IMMUNOLOGICAL SIGNIFICANCE OF ANTISTREPTOLYSIN O (ASL) IN STREPTOCOCCAL INFECTIONS

IV. THE ANTIBODY DECLINE IN SCARLET FEVER CONVALESCENTS

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ASL once developed in man after a streptococcal infection tends to decline gradually with the lapse of time. However, he may be infected again when exposed to another type of Group A streptococcus different from the type of the previous infection, since various antibodies to extra- and intracellular components of streptococci, except the type-specific antibody, are not responsible for the antibacterial immunity. Although the reinfection with the same type of Group A streptococcus has been generally considered to be very unusual (Wannamaker, 1954; Lancefield, 1959), there are many types in Group A streptococci and prevalent types in a community have been shown to change from time to time (Schwentker *et al.*, 1943; Coburn, 1949; Wannamaker, 1954; Fukumi and Kusama, 1958; Noguchi *et al.*, 1962). Thus, one may undergo repeated infections many times during his life, eventually resulting in wax and wane of the ASL amplitude.

In the preceding report (Kusama *et al.*, 1962a), the mechanisms of the ASL response were investigated in scarlet fever patients from the onset of the disease to the stage of the maximum response. In the present studies, patients were examined at appropriate intervals after released from the hospital until one year after the onset of the disease. Some of them were shown to be reinfected, and efforts were made to envisage the dynamics of fluctuation of ASL titers and how ASL is being maintained in a population.

MATERIALS AND METHODS

1. *Persons examined*: The 241 scarlet fever patients studied in the preceding report (Kusama *et al.*, 1962a), were asked to visit the hospital at 3 months' intervals until one year

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after the onset of the disease. One hundred and twenty persons who came to the hospital after 9–13 months were subjected to the present study, although some of them did not attend regularly. Upon their visit to the hospital, they received usual clinical and bacteriological examinations, and their blood specimens were collected. Their throat swabs were cultured on horse blood agar plates.

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

3. *ASL titration*: The method reported by one of the present authors (Kusama, 1958) was employed for titrating ASL. All the sera were heated at 56°C for 30 minutes, and kept at –20°C until examined.

4. *Statistical analysis*: Comparison of ASL distributions in two or more positive groups was made by analysis of variance, and a test for significance was made at the 5% level.

RESULTS

Change in the Frequency Distribution of ASL Titers During One Year Period After the Onset of Scarlet Fever

Although the initial schedule was to examine convalescents at 3 months' intervals, their irregular attendance has inevitably made us to classify the time of observation as at the 3rd–4th month, at the 5th–7th month and at the 9th–13th month after the onset of scarlet fever. Among 120 persons who were so co-operative to visit the hospital until the end of one year, 26 persons and 21 persons were absent at the 3rd–4th month and at the 5th–7th month, respectively. Changes in the frequency distribution of their ASL titers are illustrated in Fig. 1. The shaded area represents the number of sera from those who were assumed to have been reinfected with other type or group of streptococci than that in the preceding infection after released from the hospital. The criterion for the possible occurrence of reinfection is either the recovery of streptococci (mostly Group A, sometimes Group C or G) from throat cultures or the ASL titer at one time which is elevated from the titer at the preceding time by log increment of more than 0.2. Some comments may be necessary as to the reason for adopting the latter criterion. It is primarily intended to recognize as accurately as possible those who had had no reinfection during the study period, by excluding persons who had any indication for possible reinfection from the study group. In the preceding report (Kusama *et al.*, 1962a), an increase in titer more than two-fold (log increment of 0.3) was considered to be significant, and 26% failed to show significant antibody rises in spite of the persistence of Group A streptococci in their throat for a certain period. When the detection of those who had any doubt for infection is necessary, more severe criterion should be used. Moreover, ASL titers of the persons under study were gradually decreasing, if there were no reinfections, and the titer at one time would be less than that found at the preceding time of observation. Thus, log increment of 0.2 is considered to be appropriate for assessing a possible reinfection. Sixty-nine out of 94, 66 out of 99 and 61 out of 120 were supposed to have escaped from infection at the 3rd–4th month, the 5th–7th month and the 9th–13th month, respectively. The geometric mean titer of persons without reinfection at each study time is indicated in the figure as well as that of initial titers of those who were in the positive group (cf. Kusama *et al.*, 1962a) at the onset and that of titers at the 4th week. The mean titer considerably elevated at the 4th week of the disease decreased somewhat remarkably at the 3rd–4th month, and then decreased more slowly to reach the approximately same level after one year as that of the initially positive group at the time of hospitalization.

