Dynamics of the Immune Response against Extracellular Products of Group A Streptococci during Infection

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The immune response against the infecting group A streptococcus (GAS) extracellular products (EP) was determined in acute- and convalescent-phase sera from 75 patients with different clinical manifestations of GAS infection. All EP elicited a high proliferative response in human peripheral blood mononuclear cells. In patients with bacteremia, low neutralization in acute-phase sera was associated with development of streptococcal toxic shock syndrome. Lack of neutralization in acute-phase sera was more common in patients infected with the T1emm1 serotype. The majority of patients did not develop the ability to neutralize the mitogenic activity of their infecting isolate despite a significant increase in enzyme-linked immunosorbent assay titer in early convalescent-phase sera. In patients with the ability to neutralize GAS EP, the immune response remained high over at least 3 years. In contrast, the neutralization capacity conferred by intravenous immunoglobulin and/or plasma treatment disappeared within 3 months.

Group A streptococcus (GAS) is a common human pathogen that can cause a spectrum of syndromes ranging from asymptomatic carriage through trivial superficial infections to life-threatening invasive clinical conditions, such as necrotizing fasciitis and streptococcal toxic shock syndrome (STSS). STSS is a severe outcome of GAS invasive infection with mortality rates ranging from 30 to 70% (9, 10, 12, 21). During the late 1980s, several outbreaks of severe invasive infections due to T1M1 GAS were reported worldwide. In Sweden, two outbreaks of streptococcal invasive disease occurred during the time span of the current study (1986 to 1996), one in 1988 and 1989 and a second in 1994 and 1995 (33, 34).

The M-protein and streptococcal pyrogenic exotoxins (Spe) are considered important virulence factors in the pathogenesis of invasive disease. Spe belong to the superantigen (SAg) protein family that induces massive T-cell activation and subsequent release of high levels of cytokines through cross-linking of the major histocompatibility complex class II proteins and T-cell receptor molecules bearing certain Vβ chains (32). Several lines of evidence support the hypothesis of SAg involvement in the pathogenesis of STSS. In sera from patients with shock, elevated levels of Th1 cytokines have been noted, along with depletion and suppression of certain Vβ-expressing T cells (26, 29, 35). Moreover, studies with animal models have shown that SAgS induce shock-like symptoms in rabbits and rodents (6, 22, 31).

The isolation of genetically identical GAS from patients with different degrees of severity of infection implies a role for host factors in determining the outcome of microbe-host interaction (7, 24). Several studies of patient humoral immunity have demonstrated that the lack of protective antibodies to M-protein and SAgS are risk factors for development of invasive disease (4, 28). Furthermore, the magnitude of Th1 cytokine response mounted during infection has been shown to be related to the severity of disease (26). SAgS of streptococcal origin have been shown to preferentially bind HLA-DR and HLA-DQ, and in more recent studies, polymorphism within these genes has been shown to influence the host response to SAgS (19, 20, 27).

Early administration of intravenous immunoglobulin (IVIG) has shown promising results in treatment of GAS invasive disease (8, 18). However, IVIG preparations differ in their capability to neutralize SAgS (25). A possible future therapeutic approach involves identification of common epitopes on SAgS important for T-cell activation and HLA recognition, which could be used in developing a vaccine against STSS (3). These different approaches for treatment raise questions regarding the level and specificity of the individual humoral immune response induced during a GAS infection.

In this retrospective study, we monitored the humoral immune responses in patients with different clinical manifestations of GAS infections over time. The purpose of the study was to measure the neutralization of the mitogenic activity and the enzyme-linked immunosorbent assay (ELISA) titers against EP produced by the patient’s own infecting isolate during in vitro culture. Furthermore, we also wanted to investigate whether GAS infections could trigger the development of SAg-inhibiting humoral response and to analyze whether the acquired immunity is general or limited to the infecting isolate.
MATERIALS AND METHODS

