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Research Article

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the Immune Response to Group A Streptococci

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ABSTRACT The immune response after strepto-
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INTRODUCTION

Previous studies have suggested that antistreptolysin O
(ASO) levels in children with group A beta hemolytic
streptococcal pyoderma are not significantly higher than

ASO levels in a control group without pyoderma (1–3).
These may be spurious observations, resulting from
comparison of single titers rather than changes in titer.
On the other hand, if true, they raise a serious practical
question about the traditional reliance upon ASO as a
general serological indicator of streptococcal infection.
More importantly, from a conceptual point of view, the
finding may necessitate some fundamental revision in our
thinking about immunologic responses to streptococcal in-
fections (4), depending upon which of the following sev-
eral possible explanations is responsible for this observa-
tion: (a) a difference in the quantity of the antigenic
mass or in the duration of the antigenic stimulus as com-
pared with streptococcal infections of the upper respira-
tory tract; (b) bacterial or host factors resulting in a
broad unresponsiveness to all group A streptococcal an-
tigens when infection occurs in the skin; (c) a specific
deficiency in the capacity to produce streptolysin O on the
part of streptococci which commonly infect the skin;
or (d) peculiarities of the skin site per se which may
influence the local production of, or the host response
to, streptolysin O.

Events occurring at the Red Lake Indian Reservation
in Minnesota during the summer and autumn of 1966
made possible an attempt to establish the validity of
these previous observations by studying antibody re-
sponses in paired sera from children who were care-
fully followed in the interval between bleedings and to
differentiate among these several possible interpreta-
tions. These events also afforded an opportunity to com-
pare ASO responses with responses of two other
streptococcal antibodies, antideoxyribonuclease B (anti–
DNAse B) and anti–nicotinamide adenine dinucleoti-
dase (anti-NADase), in relationship to the site of infec-
tion and also to compare titers of patients who developed
acute nephritis with those who did not.

Studies of the epidemiology of streptococcal infections
and their nonsuppurative sequelae, which had been

underway for over 2 yr at Red Lake (2), were enlarged at the beginning of July 1966 to include a group of normal children attending an Operation Headstart Program at the reservation. During the 1st wk of July the Type 49 streptococcus, which had been responsible for a major epidemic of nephritis at Red Lake in 1953 but had apparently been absent from the reservation in recent years, reappeared and spread through the population, resulting in a second outbreak of acute nephritis (5). This nephritogenic type is very commonly associated with pyoderma (6) but may also be associated with upper respiratory tract infection (7, 8), a versatility of some importance for the purpose of this study.

The present paper describes observations on the immune response to streptococcal infection during this outbreak. The clinical, bacteriological, histopathological, and epidemiological features of this outbreak have been or will be described in other communications (5, 8-11).

METHODS

Weekly visits were made to examine children attending the Headstart Program, and cultures of the anterior nares (nose), posterior pharynx (throat), and infected skin lesions (when present) were obtained and processed as previously described (5, 9). All strains of beta hemolytic streptococci were serologically grouped (12) and group A strains were further classified by both the precipitation technique for M protein (13) and the agglutination technique for T protein (14).

Sera were obtained at the initial visit during the 1st wk of July and again during the 3rd wk of September. The serum was separated from the cells and stored in sterile glass tubes at 4°C. Paired sera from each child were analyzed simultaneously for three different streptococcal antibodies: antistreptolysin O (ASO); antideoxyribonuclease B (anti-DNAse B); and anti-nicotinamide adenine dinucleotidase (anti-NADase) by methods previously described (15-17). These antibody determinations are generally reproducible within 0.1 log (one dilution) and therefore a 0.2 log increase between the initial and final bleedings from any one individual is considered a significant antibody rise for that individual.

In order for a child to be considered in this analysis, a serum from both the beginning and the end of the study must have been available. He also must have been seen regularly during the study period (July, August, and the 1st wk of September) and must not have gone longer than 3 consecutive wk without being cultured. Attendance at the Headstart Program was not compulsory and varied from week to week. For this reason many of the 100 children initially enrolled either were not seen frequently enough to be included in this analysis or did not have sera from both the initial and final bleedings available.

