

DYNAMICS OF THE IMMUNOLOGIC RESPONSE OF
Macaca rhesus INFECTED WITH L-FORMS OF
HEMOLYTIC STREPTOCOCCUS

N. V. Chumachenko, G. Ya. Kagan,
Zh. I. Shmitt-Slomska, E. I. Koptelova,
I. V. Rakovskaya, Yu. V. Vul'fovich,
and S. A. Goncharova

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Acute and subacute infection of *Macaca rhesus* with L-forms of hemolytic streptococcus and its bacterial form, followed by penicillin therapy, lead to the appearance of antibodies to the L-form in the animals' serum which persist for a long time. The increase in titers of antibodies against L-forms during infection with hemolytic streptococci may serve to reflect the possible induction of L-forms in vivo under the influence of penicillin.

A model of experimental tonsillitis in monkeys (*Macaca rhesus*), infected with L-forms of group A streptococcus, was developed previously. Seedings of organ homogenates at various times after infection showed that the L-forms are excreted extremely rarely [1-4]. Nevertheless, the pathological response evoked by infection with L-forms was distinguished by its prolonged course.

It was therefore decided to investigate whether this protracted infectious process is accompanied by the onset of an immunologic response. The possibility of antibody formation, in response to infection with L-forms of hemolytic streptococcus, has been demonstrated in principle by a few investigations [5-7]. Antibodies were demonstrated by means of the agglutination, gel-diffusion and complement-fixation tests. Recently, a passive hemagglutination test (PHT) has been described [8] for demonstrating antibodies against L-forms of *Proteus mirabilis*, *Escherichia coli*, and *Streptococcus haemolyticus*. Observations by the present writers have shown that this test is specific, highly sensitive, and capable of detecting even very small amounts of antibodies against L-forms of hemolytic streptococcus.

EXPERIMENTAL METHOD

Two series of experiments were carried out (1968, 1970). In series I, sera obtained from monkeys at various times after infection with L-forms and intact forms of group A hemolytic streptococcus of types 49 and 19 (GL-8) were tested; infection of some monkeys was subacute and of the others acute.

In series I, with both subacute and acute infection (1968), the monkeys were divided into three groups: 1) receiving L-forms of streptococci; 2) receiving L-forms heated to 60°C for 1 h; 3) receiving strains of intact streptococci followed by penicillin therapy (for an account of the method of infecting the monkeys and for a description of the strains and experimental groups, see [1-3]).

In series II, sera obtained from monkeys at various times after repeated infection in 1970 with L-forms of group A hemolytic streptococcus were tested [4]. The presence of antibodies in the sera of the infected monkeys was determined by the PHT. A supernatant from a suspension of homologous strains of L-forms sonicated for 20 min in a type MSE disintegrator (100 W, 18 kHz) was used as the antigen.

N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. International Children's Center, Paris. (Presented by Academician of the Academy of Medical Sciences of the USSR V. D. Timakov.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 77, No. 3, pp. 89-92, March, 1974. Original article submitted April 12, 1973.

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TABLE 1. Dynamics of Antibody Titers in Sera of *M. rhesus* after Subacute Infection with Group A Hemolytic Streptococci and Their L-Forms

Material administered	Time of obtaining sera (in days)	No. of sera	Antibody titers (in log ₂)				
			under 3,3	3,3—5,3	6,3—8,3	9,3—11,3	12,3 and over
L-forms of hemolytic streptococcus	0	1	1	0	0	0	0
	5—7	2	1	1	0	0	0
	10—14	1	0	0	1	0	0
	20—21	3	0	0	2	1	0
	27—28	2	0	0	1	1	0
Heated L-forms of hemolytic streptococcus	0	1	1	0	0	0	0
	5—7	1	1	0	0	0	0
	10—14	1	0	0	1	0	0
	20—21	1	0	0	1	0	0
	27—28	1	0	0	0	1	0
Group A hemolytic streptococci + penicillin	0	1	1	0	0	0	0
	5—7	5	3	2	0	0	0
	10—14	6	0	2	3	1	0
	20—21	7	0	0	4	2	1
	28—28	1	0	0	0	0	1

Note. Infection was repeated 4 times at intervals of 5-7 days. Serum was obtained on the day of infection and 7 days after the last infection.