Clinical material. Isolates and sera from patients with different clinical manifestations of GAS infections were collected in Sweden from 1986 to 1996 (12, 17, 28). A total of 84 isolates were used. Both acute- and convalescent-phase sera and the infecting isolate were obtained from 75 patients. Patients were grouped according to diagnosis: 20 had sepsis without shock, 23 had STSS, 21 had erysipelas, and 11 had tonsillitis; the mean ages for the four patient groups were 60, 46, 59, and 18 years, respectively. The diagnosis was made by a physician. Tonsillitis was defined as a sore throat with positive GAS culture. Erysipelas was defined as a febrile condition with a red, well-demarcated, warm, and painful area of erythema. Patients with a positive blood culture were included in the sepsis group, and STSS was defined according to standard criteria and in some cases was diagnosed retrospectively (36). Sera were collected at different time points and divided in groups. Acute-phase sera were collected on days 0 to 7 after the onset of disease; if the onset was not noted, day of admission to hospital was considered day 1. Early convalescent-phase sera were collected from days 10 to 75, intermediate convalescent-phase sera were collected from days 114 to 400, and late convalescent-phase sera were collected from days 696 to 1,512. Sera were not available at all time intervals from every patient. For one patient, sera obtained prior to the onset of disease was regarded as acute-phase sera. In the analyses of immune status in acute-phase sera, one patient was excluded from analysis, since the patient had already received IVIG and fresh-frozen plasma. Nine patients had received treatment with either IVIG and/or fresh-frozen plasma when their convalescent-phase sera were collected and were therefore analyzed separately. Supplementary sera were stored at −70°C. The study was approved by the Ethics Committees at the Karolinska Institute (Stockholm, Sweden), Umeå University, and Uppsala University, Sweden.

Clinical isolates and preparation of EP from bacterial supernatants. GAS from bacterial cultures of the throat or nasopharynx, skin lesions, skin biopsy specimens, ulcers, and blood samples from 84 patients were saved and stored at −20°C. T types were determined serologically. Extracellular products (EP) were prepared as previously described (17). Briefly, culture supernatants prepared from gas grown in Todd-Hewitt broth for 16 h at 37°C were used to assay optimal production and release of currently known Sags. The optical densities of the supernatants were adjusted prior to concentration 1:10 by ethanol precipitation. Precipitates were dialyzed extensively against distilled H2O, sterilely filtered, and stored at −20°C. EP was adjusted for volume changes during dialysis in each experiment.

dmm typing. dmm typing was performed by DNA sequencing according to the Centers for Disease Control and Prevention (CDC) guidelines (available at http://www.cdc.gov/ncidod/biotech/strep/strepindex.html), with minor modifications. In short, DNA was prepared either by a phenol-chloroform extraction method described earlier (13) or by using a DNeasy tissue kit (QIAGEN Nordic, Stockholm, Sweden), and the DNA was resolved in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0, or distilled H2O, respectively, and stored at −20°C. CDC PCR primer sets 1 and 2 and MF2 and MR1 were used at a concentration of 50 pmol/μL PCR products were purified with the QIAquick PCR purification kit (QIAGEN Nordic). Sequencing was performed using the CDC primer emmseq2 and the ABI PRISM Big-Dye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) on an ABI automated DNA sequencer. The sequences were aligned by using DNASIS MAX V1.0 (MiraiBio Inc, Alameda, Canada) for determination of the emm type.

Neutralization assay. Experimental conditions were optimized to generate conditions where the mitogenic activity could be neutralized by immune sera while the mitogenic activity seen using nonimmune sera remained high. All EP were mitogenic at a dilution of 1/1,000 (titrated 1/50 to 1/5,000). Human gamma globulin (Amersham-Pharmacia Biotech, Uppsala, Sweden) was added at a concentration of 62.5 μg/ml (equivalent to 5% sera) showed close to 100% neutralization for EP diluted 1/100 against all isolates tested, while 16.5 μg/ml human gamma globulin (equivalent to 1% sera) reached only 50% neutralization for EP diluted 1/100. Using EP at a concentration of 1/500, the neutralization was 100% with a gamma globulin concentration of 82.5 μg/ml, while it was up to 50% when 16.5 μg/ml of gamma globulin was applied. Ethanol precipitate of sterile Todd-Hewitt broth was nonmitogenic at a dilution of <1/50. A serum sample from a healthy donor showed between 20 and 100% neutralization of EP diluted 1/100 prepared from six different emm types, while none of 42 tested patient sera stimulated lymphocytes when applied to peripheral blood mononuclear cells (PBMCs) alone at a concentration of 5%. Thus, neutralization experiments, EP was used at a final dilution of 1/100 with 5% patient sera. PBMCs from six healthy blood donors were purified by use of a lymphoprep density gradient (Nycomed, Oslo, Norway); the PBMCs were then diluted to 107 CFU/ml in RPMI 1640 (Gibco-BRL, Stockholm, Sweden) containing 2 mM gentamicin (Saveen Werner AB, Stockholm, Sweden) supplemented with 10% fetal calf serum (Gibco-BRL, Stockholm, Sweden) and stored at 37°C in 5% CO2 for a maximum of 3 days. For neutralization experiments, 3 × 107 PBMCs were preincubated in 100 μl complete RPMI 1640 with 10% heat-inactivated patient sera for 1 h at 37°C in 5% CO2 before the addition of 100 μl EP diluted 1/50 (final dilution 1/100) in complete RPMI 1640 supplemented with 10% fetal calf serum. After 72 h, [3H]thymidine at 1 μCi/well (Amersham-Pharmacia Biotech, Uppsala, Sweden) was added for an additional 6 h at 37°C in 5% CO2. EP-induced PBMC proliferation was quantified in a liquid scintillation counter (Wallac, Sollentuna, Sweden). The neutralizing activity against EP-induced mitogenicity in patient sera was calculated as 1 − [(cpm of EP + sera)/cpm EP] × 100. Ptoyoheamaggulutinin (PHA-L) (5 μg/ml) (Sigma Chemical Co., St. Louis, MO) was used as a positive T-cell control and for background proliferation of control medium alone. All experiments were performed with five replicate samples.