The 49 children who met the critera for inclusion in this analysis were divided into categories depending on their experience with group A streptococci during the study period. Streptococcal pyoderma was defined as any lesion of the skin which yielded group A beta hemolytic streptococci on culture. In no instances were less than 10 colonies (1+) recovered from an infected skin lesion. Usually there were 50 colonies or more (3+) of beta hemolytic streptococci present on the plate, and often the beta hemolytic

streptococcus was the predominant or only organism present. The definition of streptococcal upper respiratory tract (URT) infection proved to be more difficult. Since parents were often not available to report symptoms and since the conditions of the study did not always allow repeated examinations for pharyngeal exudate, it was necessary to rely primarily on bacteriologic findings. Data obtained from this population have suggested that children with 1+ cultures obtained from the nose or throat are unlikely to show a significant rise in antibody titer to any of the three streptococcal antigens, while children with 2+ or greater throat cultures were very likely (86%) to exhibit a rise in titer (8). For this reason a 1+ nose or throat culture during the study period was not interpreted as reflecting URT infection. A child, then, had to have at least a 2+ nose or throat culture to be considered as having streptococcal infection.

Production of antigen in vitro. 44 strains of group A streptococci were tested for the production of streptolysin O, NADase, and DNAse in vitro. Lyophilized strains were grown overnight in Todd Hewitt broth containing 5% sheep red blood corpuscles (RBC). 0.5 ml of the culture was inoculated into 10 ml of dialysate medium (18) incubated for 8 hr at 37°C, followed by inoculation of 0.3 ml of the 8 hr growth into another 10 ml of dialysate broth which was incubated at 37°C for 18 hr. The cultures were centrifuged and aliquots of the supernatant were assayed for enzyme or hemolysin activity.

Streptolysin O was assayed by incubating 0.1 ml of the supernate dilutions with 0.2 ml of 5% rabbit RBC and 0.2 ml of 1.0 M cysteine in 0.2 M phosphate buffered saline, pH 6.6. Hemolytic activity was defined as the reciprocal of the dilution that shows complete hemolysis in the above system, after incubation at 37°C for 45 min. NADase production was assayed and the enzyme unit was defined as described previously (17). Total DNAse activity was assayed by the microtechnique method (16) and the unit of enzyme defined as the reciprocal of the dilution showing approximately 50% digestion of the DNA substrate. No attempt was made to differentiate the various DNAse isozymes produced (19).

RESULTS

There were 57 children attending the Operation Headstart Program from whom serum was obtained in both July and September; however eight of these children were not seen frequently enough to satisfy the criteria for inclusion in this analysis.[1]

This analysis is based upon data from the remaining 49 children. The average number of visits for each of these 49 children (during the 10 wk study period) was 6.7 visits (range 4-9 visits). The age range of the children studied was 3-6 yr (mean age was 4.5 yr). There were 24 males and 25 females. These children were divided into three groups depending upon their

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[1] From the data available six of the eight children excluded would have been classified as no infection (group I). Three of the six showed no rise in any of the three antibodies studied, and three demonstrated a rise in at least one antibody. Analysis of the data including the information from the excluded children does not appear to change significantly the conclusions reached in this investigation.

TABLE I

Geometric Mean Titers* and Changes in Titer as Related to the Site of Infection

	Group‡	Antistreptolysin O			Anti–DNAse B			Anti-NADase		
		July	Sept.	Net change§	July	Sept.	Net change§	July	Sept.	Net change§
I	No infection (9)	1.92(83)	1.90(79)	−0.02	2.03(112)	2.01(102)	−0.02	1.39(25)	1.39(25)	0.00
II	Skin infection (26)	2.13(135)	2.33(214)	+0.20	2.38(240)	2.67(468)	+0.29	2.07(118)	2.23(170)	+0.16
	A. Skin infection only (11)	2.25(178)	2.32(209)	+0.07	2.39(246)	2.60(400)	+0.21	2.15(141)	2.19(155)	+0.04
	B. Skin infection + URT infection‖ (15)	2.05(112)	2.33(214)	+0.28	2.37(234)	2.72(525)	+0.35	2.01(102)	2.25(178)	+0.24
III	URT infection only (14)	2.25(178)	2.41(257)	+0.16	2.32(209)	2.57(372)	+0.25	2.00(100)	2.21(162)	+0.21
Total	(49)	2.13(135)	2.27(186)	+0.14	2.30(200)	2.52(331)	+0.22	1.94(85)	2.07(118)	+0.14

* Geometric mean titers are expressed both as logarithm (base 10) and as arithmetic expression of the logarithms (in parentheses).

‡ Figures in parentheses refer to numbers of children in each group.

§ Refers to Δ log of geometric mean titers (see text).

‖ URT infection = upper respiratory tract infection (see definition in Methods section).

experience with group A beta hemolytic streptococci during the study period.

