Group A Streptococcal Antibodies in Subjects with or without Rheumatic Fever in Areas with High or Low Incidences of Rheumatic Fever


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The levels of streptococcal antibody titers in populations with or without rheumatic fever from an area with a relatively high incidence of rheumatic fever and an area with a low incidence of this disease were compared. Streptococcal antibody titers were determined for two populations, each of which included children without rheumatic fever (nonrheumatic children) and rheumatic fever patients. The two populations were derived from two separate geographic areas, one with a high incidence of rheumatic fever (Grenada) and another with a low incidence of this disease (central Florida). The results revealed an absence of consistent differences in the geometric mean antibody titers between the nonrheumatic subjects and the rheumatic fever patients from Grenada. In the population from Grenada, the mean anti-streptolysin O and anti-DNase B titers were higher in the nonrheumatic controls (P of 0.085 and 0.029, respectively). However, the mean titer of the antibody to the group A streptococcal cell wall carbohydrate was higher in the rheumatic fever patients than in the nonrheumatic controls (P = 0.047). This finding contrasted with the finding that the means of all three streptococcal antibody titers in the patients with rheumatic fever were significantly higher than those in the nonrheumatic subjects from Florida (P = 0.01–<0.001). The reason for this paradoxical finding became evident when the streptococcal antibody titers of the nonrheumatic subjects from Grenada and Florida were compared, revealing significantly higher levels of all three antibodies in the nonrheumatic subjects from Grenada than in the nonrheumatic subjects from Florida (P < 0.001). These results suggest that nonrheumatic individuals in an area with a high incidence of rheumatic fever have inordinately elevated levels of streptococcal antibodies in serum. The presence of elevated streptococcal antibody titers in such a population, which probably reflects a high background prevalence of streptococcal infections, should be taken into consideration when evaluating the role of the group A streptococcus in nonpurulent complications of infections.

Previous studies of the antibody responses of patients with acute rheumatic fever compared the antibody levels in such populations with those in healthy controls, which were defined as individuals from the same area who had no evidence of recent group A streptococcal infections or nonpurulent complications of such infections (3, 5, 8, 9, 15, 19). These studies consistently showed that the levels of the streptococcal antibodies in patients with rheumatic fever, particularly those with residual rheumatic heart valve disease (1, 8, 15), were significantly higher than those of the healthy controls. No studies have examined the levels of these antibodies in populations with rheumatic fever and those without rheumatic fever (termed nonrheumatic) from areas with a high incidence of rheumatic fever. The present study was undertaken to determine whether the streptococcal antibody levels in nonrheumatic individuals and those with rheumatic fever in an area with a high incidence of rheumatic fever show the same differences as those in populations from areas with a low incidence of this disease.

Streptococcal antibody titers (anti-streptolysin O [ASO], anti-DNase B, and the antibody to the group A carbohydrate [anti-A-Chol]) were determined for the sera from individuals with no history of rheumatic fever and for patients with a history of rheumatic fever who were from the same population of the island of Grenada, West Indies, an area where the incidence of rheumatic fever remains very high (13; P. K. Noah, R. Kopycinski, B. Nelson, unpublished data; and B. Nelson and G. F. Armstrong, personal communications). The levels of the antibodies in these two groups were then compared to those obtained for matched groups of healthy individuals and patients with a history of rheumatic fever from Florida, where the incidence of acute rheumatic fever is low (6).

MATERIALS AND METHODS

Grenada study. The island of Grenada has a population of 99,510, including about 26,000 in the age range of 5 to 15 years; about 75% of the population is of African descent (B. Nelson and G. Armstrong, personal communications). The recent annual incidence of rheumatic fever in Grenada is 52 per 100,000 in children under the age of 15 years (13; P. K. Noah, R. Kopycinski, and B. Nelson, unpublished data; B. Nelson and G. F. Armstrong, personal communications). This incidence contrasts with an incidence of 0.6 per 100,000 in individuals in the age group of 5 to 19 years in the United States (11). The patients in Grenada are
monitored through district clinics in their respective parishes to ensure compliance with prophylaxis. Recent data indicate that while the incidence of acute rheumatic fever has not changed in Grenada, the recurrence rate has declined significantly following the institution of penicillin prophylaxis in the early 1990s (13; P. K. Noah, R. Kopycinski, and B. Nelson, unpublished data; B. Nelson and G. F. Armstrong, personal communications).