ELISA. Each well of 96-well microtiter plates (Nunc, Roskilde, Denmark) was coated with 100 μl of 1/100 dilutions of EP, blocked with 0.5% bovine serum albumin (Sigma Chemical), and washed twice before the addition of patient sera. Sera were titrated with serial dilutions from 1/100 to 1/51,200. As a secondary antibody, alkaline phosphatase-conjugated anti-human immunoglobulin G (DAKO, Solna, Sweden) was used. ELISA titers were defined as dilutions corresponding to half the maximum absorbance.

Statistical analysis. In order to determine differences in the neutralizing activity and ELISA titer of acute- and convalescent-phase sera at different time intervals, the paired t test and Wilcoxon signed-rank test were performed. The Kruskal-Wallis test and two-sample t test were used to estimate differences in mean neutralizing activity and ELISA titers during the acute phase and for sera from immunoglobulin-treated patients. For evaluation of variance in the proportion of high and low responders for different diagnoses and M serotypes, one-sample t test was used. Pearson correlation and regression analysis were determined for neutralization versus ELISA titers and EP-induced proliferation (counts per minute, respectively). All statistical analyses were performed using Minitab 14.13 (Minitab, Coventry, United Kingdom). P values of ≤0.05 were considered significant.

RESULTS

PBMC proliferative response induced by EP of GAS extracellular products. To exclude the influence of possible differences in EP-induced proliferation among donor PBMCs, paired EP and sera from different patient categories with several emm types represented were tested in random order in each experiment. The PBMC proliferative response induced by EP from the individual GAS isolates varied between 24,000 and 158,000 cpm with a standard error of the mean of 4,767 cpm. No correlation was found between the level of the neutralization capacity of the acute-phase sera and the EP-induced proliferative response was found. Furthermore, no correlation was seen between the level of proliferation and the serotype of the isolate from which the EP was prepared.