Group I (no infection group) consists of nine children with no clinical or bacteriological evidence of group A streptococcal infection, either of the upper respiratory tract (URT) or of the skin. Group II (skin infection group) consists of 26 children from whom group A streptococci were recovered from skin lesions. Of these 26, 11 had only streptococcal skin infection without streptococcal URT infection (group II A) and the remaining 15 had streptococcal infection of both the skin and URT (group II B). Group III (URT infection only group) is composed of 14 children who met the criteria for streptococcal infection of the upper respiratory tract but had no evidence of streptococcal pyoderma.

The geometric means of the antibody titers for the July and September bleedings for each of the various groups, as well as the total group, are given in Table I. The changes in the geometric means of the antibody titers from the July to the September bleedings are shown for each group and are expressed as the Δ log (the difference in the logs of the geometric means).

The fact that there was no rise in the geometric mean titer for any of the three antibodies for the group of children in group I is consistent with the bacteriologic data obtained from these children.[2] In contrast, rises in geometric mean titers ranging from +0.16 log to +0.29 log were observed for all three antibodies in both of the

[2] As shown in Fig. 1, increases in titer for at least one of the three antibodies studied suggest that three children in this group may have had an undetected infection probably preceding or perhaps during the period of observation. Analysis of the data of this study after elimination of these three individuals produced differences which were even more significant than those presented but was considered invalid because of the probable introduction of bias.

major groups with bacteriologic evidence for infection (groups II and III). These data confirm the presence of streptococcal infection in children in these two groups during the study period.

No significant differences in antibody responses between groups II and III were found with respect to either ASO or anti-NADase, but responses for both of these antibodies appeared to be relatively feeble when infection was confined to the skin (group II A). Substantial rises in anti-DNAse B titers occurred regardless of the site of infection, and there was little difference in the response obtained when the infection was limited to the skin (group II A) as compared to that which occurred when infection was restricted to the upper respiratory tract (group III). For all three antibodies, the greatest rises as expressed by the difference in logs of the geometric means were recorded when skin infection was accompanied by colonization or infection of the upper respiratory tract (group II B)

These observations were confirmed when the data were examined with respect to the frequency of a significant antibody response. If a two dilution (0.2 log) increment or greater is considered a reproducible and therefore significant rise in an individual patient, only 3 of 11 children (27%) with infection confined to the skin showed such a rise in ASO titer as compared with 2 of 9 children (22%) with no bacteriological evidence of infection at any site. In contrast 8 of 14 children (57%) with upper respiratory tract infection and 8 of 15 children (53%) with evidence of infection at both sites showed a significant antistreptolysin O response. Similar tendencies in frequency of antibody response with respect to the site of infection were noted for anti-NADase. 2 of the 9 children (22%) with no bacteriological evidence of infection showed a response with this antibody and only 3 of 11 (27%) when infection

was restricted to the skin. 6 of 14 children (43%) with evidence of infection limited to the upper respiratory tract and 8 of 15 (53%) with evidence of infection at both sites developed an anti-NADase response. On the other hand, the majority of children with bacteriological evidence of streptococcal infection showed an anti–DNAse B response regardless of the site of infection. Only 1 of 9 children (11%) without bacteriological evidence of infection exhibited a response for this antibody, whereas 7 of 14 children (50%) with only upper respiratory tract infection, 6 of 11 children (56%) with only skin infection, and 9 of 15 children (60%) with evidence of infection at both sites showed a significant rise in anti–DNAse B titer.

These apparent differences were scrutinized further by examining the distributions of the three antibody responses for the individual patients in the various groups. These distributions are presented as scattergrams in Figs. 1 and 2. Values on individuals are expressed as the difference between the logarithm (base 10) of the titer in July and the logarithm of the titer in September. Values for each group were calculated as the difference between the logarithm of the geometric mean titer of the entire group in July and the logarithm of the geometric mean titer in September. These are designated as Δ log in the text and tables and are indicated by the horizontal lines in the figures.

Fig. 1 compares the distribution of titer changes in children with no bacteriologic evidence of infection (group I) with those in whom evidence of streptococcal infection was confined to the upper respiratory tract (group III). Despite considerable overlap, the distribution of titers appears to be different in these two groups of children, although the difference is less apparent with the anti-NADase titers due to the wider scatter of individual responses to this antigen. The Δ log for the mean antibody titers in July and September was significantly different in these two groups in the case of the antistreptolysin O ($P < 0.02$), of borderline significance in the case of the anti–DNAse B ($0.06 > P > 0.05$), and not significant in the case of the anti-NADase titers ($P > 0.1$).