■ Those who were supposed to have been reinfected with streptococci after released from the hospital.

* Number of those who were supposed to have had no reinfection.

↓ The geometric mean titer of persons without reinfection. In the distribution of titers at the time of hospitalization, the mean titer of those who belonged to the positive group is indicated.

Fig. 1. Change in the frequency distribution of ASL titers during one year period after the onset of scarlet fever.

Almost all of the initially negative group were transferred into the positive group at the 4th week and they did not come back to the negative group again during one year period of observation, even in those who were supposed to have had no reinfection. This is an additional evidence supporting the adequacy of demarcating a population into the negative and positive groups between 20 and 25 units/cc of ASL.

Change in the Frequency Distribution of ASL Titers in the Initially Negative and Positive Groups

Sixty-one persons who were supposed to have been free from reinfection during one year period were divided into the initially negative and positive groups. Changes in the frequency distribution of ASL titers in both groups are illustrated in Fig. 2. Although the number of the initially negative group is rather small, an approximately

the same process of the antibody decline is envisioned in both groups. The titer at the 4th week in the initially negative group was definitely lower than that in the initially positive group, and it influenced the antibody levels in later periods. However, it is entirely due to a small number of the study group, since no significant difference has been reported in the mean maximum titers of both groups, if an appropriately large number of patients were examined (Kusama *et al.*, 1962a).

Fig. 2. Change in the frequency distribution of ASL titers during one year period in those who were supposed to have had no reinfection after released from the hospital.

*Relationship of Log Initial Titer and Log Fold-Change in Titer
at the 4th Week or at the 9th—13th Months*

In 58 patients out of 61 who were supposed to have been free from reinfection during one year period (the remaining 3 who possessed initial titers less than 13 units/cc were omitted), changes in the relationship between log fold-change (log titer at a specified time of observation—log initial titer) and log initial titer were investigated. As shown in Fig. 3, the relationship at the 4th week of the disease is almost similar to that between log maximum fold-increase and log initial titer, presented in the preceding paper (Kusama *et al.*, 1962a). Patients dealt with herein are a mixture of Groups A, B and C (classified according to the length of the persistence of Group A streptococci in the throat; see Materials and Methods in the preceding paper (Kusama *et al.*, 1962a)), and those who are in Group A with the shortest persistence of streptococci tend to show less intense responses. Log fold-change is positive (the change in titer is an increase) in almost all patients, and the point of intersection between the regression line and the horizontal line, $Y=0$, corresponds to the initial titer of 400 units/cc, which is the mean response attainable as discussed previously (Kusama *et al.*, 1962a). In other words, those who have already reached that level would not show theoretically any antibody rises, when they undergo another infection.

Fig. 3. Relationship of log initial ASL titer (x) and log fold-change in titer in the 4th week of scarlet fever (Y).

However, owing to the varying length of streptococcal persistence, the slope of the regression line in Fig. 3 is less than -1.0 . It should be -1.0 in Group C, as shown previously. Therefore, the regression line intersects the ordinate at $Y=1.2$, where the titer is antilog of 2.3 ($1.1+1.2$), that is 200 units/cc. Accordingly, a theoretical level of the titers at the 4th week in this particular group would be between 200 and 400 units/cc.

With the lapse of 9-13 months, the relationship turns out to be very interesting, as shown in Fig. 4. The regression line intersects the horizontal line, $Y=0$, at the initial titer of about 100 units/cc, and intersects the ordinate at $Y=0.7$, where the titer is antilog of 1.8 ($1.1+0.7$), 63 units/cc. The situation is quite the same as revealed in the preceding paragraph, and ASL titers of those who had had a prolonged contact with streptolysin O would go down to somewhere around 100 units/cc. It is evidently shown that those who had high initial titers tend to show negative log fold-changes, and those with low initial titer tend to exhibit positive fold-changes. Thus, the existence of a certain level of stabilized titer is strongly suggested, regardless of the initial titer. Those who had higher initial titers tend to show negative log fold-change (decrease in titer), and those who had lower initial titers are likely to show positive

Fig. 4. Relationship of log initial ASL titer (x) and log fold-change in titer 9-13 months after the onset of scarlet fever (Y).

Fig. 5. Relationship of log ASL titer in the 4th week of scarlet fever (x) and log fold-decrease in titer 9-13 months after the onset of the disease (Y).

log fold-changes (increase in titer) after one year. Initial titers of the formers were so high that the patients showed only slight responses during the clinical course, and later their titers decreased below their initial titers.