Neutralization of T1 emm1 in relation to other serotypes. Since several investigators have reported an association of GAS serotype T1 emm1 with the severity of disease (5, 23) and the majority of sera included in this study originated from an outbreak of severe GAS T1 emm1 infections during 1989 and 1990 (33, 34), we wanted to investigate the nature of the noted neutralization capacity in relation to the GAS serotype and year of isolation. Two groups of patients could clearly be distinguished according to the capacity in their acute-phase sera to neutralize EP mitogenicity from their own infecting isolate. Patients had either low (below 20%) or high (above 70%) inhibition of EP-induced PBMC proliferation (Fig. 1). A few sera had intermediate neutralization, but for simplicity, patients with an inhibition level below 50% were categorized as low responders and patients with levels above 50% were categorized as high responders.
FIG. 1. Neutralization and ELISA titers of group A streptococcal extracellular products in sera from patients with different disease manifestations. Sera were grouped according to the collection day after onset of infection and tested for the ability to neutralize EP-induced PBMC proliferation from the respective infecting isolate. The manifestations of GAS infection related to the sera are indicated in the figure. (A and B) Neutralization titers for low responders with neutralizing capacity of EP mitogenicity below 50% (A) and high responders with EP mitogen neutralizing capacity at or above 50% (B). The zero level and 50% level are indicated by broken lines. The sera indicated below the line for zero all lacked neutralization. The mean EP mitogen neutralization in each time interval is indicated. Common mean values were calculated for the two last time intervals in panels A and B, respectively. In panel A, the neutralization values for the patients that gained neutralization capacity to above 50% are connected with a line, and in panel B, the patients that lost neutralization capacity (a and b) to below 50% are indicated. ELISA titers against 1/100 dilutions of EP produced by the infecting isolate were measured in acute-phase sera and one of the convalescent-phase sera. In patients with no early convalescent-phase sera, a later serum sample was used. Sera were serially diluted from 1/100 to 1/51,200. (C and D) The figure shows ELISA titers calculated as 10 log dilution × 100 at half the maximum absorption at 405 nm. Immunoglobulin G ELISA titers for patients with neutralizing capacity of EP mitogenicity below 50% (C) and patients with EP mitogen neutralizing capacity at or above 50% (D). The mean ELISA titer for sera in each time interval is indicated by the horizontal bar. Significant differences \((P < 0.01)\) in ELISA titers are indicated (†).
The EP mitogen neutralization capacity in acute-phase sera was compared for patients infected with T1emm1 GAS to patients infected with GAS of other serotypes. Sera from patients with tonsillitis were excluded, since they were all collected during 1989. Patients infected with T1emm1 isolates had a significantly lower mean neutralization capacity in their acute-phase sera against EP from their infecting isolate than patients infected with other serotypes \((P = 0.016)\). In patients with bacteremia and without bacteremia, no significant difference in the proportion of T1emm1 isolates in relation to other emm types (21 of 42 and 7 of 21, respectively) was seen within the infecting isolate. However, among the patients with bacteremia, the proportion of infection with GAS of serotype T1emm1 was significantly higher in the STSS group (16 of 22) than in the group with sepsis but without STSS (5 of 20) \((P < 0.001)\).

Serotype specificity of EP neutralization. To elucidate whether the neutralization noted in patient sera was specific for their respective infecting isolate or whether the immune response also recognized other isolates belonging to this emm genotype, sera with high neutralization were selected and tested against isolates of the same and other emm types. Overall, the spectra of neutralization in the patient sera were narrow and limited to the emm type and sometimes neutralized only the mitogenic activity of the patient's own infecting isolate. Figure 2 shows that a serum sample from a T1emm1-infected patient obtained in 1989 completely neutralized EP prepared from six other GAS of serotype T1emm1, which were isolated from 1989 to 2001. Similar results were seen for sera drawn from immune individuals infected with GAS of serotypes T8emm8 and T12emm12. However, a serum sample from a healthy donor obtained in 2003 completely inhibited the mitogenic activity of a T1emm1 isolate from 2001 but had virtually no neutralization against an earlier T1emm1 isolate from 1989. A change in mitogenicity over time was also noted among other emm types. Sera collected during 1989 to 1991 with high neutralization activity against their infecting T2emm2, T11emm89, T28emm28, and T4emm14 isolates showed significantly lower neutralization against GAS of these emm types isolated a few years later.

Two patient sera showed inhibition of EP mitogenicity from emm types other than that of their infecting isolates (T13emm73 and T22emm3). Similarly, a serum sample from a healthy donor collected in 2003 inhibited the mitogenic activity of EP from three different emm types, T1emm1, T2emm2, and T11emm89 (Fig. 2). Sera obtained from individuals infected with GAS of serotypes other than T1emm1 in general showed neutralization below 50% when EP from a T1emm1 1989 isolate was tested. One exception was a serum sample from a patient who had recently been treated with IVIG. This serum sample showed 100% neutralization against EP from GAS of all nine emm types tested, indicating the broad spectrum of neutralization that may be provided by IVIG.

Neutralization levels in relation to disease outcome. Analysis of the neutralization capacity in acute-phase sera against SAg mitogenicity revealed that the majority of patients (50 of 74) were initially low responders. No significant difference in the numbers of low and high responders was seen when patients with bacteremia (32 of 42) and patients without bacteremia (18 of 32) were compared. However, when subdividing the bacteremia group, a significantly higher number of patients with STSS (20 of 22) with low neutralization capacity in their acute-phase sera was found compared to patients with sepsis (12 of 20) \((P = 0.014)\). Analyses of patients without bacteremia showed that erysipelas patients (12 of 21) had a higher number of high responders in acute-phase sera than tonsillitis patients (2 of 11).