Fig. 2 compares the distribution of titer changes in children with no bacteriologic evidence of infection at any site (group I) with those in children with skin infection (group II). The latter group is divided into children whose infection was limited to the skin (group II A) and those with evidence of streptococcal infection at both sites (group II B). As shown in this figure, when infection was restricted to the skin (group II A), the distribution of antistreptolysin O titers was not markedly different from that of patients without infection (group I); and there was no significant difference in the Δ log values for the change in antistreptolysin O
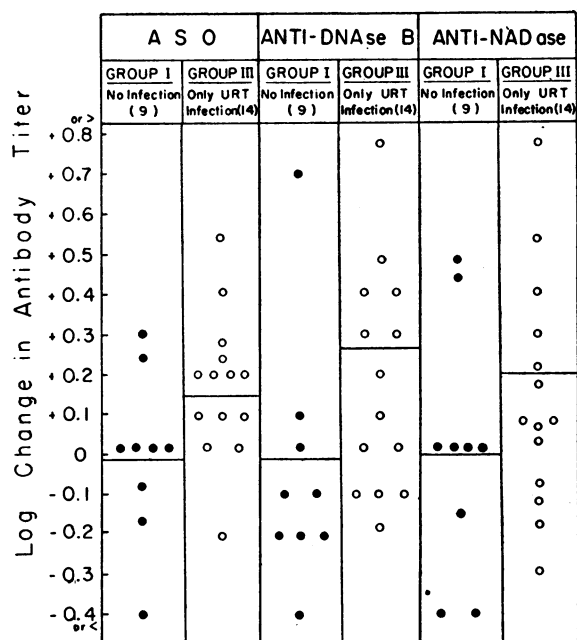

FIGURE 1 Scattergram for three different antibodies comparing the changes in titers in children with no bacteriologic evidence of streptococcal infection (group I) and children with evidence of only upper respiratory tract infection (group III). The horizontal line represents the geometric mean change in titer for each group.

titers for these two groups ($P > 0.1$). However, in the group with evidence of infection in the upper respiratory tract as well as the skin (group II B), the distribution of antistreptolysin O titers is distinctly higher than that for children without infection (group I) and the Δ log values for the change in geometric mean titers are significantly different for these two groups ($P < 0.03$).

In the middle panel of Fig. 2, the distributions of anti–DNAse B titers appear to be higher in children with skin infection than in children without bacteriological evidence of infection, regardless of whether the skin infection is accompanied by evidence of infection of the upper respiratory tract. The Δ log value is higher in children with infection at both sites (group II B) than in children with infection restricted to the skin (group II A), and each is significantly higher than the group without infection ($P = < 0.04$ and < 0.005 respectively).

As previously seen for infections confined to the upper respiratory tract (Fig. 1), the wide scatter of anti-NADase responses in children with infections limited to the skin (group II A, Fig. 2) makes interpretation difficult, but there seems to be no difference in distribution of titer changes or in Δ log value from children in group I ($P > 0.1$). The distribution of anti-

Figure 2 — chart with axis "Log Change in Antibody Titer" ranging from +0.8 (or >) to −0.4 (or <), and column headers: A S O, Anti-DNAse B, Anti-NADase; each with GROUP I (No Infection (9)) and GROUP II A Skin only (11), B Skin + URT (15).
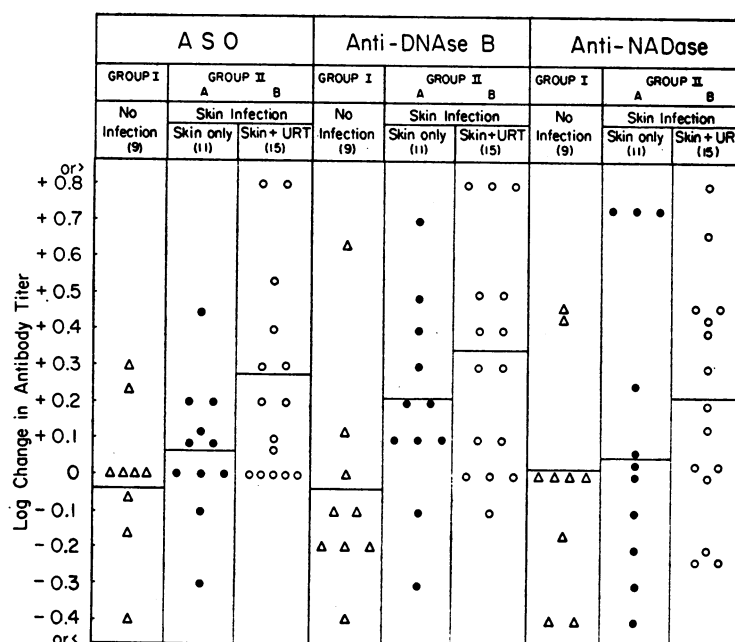
FIGURE 2 Scattergram for three different antibodies comparing the changes in titers in children with no bacteriologic evidence of streptococcal infection (group I), children with streptococcal infection limited to the skin (group II A), and children with streptococcal infection of both the skin and the upper respiratory tract (group II B). The geometric mean change in titer for each group is represented by the horizontal line.