(i) Nonrheumatic subjects. Nonrheumatic subjects consisted of 30 children of African ancestry: 16 were healthy children, 8 children had congenital heart disease, 4 children had Marfan syndrome, 1 child had congenital rubella, and 1 had congenital hemolytic anemia. These subjects had no history of recent pharyngitis and no evidence of active impetigo or complications of group A streptococcal infections. They were seen in the clinic at the same time as the patients who had rheumatic fever and were matched with these patients for age (range, 4 to 24 years; median age, 14 years).

(ii) Rheumatic fever patients. Patients previously diagnosed as having acute rheumatic fever were scheduled to return to the clinic, where they were reevaluated by one of the physicians involved in this study. The diagnosis of acute rheumatic fever had been established at the time of the original presentation based on the updated Jones criteria (7). The history of their illness and their adherence to prophylaxis were reviewed. The patients received a complete physical examination. Electrocardiogram and echocardiographic studies were performed on patients with suspected cardiac disease, and the results were reviewed by a pediatric cardiologist.

Thirty-two patients were included in the Grenada study. Their ages ranged from 6 to 32 years (median age, 13 years). All patients were of African descent. None of the patients had acute disease at the time of evaluation. The interval from the time of acute disease to the present evaluation for the patients ranged from 0.2 to 20 years, with a mean of 5.8 years. This interval was <1 year for 3 patients, 1 to 5 years for 16 patients, 6 to 10 years for 7 patients, and 11 to 20 years for 6 patients. Twenty-six of the patients had clinical evidence of chronic valvular heart disease, and five of these patients had undergone surgical repair of their mitral valves. The nature of the valvar disease was confirmed by echocardiography, which was performed at the time of this evaluation on all but two of the patients who had undergone mitral valve replacement. Mitral valve abnormality was present in only 15 patients, combined mitral valve and aortic valve disease was present in 8 patients, and isolated aortic disease was present in 3 patients. Six patients had no clinical or echocardiographic evidence of cardiac disease; five patients had arthritis and one patient had Sydenham chorea as the major manifestation of their disease. Twenty-five of the patients were receiving penicillin prophylaxis: 22 were receiving monthly intramuscular benzathine penicillin G, and 3 were receiving daily oral penicillin.

After the consent of the patients or their parents was obtained, blood was procured from all Grenada subjects for streptococcal antibody tests. Gainesville, Fla., study. Antibody titers obtained for the Grenada nonrheumatic subjects and the rheumatic fever patients were subsequently compared with titers obtained for 32 nonrheumatic individuals and 32 patients with rheumatic fever from Florida. Only African-American subjects were included.

(i) Nonrheumatic subjects. The nonrheumatic individuals from Florida consisted of healthy school-age children and young adults from whom sera had been procured for previous studies to serve as healthy controls. These subjects were screened at the time of blood procurement and were found to have no history of recent pharyngitis or complications of streptococcal infection. They were matched for age and race with the rheumatic fever patients. The ages of the subjects ranged from 5 to 26 years (median age, 13 years).

(ii) Rheumatic fever patients. The rheumatic fever patients included in the Florida study were age matched (range, 5 to 32 years; median age, 13 years) with the Grenada patients. They were also matched with respect to cardiac involvement. They included 9 patients without cardiac disease and 23 with valvular disease: 14 patients had mitral valve disease, 6 had combined mitral and aortic valve disease, and 3 had isolated aortic valve disease. The interval between the time of acute disease and procurement of blood samples ranged from 0.2 to 24 years, with a mean interval of 5.9 years. The difference between this interval and that of the Grenada patients was not significant (P = 0.49). Data on prophylaxis available for 21 patients, revealed that 8 of the patients were receiving monthly intramuscular benzathine penicillin G prophylaxis and 13 were receiving oral penicillin prophylaxis.

Streptococcal antibody tests. Serum was separated aseptically from the blood samples obtained from the Grenada subjects prior to transportation for assay in our laboratory (University of Florida, Gainesville). All sera were stored at −20°C. Proportional numbers of sera from each of the study groups were assayed in tandem for the three streptococcal antibodies, namely, ASO, anti-DNase B, and anti-A-Cho. The assays were performed as previously described (4).

Statistical analysis. Analysis of the significance of the difference between means and geometric means of the antibody titers for the different groups was assessed by two sample t tests that assume unequal variance provided in the Excel program in Office 97 (Microsoft Corporation, Redmond, Wash.). Differences were considered significant if the P value obtained by one-tailed analysis was <0.05.

This study was approved by the University of Florida Medical Center Review Board for the protection of human subjects.