Immune development. The main purpose of this study was to investigate whether GAS infections could trigger the development of SAg-inhibiting humoral response. The neutralizing capacities in acute- and convalescent-phase sera of lymphocyte proliferation induced by EP from the infecting isolate was compared for each patient. Patients were grouped according to the neutralization capacity in the acute-phase sera, and patients that received IVIG and/or fresh-frozen plasma were excluded. Sera were divided into time intervals according to the expected immune development following a normal immunization. In neither high nor low responders did the initial mean levels of neutralization in acute-phase sera change significantly compared to that in early or late convalescent-phase sera (Fig. 1). However, when comparing individually paired serum samples, 6 of 42 patients showed significant increases in their low neutralizing levels in acute-phase sera to levels above 50% in their convalescent-phase sera. Of these patients, three were infected with GAS of the T1emm1 serotype. Two patients were diagnosed with sepsis, two with STSS, and two with tonsillitis. In the patient group with high levels of neutralization in their acute-phase sera, 2 of 24 had a decreased level below 50% in their convalescent-phase sera, and only for one of these was the decrease significant \((P = 0.001)\).

ELISA titers in relation to neutralization. ELISA titers against EP in acute- and convalescent-phase sera were determined to rule out whether the noted low levels of mitogen neutralization in acute-phase sera were due to a general immune deficiency in patients with sepsis and STSS. Despite a significant difference in the EP-neutralizing capacity in the acute-phase sera, the neutralization capacity in the acute-phase sera, and patients with STSS (20 of 22) with low neutralization capacity in their acute-phase sera was found compared to patients with sepsis (12 of 20) \((P = 0.014)\). Analyses of patients without bacteremia showed that erysipelas patients (12 of 21) had a higher number of high responders in acute-phase sera than tonsillitis patients (2 of 11).

To evaluate the relationship between ELISA titers and mitogen neutralization capacity in patient sera, a correlation analysis was performed. The results showed that sera with ELISA titers between 1,000 and 10,000 revealed both high and low neutralization capacity. Five sera with ELISA titers below 500 inhibited the mitogenic activity of EP at or above 50%. In contrast, 12 sera with ELISA titers above 10,000 lacked the capacity to neutralize the EP-induced mitogenicity. Determination of the Pearson correlation coefficient revealed no correlation between ELISA titer and neutralization of EP mitogenicity \((R^2 = 0.0124)\). In summary, our data show that very few patients with initial low SAg neutralization developed the ability to inhibit the mitogenic activity of EP in response to their infection, despite the development of high ELISA titers against GAS EP. The neutralizing activity found was very spe-
specific and typically limited to the infecting GAS serotype, and sometimes to the infecting isolate only.

**Treatment with immunoglobulin or plasma.** Nine patients who had received IVIG and/or fresh-frozen plasma treatment were analyzed separately for the presence of neutralizing activity before treatment and soon after treatment and for the persistence of the activity (Fig. 3). One patient had received treatment with fresh-frozen plasma before the acute-phase serum sample was drawn and was not included in the acute-phase serum analyses. All of the treated patients lacked neutralization capacity in their untreated acute-phase sera. In four patients given ≤9 units of fresh-frozen plasma, the neutralization levels remained low, while in the two patients who received ≥15 units of fresh-frozen plasma and the two patients given IVIG and fresh-frozen plasma, the neutralization levels increased above 50%. The increase in neutralization capacity in these patients was noted immediately after they had received the immunoglobulin and disappeared within 3 months. No correlation was seen between neutralization capacity and ELISA titers among the treated patients. Table 1 shows that the ELISA titers against total EP increased in seven of eight sera and that the increase was significant ($P = 0.027$).
The EP from all GAS isolates tested were highly mitogenic to human PBMCs, confirming the previous findings that STSS is not caused by a specific strain with particularly strong induction of T-cell proliferation. Instead, the results showed that GAS of various serotypes isolated in association with GAS bacteremia and nonbacteremic GAS infections all had the capacity to produce T-cell mitogenic substances when cultured in vitro. To exclude the possible presence of yet unidentified mitogens produced by the isolates, we chose to study the immune response against the entire supernatant from in vitro cultures of GAS, all grown under identical conditions.