NADase titer changes in patients with evidence of infection at both sites (group II B, Fig. 2) tended to be higher than that of children in group I, but the Δ log values were not statistically different in these two groups ($P = > 0.2$).

Since dissimilarities in strains infecting different sites might explain the irregularities in immune response observed in this study, an analysis was made of the serotypes associated with infection at different sites (Table II).

TABLE II

Types of Group A Streptococci Associated with Infection at Different Sites*

Group	Total with Type 49	Type 49 only	Type 49 + other group A	Other group A only	Distribution of other types						
					11	12	22	25	31	33	NT‡
II A Skin infection only (11)	7 (64%)	7	0	4	1	0	0	0	1	0	2§
II B Skin infection + URT infection (15)	10 (67%)	5	5	5‖	3	0	0	1	1	2	4
III URT infection only (14)	9 (64%)	6	3	4	0	1	1	0	1	0	5
Total (40)	26 (65%)	18	8	13	4	1	1	1	3	2	11

T-agglutination patterns on these strains were as follows: 9 (three children), 3/13/B3264 (two children), 27/28 (two children), 5/27/44 or 5/11/12/14/27/44 (two children), 8/25/Imp. 19 and not classifiable (one child each).
* See footnotes for Table I.
‡ NT = Not typable.
§ One of these two had a strain of T-agglutination pattern 5/11/12/14/27/44 which precipitated with both Type 11 and Type 49 antisera and is considered not typable in this tabulation.
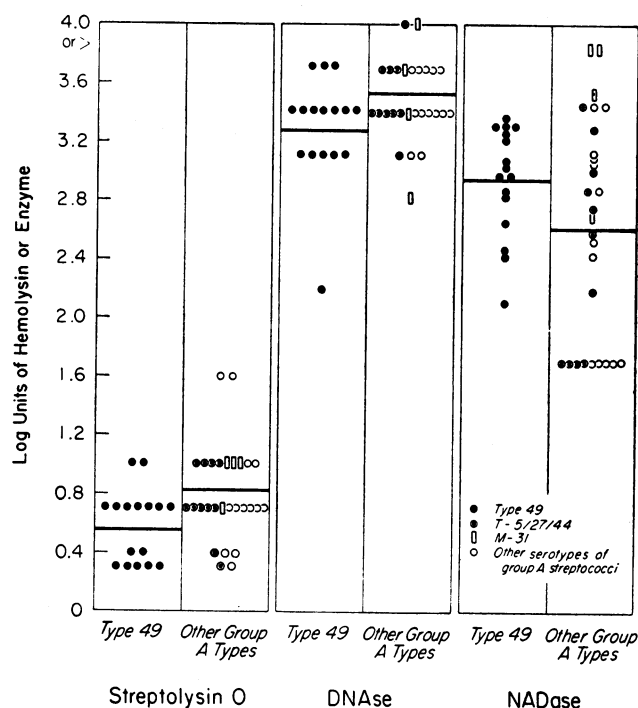‖ One of these five had strains of two different serological types.

FIGURE 3 Scattergram comparing in vitro antigen production by Type 49 and other serotypes of group A streptococci. The geometric means are shown by horizontal lines.

Of the 11 patients with infection confined to the skin, 7 (64%) showed the epidemic strain (Type 49). Of the 15 patients with infection at both sites, 10 (67%) had the epidemic strain. Of the 14 patients with infection limited to the upper respiratory tract, 9 (64%) showed the epidemic strain. This even distribution of Type 49 streptococci by site of infection indicates that there were no contrarieties in this respect which could have contributed to the differences in antibody responses noted in the various groups. Similarly, in all groups there was a rather wide scatter of other types, suggesting that their distribution was unlikely to have played a significant role in the antibody response recorded.

