Type-Specific Opsonic Antibodies in Streptococcal Pyoderma

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Prospective studies of streptococcal pyoderma were carried out among black children enrolled in Project Headstart centers in Holmes County, Miss. Sera collected from 28 of these children in early October were tested for opsonic antibodies to one of two prevalent skin strains of group A streptococci isolated from them on one or more occasions over the preceding 3 months. The two streptococcal strains (A and B) belong to M-types previously unrecognized. Ten subjects (36%) had antibody to their homologous serotypes detectable by the indirect bactericidal test: this included 6 of 10 subjects infected with strain B but only 4 of 18 infected with strain A ($P < 0.05$). Of 17 children who had strains A or B isolated from skin lesions only, 12% developed type-specific antibodies (TSA) against the infecting serotype. In contrast, 11 subjects had these strains isolated from throat cultures (either with or without associated pyoderma), and 72% had detectable TSA ($P < 0.01$). There was no demonstrable relationship between the development of antibodies to streptococcal extracellular products or to non-type-specific cellular antigens and the development of TSA. These results demonstrate that type-specific immune responses do occur following infection with pyoderma streptococci. The frequency with which such antibodies develop is variable and appears related to a number of factors, including the immunologic properties of the infecting strain and the site of bacterial colonization. Pharyngeal carriage may represent an important mechanism for development of acquired immunity to skin strains of group A streptococci.

It has been 3 decades since the studies of Kuttner and Lenert (10) and of Rothbard (17) confirmed the appearance of type-specific opsonic antibodies in the serum of children convalescent from streptococcal pharyngitis. Over the ensuing years, studies by a number of investigators have done much to characterize the nature of the M-antibody response after pharyngeal infection. It is known, for example, that M-antibodies appear 4 to 8 weeks after throat infection, that they may persist for many years, that their development may be prevented by prompt, effective antibiotic therapy, that their frequency of development is greater in patients with prolonged convalescent pharyngeal carriage, and that there is no definite relationship between the development of streptolysin O antibodies and M-antibodies (8, 9, 12, 16, 18). In contrast, very little is known about the development of type-specific antibodies (TSA) following cutaneous streptococcal infection.

Potter and associates (15) detected TSA to a number of different pyoderma types in patients with acute glomerulonephritis and in well school children in Trinidad. The percentage of sera which were positive varied over time, and the opsonic power of many of the sera tested was weak. Bergner-Rabinowitz et al. (4) found TSA in the majority of patients convalescing from glomerulonephritis due to M-type 55 streptococci. Antibodies were detectable in 2 to 4 months after the disease and waned gradually over the ensuing 1 to 3 months.

No prospective data are available, so far as we are aware, relating to the development of type-specific opsonic antibody in uncomplicated streptococcal pyoderma. The studies reported here represent an attempt to provide at least partial answers to the many outstanding questions relating to type-specific immunity in cutaneous streptococcal infection.

MATERIALS AND METHODS

Plan of study. These investigations were carried out in Holmes County, Miss., a rural, agricultural area where streptococcal pyoderma is highly endemic. Subjects were indigent black children, ages 2 to 6, enrolled in Project Headstart, a government-supported preschool day-care program. Observations re-
ported here were made during the summer of 1970, at the peak of the pyoderma season, but during a period when the incidence of acute glomerulonephritis was low (14).

Three Project Headstart centers were selected for intensive surveillance. Each of the centers was visited weekly, and all children in attendance had the following studies: throat culture, examination for skin lesions, culture of at least one pyoderma lesion if present, and dipstick urinalysis (Hema-Combistix, Ames Co., Elkhart, Ind.) for hematuria and proteinuria. Microscopic examination of urine was performed if hematuria or greater than 1+ proteinuria was present. Antibiotic therapy was not administered to children with uncomplicated streptococcal pyoderma; control measures focused upon improving the level of personal hygiene in study subjects. Serum samples were obtained at the initiation (week of July 29, 1970) and periodically throughout the course of the study.

Serogrouping and M and T serotyping of group A isolates were performed by standard methods (13, 20). Complement-fixing antibodies to non-type-specific moieties of M-protein were measured by the method of Widdowson, Maxted, and Pinney (22). Further details as to the plan of study, bacteriologic and serologic procedures, and antibody responses to streptococcal extracellular products have been published elsewhere (5).

During the course of the study, over 4,000 patient-weeks of observation were performed on 444 different subjects. Attendance was not mandatory, however, and absences were frequent. Moreover, a large turnover of students occurred at the end of the Headstart year in August. Only a small group of approximately 75 subjects was observed frequently enough to provide a basis for detailed epidemiologic analysis.