*Relationship of Log Titer at the 4th Week and Log Fold-Decrease
in Titer at the 9th—13th Months*

As shown in Fig. 5, a positive correlation can be seen between log titer at the 4th week of scarlet fever and log fold-decrease (log titer at the 4th week—log titer at the 9th—13th months) in 58 persons who were studied in the preceding chapter. Those with higher titers at the 4th week tend to show larger fold-decreases, and a tendency to converge to a certain level of ASL titer during 9–13 months is evidently observed here again.

If our theory of the existence of the maximum mean response attainable and the stabilized level to be settled is applied to the present situation, titers at the 4th week should be theoretically on a vertical line crossing the abscissa at 400 units/cc (2.6 on log scale), and titers after one year should converge at the point ($Y=0.6$, $x=2.6$) (corresponding to a titer of 100 units/cc; $2.6-0.6=2.0$) which is indicated with a circle in the figure. As shown in the preceding chapter, however, a theoretical level of the titers at the 4th week in this particular group of persons would be between two vertical lines passing $x=2.6$ and 2.3 , as indicated in Fig. 5. Those having 200 units/cc of ASL at the 4th week would converge at the point, $Y=0.3$ ($2.3-2.0$) and $x=2.3$. Thus, a theoretical position of the study group would be within an elliptical orbit illustrated in the figure. However, being influenced by those with titers less than 100 units/cc at the 4th week, the slope of the regression line for overall points turns out to be smaller than the inclination of the theoretical ellipse. Those who belonged to the negative group at the time of hospitalization are indicated with open circles, and there seems to be no significant difference in the distribution pattern in the diagram between the initially positive and negative groups.

DISCUSSION

The present report deals with the maintenance of ASL, which was once developed or elevated during the clinical course of scarlet fever, in a group of persons who had passed a later period of one year without streptococcal reinfections. At the convalescent stage, ASL titers were elevated to approach the theoretical mean response attainable (400 units/cc). Thus, those who had high initial titers showed slight increases at the 4th week, but their titers were apt to decrease greatly in a later period, and became below the initial titers after one year. On the other hand, great increases were expected during the clinical course in those who had low initial titers, and their titers were still above the initial level after one year. The ASL titers once developed or elevated through the experience of scarlet fever were thus converged to a stabilized level. Following repeated infections, the ASL titer may be amplified from time to time, but, sooner or later, it will be settled down to this level. There is no apparent boosting effect of a repeated infection. This is in accordance with previous findings (Kusama *et al.*, 1962b) that the geometric mean titer of the positive group in normal populations from 5 years to adult does not change with advancing age.

Almost all of those who belonged to the negative group at the onset of scarlet fever entered into the positive group during the clinical course, and did not come back

again to the negative group during a period of one year, even in those who were supposed to have been free from reinfection. A concept was presented in the previous report (1962b) that a population can be divided into two groups, negative and positive, with respect to the distribution of ASL titers, indicating negative and positive histories of streptococcal infection in the past. The results of the present study seem to support the adequacy of this concept.

In the preceding report (Kusama *et al.*, 1962a), no significant differences were observed in the process of antibody production between the initially positive and negative groups. As far as studied in the present investigations, the process of antibody decline also does not seem to be different in both groups. This eventually means that the mechanisms involved in the antibody response and decline may be the same whether one undergoes a primary or secondary infection, when he has reached a certain age.

SUMMARY

1. In scarlet fever patients who had escaped from reinfection with a different type or group of streptococcus after released from the hospital, the geometric mean titer elevated considerably at the 4th week of the disease, decreased gradually thereafter and reached approximately the same level after one year as that of the positive group at the onset of the disease.

2. Almost all of the initially negative group were transferred into the positive group at the 4th week and they did not come back again to the negative group during a period of one year, even if they did not undergo reinfection.

3. As far as studied, no significant difference in the process of antibody decline was found between the initially positive and negative groups.

4. With the lapse of one year, log fold-change (log titer at the 9th–13th months – log initial titer) was found to be dependent upon log initial titer. Those who had high initial titers tended to stabilize to titers below the initial levels, and those with low initial titers tended to be settled to titers higher than the initial levels. Thus, a certain level of ASL titer was suggested to exist to which any initial titers were likely to converge during one year.

5. A positive correlation was observed between log titer at the 4th week and log fold-decrease in titer after one year (log titer at the 4th week – log titer at the 9th–13th months).

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