The possibility that strains of different types encountered in this study might show deficiencies or marked deviations in their capacity to produce certain extracellular antigens was examined in vitro. Yields of such products by 44 strains of group A streptococci recovered during this study were compared under identical conditions of growth.

Fig. 3 is a scattergram showing the units of hemolysin and enzymes produced by these strains and compares the geometric means for the Type 49 strains with those for other group A streptococci. In the latter group results are also differentiated by appropriate symbols for strains of two serological classifications (T-5/27/44 and M-31) which were of special interest.

A low yield of streptolysin O was obtained with all strains. This made it difficult to detect differences in production of this hemolysin, but no marked differences were apparent. NADase was produced in generally higher quantities by Type 49 strains than by other serotypes (except Type 31), whereas the opposite was true of DNAse production. Strains of M-type 31 tended to produce relatively high levels of all three of the biologically active factors tested. In some instances in vitro production was as variable among strains of the same serological classification (e.g., Type 49 and those with a T-agglutination pattern of 5/27/44) as among strains of different types. Within the same type, there was no apparent correlation between the amount of hemolysin or enzymes produced in vitro and the site from which the organism was isolated.

A modifying influence of antibiotic therapy on the antibody response to streptococcal infection has been reported in some studies (20, 21). In order to examine the possible effects of antibiotics on the antibody responses of children in this analysis, the medical records of all children were reviewed with respect to the kind of treatment administered.[3]

[3] The medical care of the population living on the reservation was the responsibility of the U. S. Public Health Service Medical Officers of the Red Lake Hospital, and the investigators involved in this study served only as sources of reference to the clinic in the case of obviously ill children.
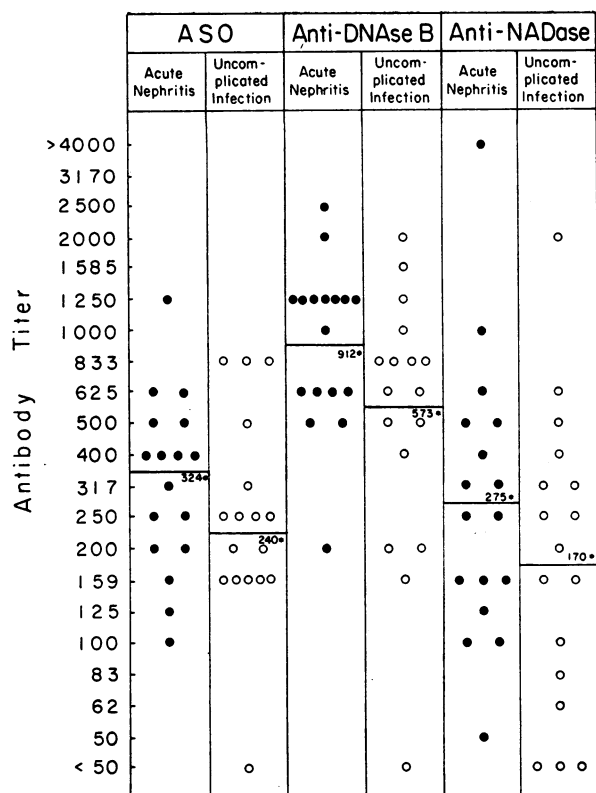
	ASO		Anti-DNAse B		Anti-NADase	
Antibody Titer	Acute Nephritis	Uncomplicated Infection	Acute Nephritis	Uncomplicated Infection	Acute Nephritis	Uncomplicated Infection
>4000					●	
3170						
2500			●			
2000			●	○		○
1585				○		
1250	●		●●●●●●●	○		
1000			●	○	●	
833		○ ○ ○	912●	○○ ○○		
625	● ●		●●●●	○ ○	●	○
500	● ●	○	● ●	○ 573● ○	● ●	○
400	●●●●			○	●	○
317	● 324●	○			● ●● 275●	○ ○
250	● ●	○○○○			● ●	○ ○
200	● ●	○ 240● ○	●	○ ○		○ 170●
159	●	○○○○○		○	● ● ●	○ ○
125	●				●	
100	●				● ●	○
83						○
62						○
50					●	
<50		○		○		○ ○ ○

FIGURE 4 Scattergram comparing the titers of three different antibodies in children with acute nephritis with those in matched controls covalescent from uncomplicated streptococcal infections (see text). The geometric mean titers are shown by the horizontal lines and by the figures with an asterisk (*).

