IMMUNOLOGICAL SIGNIFICANCE OF ANTISTREPTOLYSIN O (ASL) IN STREPTOCOCCAL INFECTIONS

III. THE ANTIBODY RESPONSE IN SCARLET FEVER PATIENTS

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The study of antibodies in man following streptococcal infections has indicated the presence of some antibodies which play a certain role of immunological importance in the infection process. The type-specific antibody to the M antigen of Group A streptococci has been generally considered to be responsible for the antibacterial immunity, and the antibody to the erythrogenic toxin has been assumed to be capable of preventing the occurrence of scarlet fever. However, the immunological significance of antibodies to various extracellular products, such as antistreptolysin O (ASL), antistreptokinase, antihyaluronidase, etc., in human infections has not been well defined, although numerous previous studies have contributed a great deal to the knowledge of the antibody response.

In the first report of the present series of investigations (Kusama et al., 1962), the fundamental aspects of the distribution of ASL titers in various age groups were presented, and it was shown that the experience of streptococcal infections in the past has divided a population into the positive and negative groups with respect to the ASL response. With these informations in mind, investigations were undertaken to disclose such mechanics of ASL response in scarlet fever patients as the relationship of the amplitude of the response to the initial titer and to the serologic types of infecting organisms. Furthermore, a special attention was paid to the difference in the magnitude of the antibody response following a newly established infection between those who belonged to the positive and negative groups at the time of infection.
MATERIALS AND METHODS

1. Patients examined: Among the patients with scarlet fever admitted to the Metropolitan Toshima Hospital from March, 1958 to June, 1959, and to the Metropolitan Toyotama Hospital from November, 1958 to June, 1959, 241 who were from 3 to 19 years old (92% of them were from 4 to 13 years old) were selected for the present study. Since the early initiation of antibiotic therapy tends to suppress the antibody response, most of the patients did not receive antibiotic therapy during the 1st week of illness, but received during the 2nd or 3rd week or even later. Sera were obtained from patients at the time of admission to the hospital and at weekly intervals thereafter until released. Throat (and sometimes nasal) swabs from patients were cultured on horse blood agar plates every or every other days after admission. From the duration of the persistence of Group A streptococci in the upper respiratory tract, the patients were divided into Group A (33 patients: Group A streptococci were not recovered from their throats after the 5th day of illness, owing to early antibiotic therapy), Group B (102 patients: Group A streptococci had continuously been recovered until the 5th to 10th day of illness before eradicated by antibiotic administration) and Group C (106 patients: Group A streptococci had continuously been recovered beyond the 11th day of illness).

2. Identification of streptococcal strains: Representative streptococcal strains isolated from patients were grouped and typed according to the method previously described (Swift et al., 1943).

3. ASL titration: ASL titers of sera were determined according to the method previously described by one of the present authors (Kusama, 1958). All the sera were heated at 56°C for 30 minutes, and stored at -20°C until examined.

4. Statistical analyses: Comparison of ASL distributions in two groups was made by (1) chi-square test for the percentages of the negative and positive groups, and (2) analysis of variance for the distribution of the positive group, as in the first report. A test for significance was made at the 5% level.

RESULTS

Distribution of ASL Titers in Sera from Scarlet Fever Patients Drawn During the First Week of Illness (Distribution of Initial Titers)

The distribution of ASL titers in sera from scarlet fever patients (their age ranged from 3 to 19 years) drawn during the 1st week of illness is shown in Fig. 1. Since the rise in ASL titer is not generally observed during this period of the disease, the

![Fig. 1. Frequency distribution of initial ASL titers in 241 scarlet fever patients (Groups A, B and C) (from 3 to 33 years of age).]
titers of these sera can be regarded as those at the time of infection, and they will be referred to the initial titers. The shaded area in the figure indicates the number of sera from those who were from 6 to 12 years old (corresponding to primary school age). They happened to consist of about 30 children in each age group, except the 8-year age group (41 children) and the 12-year age group (12 children). The figure clearly demonstrates that this population can also be divided into two heterogeneous groups, positive and negative, according to the interpretation in the previous report (Kusama et al., 1962). A question now arises as to whether those who are on a certain level of ASL titer are liable to contract scarlet fever. It may, therefore, be interesting to examine this distribution with reference to that in a normal population of the same age group. For this purpose, the percentage distribution of ASL in primary school children presented in the previous report (Kusama, 1962) is compared with that in the patient children of school age, as shown in Fig. 2. The percentage of the negative group and the mean value of ASL titers in the positive group are not significantly different in both groups. The log standard deviation of the positive group in normal and patient children are 0.314 and 0.290, respectively. Thus, it is strongly suggested that those who will be infected and develop scarlet fever do not belong to any special position in the ASL distribution, and that ASL does not confer any protection against the manifestation of scarlet fever following the streptococcal infection.