TABLE 2. Dynamics of Antibody Titers in Sera of *M. rhesus* after Acute Infection with Group A Hemolytic Streptococci and Their L-Forms

Material administered	Time of obtaining sera (in days)	No. of sera	Antibody titers (in log ₂)				
			under 3,3	3,3—5,3	6,3—8,3	9,3—11,3	12,3 and over
L-forms of hemolytic streptococcus	0	5	3	2	0	0	0
	1	4	3	1	0	0	0
	2	3	0	0	2	1	0
	3	7	0	1	5	1	0
	4—7	5	0	0	0	4	1
Heated L-forms of hemolytic streptococcus	0	1	0	0	1	0	0
	1	1	1	0	0	0	0
	2	2	0	0	2	0	0
	3	2	0	0	2	0	0
	4—7	1	0	0	1	0	0
Group A hemolytic streptococci + penicillin	0	4	4	0	0	0	0
	1	—	—	—	—	—	—
	2	—	—	—	—	—	—
	3	—	—	—	—	—	—
	4—7	3	0	0	1	2	0

Note. Infection carried out 4 days in succession. Serum was obtained on the day of infection.

EXPERIMENTAL RESULTS

Antibodies against L-forms of streptococci were absent in the sera obtained before infection. Consequently, the increase in antibody titers after infection was evidence of an immune response evoked by an antigen of the L-forms.

Analysis of the results in Table 1 shows that subacute infection with L-forms of streptococcus causes the appearance of antibodies with maximal titers ($\log_2 = 13.2$) in the period from the 10th to the 28th day. Antibodies also were found in a monkey infected with a heated culture of L-forms, but in a comparatively low titer. Heating under these conditions evidently did not completely abolish the antigenicity of the injected L-culture.

In monkeys infected with the bacterial form of streptococcus and subsequently treated with penicillin, antibodies against L-forms were found in the PHT starting from the 5th-7th day after infection. Their titer gradually increased on the 10th, 14th, and 28th days.

TABLE 3. Dynamics of Antibody Titers in Sera of *M. rhesus* after Reinfection with L-Forms of Group A Hemolytic Streptococcus

Material administered	Time of obtaining sera (in days)	No. of sera	Antibody titers (in log ₂)				
			under 3,3	3,3-5,3	6,3-8,3	9,3-11,3	12,3 and over
L-forms of hemolytic streptococcus	0	16	10	4	2	0	0
	7	16	1	6	4	5	0
	14	13	0	5	1	4	3
	21	8	1	3	1	3	0
	28	9	0	4	2	3	0
Heated L-forms of hemolytic streptococcus	0	8	8	0	0	0	0
	7	8	8	0	0	0	0
	14	4	2	1	1	0	0
	21	3	1	1	1	0	0
	28	2	1	1	0	0	0
Broth	0	1	1	0	0	0	0
	7	1	0	1	0	0	0
	14	1	1	0	0	0	0
	21	1	1	0	0	0	0
	28	1	1	0	0	0	0

Note. The monkeys were infected 4 times at intervals of 6-7 days. Blood was taken on the day of infection and 7 days after the last infection.

In the case of acute infection of the monkeys with L-forms (Table 2), antibodies were found after 2-3 days and the maximal titers were reached by the 7th day.

The appearance of antibodies, in the writers' view, can be attributed to the fact that the L-form, lacking a cell wall, and also streptococci exposed to penicillin *in vivo*, induce the formation of antibodies against antigenic components of L-forms localized outside the cell wall.

Antibody formation against L-forms following streptococcal infection and subsequent penicillin therapy may evidently be an indirect indicator of the induction of L-forms *in vivo*.

On reinfection of monkeys with L-forms of streptococcus (experiments of series II), antibody formation was found in some monkeys on the first days after administration of the culture. This was probably because the monkeys used in the experiments had previously (1968) been infected with L-forms of hemolytic streptococcus. Starting on the 7th and continuing until the 28th day (end of observations) after infection, practically all the monkeys contained antibodies against L-forms (Table 3).

After injection of the control group with heated L-forms of streptococcus only solitary monkeys showed antibodies in low titers at these times.

Antibody-formation against L-forms thus takes place both in monkeys infected with the L-form itself and also in monkeys infected with the bacterial form of streptococcus and subsequently treated with penicillin.

Reinfection with L-forms of hemolytic streptococcus leads to the early appearance and prolonged preservation of antibodies in the monkeys' sera.

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