**Measurement of type-specific antibodies.** Type-specific anti-M opsonic antibodies were assayed by the indirect bactericidal method of Lancefield (11) as modified by Stollerman et al. (19). This method measures the ability of test serum to inhibit the multiplication of M-rich group A streptococci during a period of rotation at 37°C in fresh human blood. The test mixture consisted of 0.3 ml of lightly heparinized blood, 0.1 ml of test or control serum, and 0.1 ml of bacterial inoculum. Two different bacterial inocula, consisting of dilutions of log-phase cultures of group A streptococci, were used in each test.

In most experiments, these inocula consisted of approximately 100 and 50 colony-forming units, respectively. Results were expressed as the bactericidal index (19), which represents the ratio of growth of the inoculum in control tubes containing normal serum to that in tubes containing test serum. For purposes of the analysis, tests were considered valid only if the bacterial inoculum multiplied 16-fold or more in control tubes during the 3-h incubation period. Bactericidal indices were graded semi-quantitatively as follows: 25 or less, negative; 26 to 50, trace positive; 51 to 100, 1+; 101 to 250, 2+; 251 to 500, 3+; > 500, 4+.

**RESULTS**

**Serosurvey.** Initial data concerning the distribution of TSA were derived from a serosurvey carried out among children from a single Headstart center. Sera obtained from these children at the end of the summer were used in the indirect bactericidal test against three group A streptococcal strains isolated from the same center during the preceding months. Serotypes of the three test strains were T-11 and T-6, neither of which were M-typable, and M-43. The prevalence of TSA to these three strains ranged from 13 to 27% (Table 1). Twenty-one of these subjects sera were also tested against a serotype (M-24) not found in Holmes County to date. None had evidence of opsonic activity.

**Prospective observations.** The second phase of this investigation was limited to those subjects who attended faithfully and who were observed and cultured on a regular basis. As test organisms, representatives of the two most prevalent T-serotypes were selected. Strain A typed as T-14, and strain B gave the complex T pattern, 3/13/B3264/28/56/9. Neither strain could be identified with M-antisera available in our own laboratory or at reference laboratories.

Both strains probably represent previously unrecognized serotypes. Specifically, strain A is not a representative of M-type 49 (W. R. Maxted, personal communication) and, despite its agglutination with T-56 antisera (6), strain B does not belong to M-type 56 Representatives of both strains were found that grew well in human blood and could thus be employed in bactericidal tests.

In this phase of the study, positive results were confirmed by repeated testing. Results of repetitive experiments were averaged and expressed semi-quantitatively as indicated above. Each serum sample was also tested to assure that it failed to inhibit the growth of a heterologous streptococcal strain of either M-24 or M-30.

Twenty-seven children met the criteria for inclusion, e.g.: (i) a minimum of 6 weekly observations during the 13-week period between June 29 and September 27, 1970; (ii) no more than four consecutive absences; (iii) at least one culture positive for either strain A or strain B; (iv) a serum sample obtained during the first week in October. (One child, with positive

<table>
<thead>
<tr>
<th>Table 1. Survey for type-specific antibody in a single Headstart center</th>
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<tbody>
<tr>
<td>Serotype of test organism</td>
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<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>T-6</td>
</tr>
<tr>
<td>T-11</td>
</tr>
<tr>
<td>M-43</td>
</tr>
<tr>
<td>M-24</td>
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</tbody>
</table>
cultures for both strain A and strain B, has been counted twice in subsequent calculations.)

The period of observation began in mid-summer, at which time pyoderma was already highly prevalent in the study population. Two patients had detectable antibody at the outset to strains with which they subsequently became colonized (Table 2). Subject number 1 had only a single throat culture positive for strain A in late July. Subject number 25, however, had multiple skin and throat cultures positive for strain B over a 6-week period despite the presence of bactericidal antibody.

For subjects 8, 20, 21, and 28, it was possible to ascertain the time between first culture positive for strain A or B and first documentation of TSA. These intervals were 3, 8, 11, and 10 weeks, respectively.

By early October, 10 (36%) of the children had demonstrable opsonic antibody to the strains which had infected them during the preceding summer. This included 4 of 18 subjects from whom strain A was isolated and 6 of 10 subjects from whom strain B was isolated ($P < 0.05$).

