lower levels of these anti-bodies in plasma than controls, and there was no significant difference between the patients with the severe and nonsevere infections.

Similarly, levels of antibodies to SpeA, SpeB, and SpeF (determined by ELISA) were significantly lower in plasma specimens of patients with invasive infections than in those of healthy controls, and equally low anti-Spe antibody levels were found for the severe and nonsevere invasive cases. An important role for anti-Spe antibodies in invasive streptococcal infections has been suggested (30, 37–39, 42), and we show here that the low levels of these antibodies are not a factor in disease severity. Furthermore, we and others (38, 42) have shown that the quality (neutralizing activity) rather than the quantity of anti-Spe antibodies is more relevant to disease pathogenesis. Thus, in addition to determining the levels of anti-Spe antibodies by ELISA, we also assessed the levels of antibodies that can neutralize the mixture of superantigens produced by these isolates. Although there was no statistical difference in the levels of neutralizing antibodies to pure Spe proteins between patient and control groups (Fig. 3B), we found a significant difference between patients and controls with respect to levels of neutralizing antibodies against the mixture of superantigens produced in the supernatants of the patients’ isolates (Fig. 3C). The findings illustrate the point that streptococcal isolates produce a mixture of known and novel superantigens. Patients who had high levels of neutralizing antibodies to a specific pure Spe but not to the mixture of superantigens produced by their infecting isolate may lack protective humoral immunity against the novel superantigens produced by these MIT1 isolates. This underscores the clinical relevance of evaluating the plasma neutralizing activity of the patient against the mixture of superantigens produced by the respective isolate.

Importantly, we found no difference in the levels of isolate-specific neutralizing antibodies between the patients with severe and nonsevere invasive infections; both were significantly lower than those in controls. The data suggest that the low levels of isolate-specific neutralizing antibodies may have contributed to the risk of invasive group A streptococcal disease but that they are not a major factor in determining disease severity. The mechanism by which lack of these antibodies may contribute to increased invasiveness of the organism is at present not clear. However, the superantigens are known to cause tissue damage and are capable of activating resident macrophages to produce inflammatory mediators and chemotactic factors; in the absence of neutralizing antibodies, the superantigen-mediated inflammatory reactions may facilitate bacterial invasion of host tissue.

Recent work from our laboratory has demonstrated that pooled human Ig (IVIG) preparations contain high levels of opsonic antibodies to several M serotypes (2), including MIT1 strains, as well as high levels of antibodies that can neutralize the immune stimulatory activity of a wide variety of streptococcal superantigens (37–39). Importantly, these protective antibodies were transferred to patient plasma, and their presence appeared to help halt disease progression (2, 23, 37). Patients who lack protective antibodies may benefit from IVIG adjunctive therapy, since these antibodies appear to help in the elimination of the bacterium and the neutralization of its toxins.

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