An important host factor which influences the outcome of GAS invasive infections is the humoral immune defense. Our results support the hypothesis that neutralization of SAg mitogenicity confers protection against STSS, since there was a strong correlation between the absence of neutralization against EP-induced mitogenicity in acute-phase sera and the development of STSS among patients with sepsis. In contrast, a study by Basma et al. (4) showed no difference in the levels of protective antibodies against SAgs in patients with severe and nonsevere invasive disease. They concluded that low levels of SAg neutralizing antibodies might contribute to host susceptibility to invasive streptococcal infections, but unlike our present findings, they do not modulate disease outcome. However, the neutralizing capacity of the mitogenic activity of SAgs is not the only factor that modulates the outcome of infection. In the current study, 12 of 32 sepsis patients with low EP inhibitory activity in acute-phase sera did not develop STSS. Host factors, such as the presence and specificity of SAg binding epitopes among immune cells (HLA and T-cell receptor), underlying disease, and the physical status and age of the patient might also contribute. In the present study, the mean age of patients with high EP neutralization in acute phase was

![Image](http://cvi.asm.org/)  

**FIG. 3.** Change in EP mitogen neutralization capacity in patient sera after treatment with IVIG and/or fresh-frozen plasma. Sera were collected before and at different time points after treatment, and each line represent an individual patient. From one patient (patient a), no serum was drawn before treatment. The zero and 50% levels are indicated by broken lines. The values indicated below the line for zero all represent sera that lack neutralization. To include the last serum sample, the x axis is broken at day 85. One unit of plasma is equivalent to 270 ml of plasma from a healthy blood donor.

### TABLE 1. Neutralization and ELISA titers against GAS extracellular products in acute- and convalescent-phase sera from immunoglobulin-treated patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Treatment</th>
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<th>Convalescent-phase sera</th>
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<tr>
<td></td>
<td></td>
<td>% Neutralization</td>
<td>ELISA titer</td>
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<tr>
<td>12</td>
<td>IVIG + plasma</td>
<td>0</td>
<td>333</td>
</tr>
<tr>
<td>349</td>
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<td>833</td>
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<td>85b</td>
<td>Plasma (≥15 units)</td>
<td>61</td>
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<td>241</td>
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</table>

* One unit of plasma is equivalent to 270 ml plasma from a healthy blood donor.  

b The patient had received plasma before the acute-phase sera was drawn.  

c NA, not available.
higher than that of patients with low EP neutralization within all patient groups, suggesting that the levels of neutralization increase with age. When comparing the EP neutralization in relation to diagnosis, we found higher neutralization in patients with erysipelas (mean age, 59 years) than in patients with tonsillitis (mean age, 18 years). Since neutralization was found to last for a long time, the high levels of neutralization among erysipelas patients compared to tonsillitis patients might be explained by the higher age of the erysipelas patients in the present study.

In many reports, GAS of T1 emm1 serotype and its related SAgs have been described as more virulent and more often causing severe forms of disease (5, 23). Of the patients with bacteremia and low levels of neutralization, 16 of 20 infected with T1 emm1 developed STSS compared to only 4 of 12 infected with GAS of other emm types. These results imply that T1 emm1 GAS is more virulent than other serotypes and may in addition imply that the SAgs express unique virulence factors which contribute to its high pathogenicity. Recent findings have identified other factors in M1 isolates important for virulence. The M1 protein has been shown to be involved in the induction of excessive vascular leakage seen during shock (15). In addition, protein SIC which has been shown to be expressed only by M1 and M57 isolates (1) has been shown to inactivate antimicrobial peptides such as LL-37 and human α-defensin (14). Experimental studies using a mouse model of subcutaneous infection showed that the SIC-deficient mutant strain was significantly attenuated for virulence compared to its wild-type strain (16).

When comparing the neutralization capacity in relation to GAS emm type, we found that the proportion of patients with high neutralization levels in their acute-phase sera was significantly lower among patients infected with GAS of the T1 emm1 serotype than in patients infected with other serotypes. Patients who were infected with T1 emm1 GAS after the 1988–1989 T1 emm1 outbreak in Sweden did not reveal higher levels of neutralization against EP from T1 emm1 than sera collected during the outbreak.