RESULTS

The distributions of the streptococcal antibody titers obtained for the nonrheumatic subjects and the patients from Grenada and Florida are illustrated in Fig. 1 and 2, respectively. No marked differences in the distributions of the three antibodies are noted between the different groups. However, a comparison of the geometric mean titers obtained for the nonrheumatic subjects and the rheumatic fever patients from Grenada and Florida revealed a number of significant differences.

For the Grenada subjects, the geometric means of the ASO and anti-DNase B titers were higher in the nonrheumatic population (Table 1). The difference was not significant for the ASO titers (P = 0.085), but it was significant for the anti-DNase B titers (P = 0.029). In contrast to the higher ASO and anti-DNase B titers in the nonrheumatic subjects, the mean anti-A-Cho titer was significantly higher in the rheumatic fever population than in the nonrheumatic controls (P = 0.047). These results contrasted with those for the populations from Florida where, in keeping with the findings of previous studies (1, 8, 15), the mean titers of all three antibodies among the rheumatic fever patients were significantly higher than those for the nonrheumatic group (P = 0.01–<0.001).

In order to explain the unexpected findings for the streptococcal antibody titers in the nonrheumatic subjects and the rheumatic fever patients from Grenada, the antibody titers of the nonrheumatic subjects from Grenada were compared with those of the nonrheumatic individuals from Florida. As shown in Table 2, the means of all three antibody titers were significantly higher in the Grenada nonrheumatic group than in the Florida nonrheumatic population (P <0.001). A similar analysis of the geometric means of the antibody titers for the rheumatic fever groups from Grenada and Florida (Table 1) revealed no significant differences.

DISCUSSION

Previous studies that established serologic evidence for an etiologic association between group A streptococcal infection and rheumatic fever did so by demonstrating significant differences in the antibody levels of streptococcal antibodies between healthy controls and patients with rheumatic fever (5, 8, 9). The healthy controls were individuals from the same population or geographic area as the patients with rheumatic fever but who had no clinical evidence of recent group A streptococcal infection. Because the majority of these studies were carried out in areas with a low incidence of rheumatic fever (6, 11), the present study was conducted to determine if the streptococcal antibody titers of rheumatic fever patients and nonrheumatic individuals in populations with higher incidences of rheumatic fever demonstrate the same differences as those
seen in corresponding populations in areas of endemicity where the prevalence of rheumatic fever is low.

The data obtained in this study confirmed prior observations regarding the presence of significant differences in the levels of streptococcal antibodies between healthy individuals and patients with rheumatic fever in Florida, an area with a low prevalence of rheumatic fever (6). However, this difference did not hold when we compared the streptococcal antibody titers of nonrheumatic individuals to the titers of patients with rheumatic fever in Grenada, an area with a high incidence of...
rheumatic fever (13; P. K. Noah, R. Kopycinski, and B. Nelson, unpublished data; B. Nelson and G. F. Armstrong, personal communications). The reason for this discrepancy became apparent when the antibody titers of the nonrheumatic population when the antibody titers of the nonrheumatic population from Grenada and Florida were compared. This revealed that the streptococcal antibody titers in the nonrheumatic group from Grenada were significantly higher than those of the healthy individuals from Florida (Table 2).

The most likely explanation for this finding is that children in Grenada are subjected to a high frequency of group A streptococcal infections in the form of pharyngitis or impetigo. As in other tropical regions, impetigo is reported to be highly prevalent in Grenada (B. Nelson, personal communication). While this may account for the high levels of anti-DNase B in the nonrheumatic population in Grenada, the presence of ASO titers in the Grenada nonrheumatic population that are significantly higher than those of the healthy population in Florida favors the probability that streptococcal pharyngitis also contributed to the elevated streptococcal antibodies encountered in the nonrheumatic population from Grenada. This conclusion is based on previous observations that show that patients with impetigo most often show a brisk anti-DNase B response but a feeble ASO response (10).

It is of interest that, despite the significantly lower anti-DNase B levels in Grenadian rheumatic fever patients than in the local controls, a finding that reflects more recent streptococcal infections in the latter group, the anti-A-Cho levels were significantly higher in the rheumatic fever population. This is consistent with earlier reports (1, 8, 15) which showed that the anti-A-Cho levels remain elevated for several years in patients with persistent rheumatic mitral valve insufficiency but not in patients without cardiac sequelae or with transient carditis.