Fig. 2. Comparison of the percentage distributions of ASL titers in primary school children* and scarlet fever patients in the corresponding age groups at the time of hospitalization. The arrow indicates the geometric mean titer of the positive group.

* Cited from (Kusama et al., 1962a).
Relationship of Maximum ASL Titer During the Course of Scarlet Fever and Initial Titer

The highest titer of serum specimens collected at weekly intervals was considered as the maximum attained during the clinical course, usually being the titer of a 3rd or 4th week’s serum. In Fig. 3, the maximum titers of those who were classified as

**GROUP A**

![Graph of Group A relationship between initial and maximum ASL titers.](image)

Fig. 3. Relationship of the initial and maximum ASL titers in scarlet fever patients (Group A).

Group A are plotted against their initial titers. If an increase in titer more than two-fold is considered to be significant, only one third showed significant antibody rises, and the remaining two thirds showed unchanged titers or dubious elevations, being within the diagonal belt in the figure. The same relationship was then examined in Groups B and C where patients harbored Group A streptococci for longer periods of time. As shown in Fig. 4, 154 out of 208 (74%) exhibited significant antibody rises. There were 41 patients who possessed titers below 20 units/cc at the time of hospital admission (the initially negative group), but all of them, except 2, entered into the positive group during the course of illness. There is a distinct tendency observed that the distribution of the maximum titers becomes smaller as the initial titer goes up, suggesting that a certain level of the mean response attainable after infection exists somewhere around 400 units/cc. When one has a high initial titer near to the attainable mean, he has little or no capacity to respond further to the antigenic stimulus of repeated infections, and host and agent factors related to the antibody response are likely to show little influence. On the other hand, when one has a low initial titer, he has a greater capacity to respond to the antigenic stimulus,
Fig. 4. Relationship of the initial and maximum ASL titers in scarlet fever patients (Groups B and C).

and individual variations in host and agent factors can influence to a greater extent the magnitude of the antibody response. This mechanism will be more clearly demonstrated in the next chapter.

Serological types of Group A streptococci isolated most frequently from patients during the period of observation were types 6, 3, 1 and 12, in order of frequency. In Figs. 3-7, patients from whom type 3 organisms were isolated at the time of hospitalization are indicated with open circles.

Relationship of Log Fold-Increase and Log Initial Titer

In the preceding chapter, examinations were made as to how maximum titers are related to initial titers. In this chapter, log fold-increase (log maximum titer—log initial titer) is analyzed instead of the maximum titer. In Fig. 5, the relation between log fold-increase and log initial titer in Group A is illustrated, but it is difficult to see any definite relation, owing to a small number of patients as well as to slight
GROUP A

Fig. 5. Relationship of log initial ASL titer (x) and log fold-increase in titer (Y) in scarlet fever patients (Group A).

GROUP B

Fig. 6. Relationship of log initial ASL titer (x) and log fold-increase in titer (Y) in scarlet fever patients (Group B).

\[ Y = 1.97 - 0.79x \]

\[ r = -0.66 \]
fold-increases. However, there can be seen a definite negative correlation in Group B (Fig. 6) and Group C (Fig. 7), and log fold-increase is dependent on log initial titer. The departure from linearity is found statistically non-significant at the 5% level.

Smaller increases may be expected in patients with high initial titers, whereas greater increases may be found in those with low initial titers. The slope of the regression

| GROUP C
<table>
<thead>
<tr>
<th>Initial ASL titer (x)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Units/cc</td>
</tr>
<tr>
<td>13</td>
</tr>
<tr>
<td>Log</td>
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**Fig. 7.** Relationship of log initial ASL titer (x) and log fold-increase in titer (Y) in scarlet fever patients (Group C).

The departure from linearity is found statistically non-significant at the 5% level.

When patients were infected with type 3 organisms, their fold-increases tended to be higher than those infected with other types of Group A streptococci, as indicated with open circles in Figs. 6 and 7. This is in accordance with several reports of previous workers (Rantz et al., 1948; Iimura, 1958; Habu, 1960).
Comparison of the Antibody Responses in the Initially Negative and Positive Groups

Five patients in Group B and 13 patients in Group C belonged to the negative group at the time of hospitalization with respect to their distribution of ASL titers, as shown in Figs. 6 and 7 (those who had titers less than 13 units/cc are omitted from the diagrams). Although their number is rather small, their position in the diagrams seems to be well fitted to the regression line drawn for the overall patients. It is, therefore, suggested that the mechanics of ASL response may be the same, whether the infection is primary or repeated in patients from 3 to 19 years old.