In general, the acquired immune response after infection was found to be serotype specific and sometimes isolate specific. This was illustrated by the finding that none of the sera with high neutralization capacity against their own infecting GAS, representing several serotypes, could inhibit the EP-induced mitogenicity from the T1 emm1 GAS isolated during 1989 to the 50% level. Moreover, a serum sample from a healthy blood donor totally inhibited the mitogenicity of EP from a T1 emm1 serotype isolated in 2000, while the serum had no neutralizing capacity towards the T1 emm1 serotype isolated in 1989. Since all EP were equally mitogenic, it is very unlikely that this is due to proteolytic degradation of mitogens in EP. The total lack of neutralizing capacity towards the GAS of T1 emm1 from 1989 among the sera tested more likely reflects differences in SAgs production between the different isolates. Thus, results in the present study indicate that the SAg profile of GAS vary both between emm types and within emm types and also tend to vary over time. Similar findings were reported in a recent nationwide Danish study (11). Their results showed a significant decrease in the occurrences of speA and ssa and an increase of spec among GAS isolated from 1999 to 2002.

Severity and site of infection did not seem to be important for triggering development of EP neutralizing activity, since patients with tonsillitis were also found to develop SAg neutralization capacity. Very few patients with low inhibitory capacity in acute-phase sera (6 of 42) developed neutralization of EP-induced mitogenicity in response to the infection. There might be different explanations for this, such as either SAg immune modulation inhibits development of neutralization or the humoral immune response is directed against nonprotective epitopes. SAg-induced T-cell proliferation is unspecific and predominantly leads to a Th1 response which may influence the ability to develop a highly specific humoral immune response. In an experimental mouse model, it has been shown that induction of a broad-spectrum protective immunity against SAgs requires suppression of SAg-induced Th1 response by treatment with a SAg antagonistic peptide (2). In the present study, two patients with STSS developed high levels of neutralization following infection. In contrast, all STSS patients with initially low neutralization developed high ELISA titers against EP from their respective infecting isolates, reflecting a strong Th2 response. Thus, blocking the protective B-cell response through the action of a vigorous Th1 response induced by SAgs during infection does not seem to be the mechanism behind the lack of development of GAS-specific antibodies during the convalescent phase. Absence of EP neutralizing activity in sera following GAS infection may rather be related to the specificity of the selected immunoglobulin G. In a previous study, patients with low neutralization of SpeA-, SpeB-, and SpeF-induced mitogenicity in acute-phase sera were found to have high ELISA titers against the purified superantigens (27). The absence of a correlation between ELISA titers and SAg neutralization observed in the present study supports these findings. This apparent restriction in the ability to respond to certain antigens or epitopes has previously been seen in connection with response against Haemophilus influenzae polysaccharide and cytomegalovirus where allelic variation in the V-gene loci were shown to influence the diversity of the antibody response (30). Another factor that might influence the outcome of GAS infection is the immune receptor repertoire of the infected individual. The interactions between SAgs and immune cells have been shown to differ depending on the subset of receptors present on antigen-presenting cells and T cells (19, 20, 21, 27).

Only one-third of the patients in this study showed high inhibition of EP in their acute-phase sera, whereas full neutralization occurred when patients were given IVIG and/or fresh-frozen plasma. IVIG preparations have been reported to differ in their ability to neutralize SAg activity (25). This reflects the variable levels of neutralization capacity in the population, which is also seen among patients treated with plasma, since more than 15 units was required to obtain full neutralization. EP inhibition conferred by treatment with passive immunization disappeared within 3 months. In contrast to the neutralization conferred by IVIG and plasma, naturally acquired neutralizing activity remained above 50% in convalescent-phase sera collected more than 3.5 years after infection. This is most likely due to acquired B-cell memory, since very low ELISA titers were required for inhibition of SAg mitogenicity. That can also explain the moderate increase in ELISA titers noted among patients with initially high neutralization.

In conclusion, we show that patients develop antibodies
against GAS extracellular products following streptococcal infection but these antibodies do not always neutralize the mitogenic activity produced by the infecting isolate. In examining the nature of the EP neutralization, we found that it is largely specific for one particular GAS isolate and in some cases GAS of the same emm type. The findings in this study indicate that EP neutralization does not confer protection against GAS infections but may rather modulate the outcome of disease.

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