Of the 49 children included in the analysis, 13 (26%) received some form of antibiotic therapy during the study period. Usually this was intramuscular benzathine penicillin or oral penicillin. 2 of the 13 were children in group I (no infection) and the remaining 11 were from groups II and III. Thus, of the 40 children with evidence of streptococcal infection by culture, 11 received antibiotics at some time during the study period and 29 did not. Of the 11 who were treated, 8 (72%) showed a significant rise in at least one of the three antibodies studied. Of the 29 who did not receive any antibiotics during the study period, 20 (68%) showed a significant rise in titer of at least one of the three antibodies studied. These data suggest that the antibiotic therapy used probably did not have a very large influence on the frequency of antibody response in the children in this study.

Acute glomerulonephritis developed in a total of 25 children during the 1966 outbreak at Red Lake (5, 9), but only a few were studied prospectively with bleedings and other observations before and after development of nephritis (8, 9). Therefore, because of small numbers, it was not feasible to compare rises in antibody titer for children in whom nephritis occurred with those in whom no complications developed. But it was possible to compare titers at onset of acute nephritis with those of comparable children whose infection was not followed by renal disease.

Uncomplicated controls were chosen from the population in the prospective study in the following manner. Each control was age-matched to a specific nephritis patient. In addition, the control must have had streptococcal infection of a similar site for a similar period of time (±10 days) before the bleeding was obtained. The controls were also matched to the patients with nephritis according to whether they were infected by the epidemic type (Type 49) or another type of group A streptococcus. Using these criteria, suitable controls were found for 17 of the 25 patients with acute nephritis.

The data from these two groups of children are shown in Fig. 4. In each of the three panels the titers of the 17 patients at onset of nephritis are compared with those of the matched controls with uncomplicated streptococcal infection. The horizontal lines represent the geometric means of the titers. In the case of all three antibodies (ASO, anti–DNAse B, and anti-NADase), the geometric mean for the patients with nephritis was higher than the corresponding geometric mean titer for children with uncomplicated infection. The difference in the geometric means for the ASO was greater than one dilution increment (0.13 log); and for both the anti–DNAse B and anti-NADase, the difference was greater than two dilution increments (0.21 log in each case). While these data show a tendency of antibody titers to be higher among patients whose infection is followed by renal complications, the differences in titers were not significantly different by statistical analysis ($P > 0.1$).

DISCUSSION

The nine children who did not develop bacteriological evidence of streptococcal infection during the study had much lower initial titers for all three antibodies than children who developed bacteriological evidence of infection. This is an interesting but rather puzzling finding. Several of them did have serological evidence of infection, which is easier to document when the initial titer is low (22). A possible explanation for the low initial titers in this group could be related to the age of the patients. It is known that younger children, particularly infants, are likely to have low antibody titers to streptococcal antigens (23), probably due to the infrequency of streptococcal infection in infancy but possibly to some age-dependent influence which modifies

antibody response. In the present study, the mean age for children without bacteriologic evidence of infection (group I) was 3.8 yr as compared to a mean age of 4.7 yr for children with streptococcal skin infection (group II), and a mean age of 4.6 yr for children with streptococcal URT infection (group III). However, these differences are not statistically significant. It is difficult, therefore, to invoke age as an adequate explanation for the low initial titers. Perhaps the undefined factors which prevented this group of children from developing streptococcal infection before the onset of this study continued to operate during the course of this investigation.

The relatively feeble ASO response after streptococcal infections of the skin is confirmed in the studies reported here. Earlier studies (1–3), based primarily on comparison of single titers rather than changes in titers, were subject to the usual hazards of single titer evaluations, including the problems of selection of well-matched controls, the rather wide variations in the distribution of normal titers, and consequently in the definition of upper limits of normal for a particular population group (24). In addition, a special problem exists with interpretation of antibody titers, particularly single titers, obtained from patients with skin infections. Since it is often difficult to document accurately the onset of pyoderma, it may be impossible to insure that bleedings are obtained at the appropriate time for maximal opportunity to demonstrate an antibody elevation or response. Although this optimal interval is not known for skin infections, it is reassuring that these studies with paired bleedings support previous observations, including the recent preliminary report of Dillon and Reeves (3) based on single titers from an impressively large number of patients.

