serum also was observed on the intracellular digestion of *B. mesentericus* and *B. subtilis*. However, relative to pathogenic microorganisms (*E. coli* 055) (Table 2), of all the contaminating microorganisms chosen, the only one able to stimulate opsonic activity was *S. faecalis*.

The results indicate that there is a connection between the composition of the microflora and the opsonic activity of the blood serum of animals. The actual value of this index also depends on the properties of the object phagocytosed.

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IMMUNE RESPONSE OF MICE OF VARIOUS INBRED LINES TO *Clostridium oedematiens* TOXOID

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Mice of various inbred lines were immunized intradermally with *Clostridium oedematiens* α toxoid. The immunization was repeated 30 days later. The titer of antibodies against toxoid was determined by the passive hemagglutination test in the blood of the mice 20 and 30 days after the first and 10 days after the second immunization. The maximal response to primary immunization was recorded in C3H mice, the minimal in DBA/2 mice, with a more than 30-fold difference. The remaining tested lines of mice (A, CBA, BALB/c, AKR, C57BR) occupied an intermediate position. After the second immunization the differences were reduced. The existence of genetic control of the immune response to this particular antigen is postulated in mice.

KEY WORDS: Clostridium oedematiens *toxoid*; *antibody formation*; *genetic control*.

As has been shown repeatedly, ability to give an immune response to concrete antigens is under genetic control in animals and man [2, 6]. The separate genes controlling the immune response have now been identified. These genes may be linked with the main system of genes determining histocompatibility in mice (H-2), guinea pigs (GPL-1) man (HLA), etc., or they are not linked with it (for example, the IR-3 locus in mice). The search for highly immune antigens with the minimal number of antigenic determinants on the molecule is being pursued intensively, for it is only by the use of such antigens that any further elucidation of the mechanisms of control of the immune response will be achieved.

In this investigation antibody formation was studied in mice of different lines during immunization with *Clostridium oedematiens* toxoid.

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TABLE 1. Antibody Formation against *Cl. oedematiens* in Mice of Various Inbred Lines (log_2 of titers and confidence limits)

Line of mice	Titer of antibodies		
	after immunization		10th day after 2nd immunization
	20th day	30th day	
C3H	10,83±0,31 (10,18÷11,48)	13,1±0,21 (12,66÷13,54)	13,9±0,32 (13,20÷14,60)
A	10,30±0,68 (8,63÷11,97)	12,70±0,63 (11,20÷14,12)	14,60±0,24 (14,07÷15,13)
CBA	10,4±0,32 (9,68÷11,12)	12,1±0,24 (11,56÷12,64)	14,3±0,37 (13,43÷15,17)
BALB/c	9,9±0,63 (8,32÷11,44)	11,6±0,39 (10,52÷12,68)	13,4±0,27 (12,77÷14,02)
AKR	8,9±0,48 (7,82÷9,98)	11,1±0,61 (9,40÷12,8)	13,4±0,26 (12,8÷14,0)
CC57 BR	8,88±0,35 (8,09÷9,67)	9,5±0,81 (7,67÷11,33)	12,3±0,61 (10,6÷14,0)
C57BL	10,1±0,37 (9,26÷10,94)	9,3±0,69 (7,74÷10,86)	12,5±0,39 (11,63÷13,37)
$DBA/_2$	8,78±0,49 (7,74÷9,82)	7,63±0,45 (6,67÷8,59)	12,25±0,57 (10,90÷13,60)

EXPERIMENTAL METHOD

Mice of the following inbred lines were obtained from the "Stolbovaya" nursery of laboratory animals of the Academy of Medical Sciences of the USSR: A, C57BL/6, CC57BR, DBA/2, BALB/c, AKR, C3H and CBA.