This view is also supported by the results of comparison of the percentage distributions of maximum ASL titers in initially negative and positive groups (Fig. 8). The mean maximum titers are almost the same in both groups, although the titers in the initially negative group tended to show a wide and irregular distribution, partly owing to a relatively small number of patients included. There were still 2 patients who remained in the negative group during the course of illness.

DISCUSSION

For the consideration of the immunological significance of ASL, the analysis of various host and agent factors which may influence the magnitude of the response and the understanding of fundamental mechanics of the response is of profound
importance. Scarlet fever patients, which can be easily observed from a few days after infection to the convalescent stage under the hospitalized condition, were employed for the present study, and various antibiotic regimens were applied in order to obtain patients receiving various extents of the antigenic stimulus.

Distribution of initial titers among patients of school age was found not to be different from that observed in normal school children, and scarlet fever was suggested to occur, regardless of the possession of ASL. In the preceding report of this series of investigations (Noguchi et al., 1962), ASL was assumed to have no protective effect against the streptococcal infection. The present findings further suggest that ASL is not responsible for protecting the development of scarlet fever. Although various attempts to relate the magnitude of the response or the initial titer to the nature and severity of clinical manifestations have been unsuccessful, ASL might influence in some way the development of certain clinical symptoms. This possibility may be supported by the cardiac toxicity of streptolysin O for the isolated frog heart (Bernheimer and Cantoni, 1945) and rabbit heart (Kellner et al., 1956), and cardiovascular injuries induced by the intravenous injection of activated streptolysin O into rabbits (Halbert et al., 1961).

Investigations on the relationship of the maximum ASL titer or log fold-increase to the initial titer revealed the theoretical presence of a certain level of the mean response which can be attained after infection. Those who have already maintained high titers through infections in the past are likely to respond very little to another antigenic stimulus. However, those who have had low titers are capable of showing good responses to reach the attainable mean response. In the latter cases, various agent factors, such as the ability of infecting streptococci to produce streptolysin O, the number of organisms harboring in the throat, the duration of the persistence of streptococci, etc. may influence the magnitude of the antibody response. Type 3 streptococci, many of which are usually good streptolysin O producers, have been shown to have the ability of inducing a good ASL response. One of the authors (Habu, 1960) investigated the ability of streptolysin O production in Group A streptococci isolated from scarlet fever patients admitted to the Metropolitan Toyotama Hospital, including a part of patients subjected to the present study. He found a good correlation between the fold-increase in the ASL titer and the ability of infecting streptococcus to produce streptolysin O in vitro, when antibiotic therapy was suspended during the first 10 days or more. When patients were infected not only with type 3 but also with good streptolysin O producers of other types, they tended to show large fold-increases.

As to the host factors which may determine the extent of the ASL response, the level of ASL titer at the time of infection seems to be of primary importance. There were no significant differences in the mean maximum titer and the time necessary for the maximum response (as far as examined with sera collected at weekly intervals) between those who were initially negative and positive. Moreover, the same relationship of log fold-increase and log initial titer may be applied to both groups. Thus, the mechanics of antibody production seems to be similar in primary and repeated infections, at least in the age groups studied (3–19 years). Repeated infections are shown here again to have little or no boostering effect, as revealed in the previous report (Kusama et al., 1962). However, the mechanics responding to the primary infection during the first or second year of life may be different from those investigated
in the present study, as discussed previously (Kusama et al., 1962).

SUMMARY

Factors in the host and infectious agent related to the ASL response have been analyzed by observing 241 patients with scarlet fever from the onset of the disease to the complete recovery. The patients were divided into three groups according to the length of the persistence of Group A streptococci in their throats.

1. Distribution of ASL titers in patients of school age at the time of hospitalization did not differ significantly from that in a normal school population. It is suggested that the possession of ASL does not influence the development of scarlet fever.

2. The lower the initial titers of patients, the more variable their maximum titers. A certain level of the mean response attainable after infection was suggested to exist theoretically somewhere around 400 units/cc.

3. Log fold-increase was found to be dependent upon log initial titer. The slope of the regression line tended to increase as the infecting streptococci persisted longer, and reached nearly -1.0 in the group which harbored streptococci for more than 11 days after the onset of the disease. Type 3 infections were associated with unusually high ASL responses.

4. There were no significant differences in the mean maximum titer and the relationship of log fold-increase and log initial titer between those who belonged to the negative and positive groups at the time of hospitalization. Thus, the same mechanics of the ASL response seem to be involved in the primary and repeated infections in patients over 3 years of age. No boosting effect of repeated infections on the magnitude of the ASL response was observed.

REFERENCES