This finding suggests that in patients with mitral valve disease who do not have a history of acute rheumatic fever, the presence of an elevated anti-A-Cho titer in the presence of normal ASO and anti-DNase B titers would favor a rheumatic fever etiology as the cause of the mitral valve disease. In areas such as Grenada, where streptococcal infections are highly prevalent, one should approach the interpretation of an elevated anti-A-Cho titer with care and should utilize the finding of an elevated anti-A-Cho titer as evidence for a rheumatic fever etiology in a patient with isolated mitral valve insufficiency and no history of a preceding episode of acute rheumatic fever but only if the elevated anti-A-Cho titer is accompanied by normal ASO and/or anti-DNase B titers.

Other than a study done by Urdahl et al. (18), our review of the published literature failed to reveal studies that evaluated the levels of streptococcal antibodies in healthy individuals from populations with a high frequency of streptococcal infections and/or a high incidence of rheumatic fever. Urdahl and his coworkers determined the serum antistreptokinase titers in the sera of nonrheumatic adult aboriginals in Australia, a population with a very high incidence of streptococcal infection (18). They found that the geometric mean titer of this streptococcal antibody was almost 20 times higher than that in nonaboriginal adults. This study, together with data derived from the present study, indicate that the levels of the streptococcal antibodies in healthy populations can vary substantially, depending on the frequency of streptococcal infections in those populations.

Susceptibility to rheumatic fever in certain individuals has been ascribed to a number of factors. These include genetic determinants, for example, HLA's, and the presence of certain markers such as the B-cell alloantigens (reviewed in reference 2). One of the factors originally considered in rheumatic fever susceptibility is an innate state of immune hyperresponsiveness, particularly to streptococcal antigens (12, 14, 16, 17). While the high frequency of streptococcal infection in a population such as the one in Grenada may promote the expression of acute rheumatic fever, an alternate explanation to consider is that individuals in such an area develop or possess an innate immune hyperresponsiveness to streptococcal antigens. Whether the high rate of expression of rheumatic fever in certain populations is related to a high frequency of streptococcal infections per se or to that in combination with an innate state of hyperimmune responsiveness to streptococcal antigens remains to be determined.

A number of variables should be taken into consideration while interpreting the results of streptococcal antibody titers in the context of their relationship to the epidemiology of group A streptococcal infections or the role of such infections in the pathogenesis of rheumatic fever. The results of this study point to yet another variable, the need to consider the background frequency of streptococcal infections in certain populations when the streptococcal antibody titers of rheumatic fever and nonrheumatic populations are being compared. Differences in the distribution of the titers of antibodies between such populations will become apparent when the titers in nonrheumatic subjects from an area with a high incidence of rheumatic fever are compared to those of a similar population from an area with a low incidence of this disease. Had this comparison not

### Table 1. Geometric mean streptococcal antibody titers and significance of differences between the means for nonrheumatic individuals and patients with rheumatic fever in Grenada and Florida

<table>
<thead>
<tr>
<th>Antibody test</th>
<th>Population source</th>
<th>Geometric mean titer for indicated individuals</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonrheumatic</td>
<td>With rheumatic fever</td>
<td></td>
</tr>
<tr>
<td>Grenada</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASO</td>
<td>2.3</td>
<td>2.16</td>
<td>0.085</td>
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<tr>
<td>Anti-DNase B</td>
<td>2.68</td>
<td>2.5</td>
<td>0.029</td>
</tr>
<tr>
<td>Anti-A-Cho</td>
<td>3.06</td>
<td>3.17</td>
<td>0.047</td>
</tr>
<tr>
<td>Florida</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASO</td>
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<td>2.18</td>
<td>0.01</td>
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<td>Anti-DNase B</td>
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<td>2.57</td>
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<tr>
<td>Anti-A-Cho</td>
<td>2.71</td>
<td>3.07</td>
<td>&lt;0.001</td>
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</tbody>
</table>

### Table 2. Geometric mean streptococcal antibody titers and significance of differences between the means for nonrheumatic individuals from Grenada and nonrheumatic individuals from Florida

<table>
<thead>
<tr>
<th>Antibody test</th>
<th>Population source</th>
<th>Geometric mean titer for indicated individuals</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grenada</td>
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<td>Anti-DNase B</td>
<td>2.68</td>
<td>2.29</td>
<td>0.0004</td>
</tr>
<tr>
<td>Anti-A-Cho</td>
<td>3.06</td>
<td>2.7</td>
<td>0.0002</td>
</tr>
</tbody>
</table>
been done in the present study, it is not inconceivable that one might have concluded on the basis of the data obtained from Grenada that group A streptococcal infection does not play a role in the pathogenesis of rheumatic fever.

ACKNOWLEDGMENTS

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REFERENCES