The incidence of streptococcal pyoderma dropped sharply in the fall. Although intensive surveillance continued throughout 1970, none of the 10 children with TSA had documented infections with strains A or B after mid-October. Follow-up bleedings obtained in January, 1974, were available from eight children who had had demonstrable bactericidal antibody in October. Only four of these eight subjects still had detectable TSA (Table 2).

The presence or absence of TSA in sera obtained during early October was analyzed in relation to the subjects’ infection experience over the preceding 3 months (Table 3). Seventeen subjects had strain A or B isolated from skin lesions only, and two (12%) developed opsonic antibody. In contrast, 11 subjects had these strains isolated from throat cultures (either with or without associated pyoderma), and eight (72%) had detectable antibody in the fall ($P < 0.01$, Fisher exact test). Thus, children with pharyngeal colonization were much more likely to develop TSA than were those with pyoderma alone.

Children were not questioned in detail concerning pharyngeal symptoms at the time throat cultures were obtained. Throats were examined, however, prior to culture. Five of the 11 subjects with positive throat cultures for strains A or B had pharyngeal erythema or tonsillopharyngeal exudate noted at least once on the day of a positive culture.

A number of sera were tested for the presence of complement-fixing antibodies to non-type-specific M-protein antigens. The titers were relatively low, ranging from 1:10 to 1:40. Such titers are similar to those seen in normal individuals and distinctly lower than those observed in patients with acute rheumatic fever (2, 22). There was no discernible relationship between development of antibodies to type-specific and non-type-specific M-antigens following streptococcal pyoderma.

Analysis of the relationship between type-specific M-antibodies and antibodies to streptococcal extracellular products was complicated by the fact that many children had multiple streptococcal serotypes isolated from skin and throat during the period of observation. No clear-cut relationship was observed, however, between changes in serum levels of M-antibodies and antibodies to extracellular enzymes.

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**Table 2. Results of indirect bactericidal tests in subjects developing type-specific antibody**

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Infecting strain</th>
<th>Bactericidal antibody*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (6/29/70)</td>
<td>6 (8/3)</td>
</tr>
<tr>
<td>A</td>
<td>++ + + + +</td>
<td>+ + + + + + + + + +</td>
</tr>
<tr>
<td>A</td>
<td>NT</td>
<td>+ + + + + + + + + + + +</td>
</tr>
<tr>
<td>A</td>
<td>+ + + + + + + + +</td>
<td>+ + + + + + + + + + + +</td>
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<tr>
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<td>+ + + + + + + + +</td>
<td>+ + + + + + + + + + + +</td>
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<tr>
<td>A</td>
<td>NT</td>
<td>+ + + + + + + + + + + +</td>
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<td>B</td>
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<td>B</td>
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</tbody>
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* Week of study (date in parentheses). NT, Not tested; tr, trace.

*This subject had a single skin lesion culture positive for strain B but did not develop bactericidal antibody to strain B.

* Negative in February and March, 1970.

* Negative in February, 1970.

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**Table 3. Relationship of site of streptococcal isolation to development of type-specific antibody**

<table>
<thead>
<tr>
<th>Infecting serotype</th>
<th>Skin lesion</th>
<th>Throat</th>
<th>Skin lesion plus throat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain A</td>
<td>1/12</td>
<td>2/2</td>
<td>1/4</td>
</tr>
<tr>
<td>Strain B</td>
<td>1/5</td>
<td>1/1</td>
<td>4/4</td>
</tr>
<tr>
<td>Total</td>
<td>2/17 (12%)</td>
<td>5/3</td>
<td>5/8 (63%)</td>
</tr>
</tbody>
</table>

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* Results shown as the number with TSA/number tested.
(antistreptolysin O, antihyaluronidase, anti-deoxyribonuclease B).

Considerable variability was observed in the patterns of development of TSA in relationship to the preceding infection, as illustrated by following examples: subject 19 (Fig. 1) had a skin lesion culture positive for strain B on week 4; throat cultures were repetitively positive for the same strain from weeks 4 to 7. She had been bled in February of 1970, at which time she lacked bactericidal antibody to strain B. Unfortunately, adequate serum was not available in July, 1970, after completion of other serologic studies, for bactericidal testing. However by October 1, she had developed TSA to strain B, which persisted at least through January, 1971.

Complement-fixation tests for non-type-specific M-antigens showed continuously low or undetectable titers. The anti-deoxyribonuclease B titer rose gradually between February and October.

Early in the study period, subject 21 (Fig. 2) had pyoderma and transient pharyngeal colonization associated with strain B. TSA was detectable in the fall but had waned by January. There were no significant elevations in serum titers of antibodies to the other cellular or extracellular antigens tested.