This report provides additional information which may aid in determining the most logical explanation for the failure of patients with streptococcal pyoderma to show the expected ASO response. Although a difference in antigenic mass or in duration of antigenic stimulus might theoretically affect the antibody response, the variation in the extent and the persistence of skin lesions is considerable in these patients and provides little to support this hypothesis. The disparity between the ASO and anti-NADase responses on the one hand and the anti–DNAse B responses on the other in patients with streptococcal infection limited to the skin is also against this possibility. Since rheumatic fever is generally believed to be an immunological complication of streptococcal infection, the failure of rheumatic fever to appear after streptococcal infection of the skin (25) makes the possibility of a generalized immunological unresponsiveness after infection at this site an unusually attractive hypothesis. However, the frequent

and sizable anti–DNAse B rises observed in this study exclude this possibility.

The present studies do not support the conclusion that the poor ASO response in patients with infection of the skin is due to a specific deficiency in the capacity of the epidemic or other infecting strains to produce streptolysin O, especially in view of the adequate ASO response demonstrated after infection of the upper respiratory tract with strains which did not differ markedly from those infecting the skin either in type distribution or in in vitro production of this hemolysin. Although production of streptolysin O in the test tube was rather meager for almost all strains examined in this study, it was of the same general order of magnitude as that previously reported for representative strains of many different types (26). Production of streptolysin O in laboratory cultures is regularly less impressive than that of certain other extracellular products, notably the deoxyribonucleases (27), despite the generally good ASO responses observed in most studies of streptococcal throat infections (24). Discrepancies in correlation between in vitro production of streptolysin O and ASO response have been noted by others (28).

A possible explanation for this modified host response to streptolysin O after skin infection is suggested by previous studies of Hewitt and Todd (29) who showed that cholesterol is a potent and specific inhibitor of some of the biological activities of streptolysin O. The possibility that lipids, such as cholesterol, which are present in abundance in the skin may modify the antigenicity of streptolysin O in the same way that certain lipids modify both its hemolytic capacity and its cardiotoxic effects deserves consideration. Although there is no direct evidence to show that cholesterol or other skin lipids have the ability to modify the antigenicity of streptolysin O, some difficulty in obtaining responses to intradermal injection of streptolysin O in experimental animals has been noted (30). Studies with experimental animals are in progress in an attempt to clarify these observations further.

Antibody levels to streptococcal NADase have not been examined in previous reports of streptococcal skin infections. The present study suggests that responses to this antigen in general tend to parallel those to streptolysin O. A common biological property shared by these two antigens is the dependence of their production on sulfhydryl concentration (31, 32) which may perhaps vary at different sites of infection. Previous studies have indicated that the in vitro production of streptococcal NADase is related to the serotype of the strain; Type 12 strains were exceptionally good and Type 49 strains moderately good producers of this enzyme (33). The present study confirms the tendency of Type 49 strains

to produce more of this enzyme in vitro than most of the other types tested, but there was no evidence in this study that Type 49 infections produce higher antibody responses to this antigen than infections with other group A streptococci.

The anti–DNAse B titer appears to be the antibody of choice in patients with streptococcal skin infections or renal complications of these infections. Earlier studies have emphasized the usefulness of this and other streptococcal antibodies as secondary tests in supporting the diagnosis of rheumatic fever and acute nephritis in patients presenting with low or borderline ASO titers (34, 35, 36). In this study the anti–DNAse B titer seemed to be a better antibody test than the other two, regardless of the site of infection. The present report and other reports on the use of anti–DNAse B and antihyaluronidase determinations in streptococcal skin infections (2, 3, 37) represent the first serious challenge of the antistreptolysin O titer as the best single test for general use in the serological detection of streptococcal infection.

Although it has been previously documented that patients with acute poststreptococcal glomerulonephritis usually have higher titers against streptolysin O, deoxyribonuclease B, and diphosphopyridine nucleotidase than do controls with no evidence of recent streptococcal infection (34), there are few data available comparing antibody titers of patients with acute nephritis with those of patients with uncomplicated streptococcal infection. Most of the available information relates to nephritis after pharyngeal (38) rather than cutaneous infection (3). The observations presented here suggest that children with nephritis tend to develop higher titers than similarly infected children without renal disease, but the differences were not statistically significant.

Since it is generally agreed that rheumatic fever does not follow streptococcal infection of the skin (25) and since it has been shown that under certain conditions acute nephritis more commonly follows skin infection than URT infection (8), variations in antibody or other host responses related to the site of infection may provide important clues to the differences in pathogenesis of these two sequelae of streptococcal infection (25).

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