Subject 8 (Fig. 3) had pyoderma (strain A) for 4 consecutive weeks without demonstrable pharyngeal colonization. She had developed TSA by week 6 of the study period. Although she continued to have multiple positive skin and throat cultures during the fall, she never again had strain A infections once TSA appeared.

Subject 11 (Fig. 4) had isolations of strain A from impetiginous lesions on 2 consecutive weeks in August but did not develop detectable TSA. His complement-fixation titers for non-type-specific antigens remained low. Initial serum titers of anti-hyaluronidase and anti-deoxyribonuclease B were elevated, whereas the antistreptolysin O titer was low. This pattern suggests recent cutaneous streptococcal infection (5). The anti-hyaluronidase and anti-deoxyribonuclease B titers declined over the ensuing 6 months.

**DISCUSSION**

Type-specific antibody to prevalent pyoderma strains of group A streptococci are present in the sera of many Holmes County children. The frequency with which such antibodies
develop following exposure is variable and is related, at least in part, to the site of bacterial colonization.

Children with throat carriage are more likely to develop type-specific immunity than those whose infection is limited to the skin. This finding is open to at least two interpretations. Throat carriage may simply serve as a marker for those patients with more intense and persistent cutaneous infection. Indeed, there was a tendency in our study for throat carriage to be detected more often in patients with repeatedly positive skin lesion cultures. However, the data were not conclusive on this point.

Alternatively, asymptomatic pharyngeal carriage may represent a major mechanism for the development of immunity to pyoderma strains. This would be analogous to the situation in streptococcal pharyngitis, in which a relationship has been demonstrated between convalescent pharyngeal carriage and the development of TSA (18).

Our results suggest that strain B may be more immunogenic than strain A. Interestingly, we have been successful in preparing bactericidal serum to strain B in the laboratory but have been unable to elicit opsonins to strain A in immunized rabbits.

The low frequency of development of TSA after untreated infection with strain A may relate to a number of factors, such as relatively weak strain virulence or the indolent nature of the lesions. No detailed observations were made of features, such as degree of regional adenitis or magnitude of leucocytosis, which might have provided an additional insight into the severity of the immunologic challenge represented by the pyoderma infections studied.

In this regard, it should be noted that most studies of type-specific immunity to pharyngeal streptococcal infection have been made in epidemiologic settings wherein severe clinical disease was the rule. Mild, sporadic cases of streptococcal sore throat would represent a closer analogy to the pyoderma lesions seen in our subjects. Studies in such mild cases of pharyngeal disease might conceivably reveal similarly low rates of development of type-specific immunity.

Our data are not adequate to document in detail the time course of development and decay of TSA. Where an assessment could be made, an interval of 8 to 10 weeks usually elapsed between colonization and development of humoral immunity. Half the subjects whose sera were positive on week 14 had become negative when retested on week 30. These findings are in general agreement with those of Bergner-Rabinowitz et al. (4) (vide supra).

The marked decrease in pyoderma incidence during the fall and winter months of our study clouded any possible conclusions which might be drawn regarding the efficacy of TSA in prevention of reinfection. Observations during the Viet Nam conflict have provided intriguing epidemiologic evidence of resistance to streptococcal impetigo among black U.S. combat soldiers (1). Whether such resistance is mediated by humoral or cellular factors remains unknown.

Results reported here must be interpreted in the light of the limitations of the test procedure. The bactericidal test works in a precise and clear-cut fashion when one is using human and rabbit serum of high opsonic potency and well-characterized laboratory strains of known virulence. There are significant problems, however, in testing sera of weak opsonic power against wild strains of pyoderma serotypes which demonstrate considerable day-to-day variability in growth characteristics, dissociate rapidly in the laboratory, and frequently will not regain virulence upon mouse passage. We have attempted to avoid these pitfalls by adopting rather conservative criteria of positivity and insisting upon clearly reproducible tests under conditions of excellent bacterial growth. Thus, our results may underestimate somewhat the prevalence of TSA among the subjects tested.

It will be difficult to settle the issues surrounding type-specific immunity in uncomplicated skin infection using current methodology. More readily standardized tests such as complement fixation or hemagglutination run the risk of confusion due to non-type-specific moieties (3). Attempts to develop M-protein subunits freed of non-type-specific M components (7, 21), in addition to their importance in vaccine development, may also lead to development of more precise tests for measuring type-specific immunity. Such tests would clearly be of great value.

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LITERATURE CITED