α Toxoid obtained from native *Cl. oedematiens* toxoid by the method of acid precipitation at the isoelectric point, using a culture fluid of high ionic strength, with subsequent fractionation by $(NH_4)_2SO_4$, adsorbed on alumina, was used to immunize the mice. Subsequent purification of the toxoid was carried out by ion-exchange chromatography on DEAE cellulose followed by gel filtration through Sephadex G-100 and G-200 [5]. The specific activity of the α toxoid was 1600 fixation units (FU) in 1 mg protein nitrogen in the agar precipitation test. One precipitation zone was found by immunoelectrophoresis, thus confirming the serological purity of the preparation.

In a preliminary investigation the optimal dose of toxoid giving an immune response in the mice was determined. In this investigation 10 to 12 mice of each line were immunized subcutaneously with *Cl. oedematiens* toxoid in a dose of 0.5 FU. The immunization was repeated with the same dose of antigen 30 days later. Blood was collected in capillary tubes from the caudal vein of the immunized mice. After a clot had formed it was removed from the capillary and the erythrocytes were sedimented by centrifugation at 1000g for 10 min and the resulting serum was separated from the residue. Sera were obtained on the 20th and 30th day after the second immunization. In the second series of experiments sera from mice which differed most in their antibody titers were obtained every 5 days. Antibodies against α toxoid were discovered in sera from individual mice heated to 56°C for 30 min, in the passive hemagglutination test. An erythrocytic diagnostic serum prepared in accordance with technical instruction MRTU-42 No. 543-73 and laboratory regulation No. 331-73 KVS of the Ministry of Health of the USSR, was used for titration of the antibodies. The erythrocytes were sensitized with a primary concentration of *Cl. oedematiens* toxoid in a dose of 5 FU to 1 ml of formalinized, tanninized sheep's erythrocytes. The reaction was carried out in a volume of 0.4 ml on polystyrene tiles. The quality of the diagnostic serum was verified by titration with standard antioedematiens horse serum; its sensitivity was 0.0025 i.u. toxoid throughout the period of work. The initial dilution of the serum was 1:16-1:32, after which double dilutions of the sera were used (1:32, 1:64, 1:128, and so on). The mean antibody titer in the group of mice was calculated as the arithmetic mean of log_2 of the last dilution of serum to give a positive reaction. The mean results were subjected to statistical analysis and the standard deviation and confidence limits for P = 0.5 were calculated by the usual method for analysis of biological data [1].

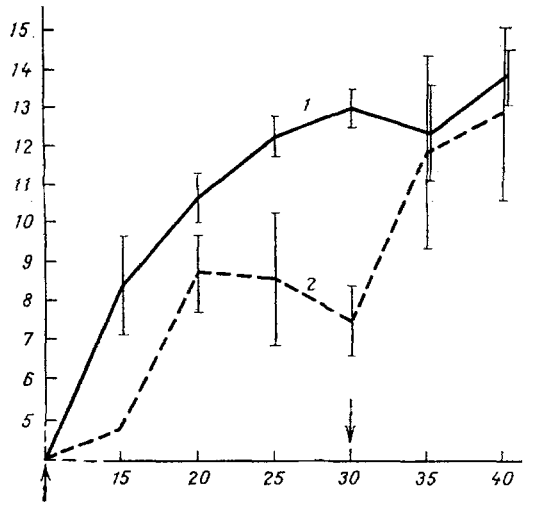

Fig. 1. Dynamics of immune response to *Cl. oedematiens* toxoid in two extreme lines of mice: 1) C3H — high response, 2) DBA/2 — low response. Abscissa, time after first immunization (in days); ordinate, antibody titer (in $\log_2$).

EXPERIMENTAL RESULTS

The mean titers of antibodies in mice of the 8 inbred lines are given in Table 1. Clearly the greatest differences in antibody titers after the first immunization were observed in DBA/2 mice (low response) and C3H mice (high response). The remaining lines of mice occupied an intermediate position between the extreme lines as regards their ability to respond immunologically to the test toxoid. Repeated immunization, as the writers observed previously [3, 4], with respect to other antigens (leptospires and sheep's red cells) greatly reduced the differences between the lines. It will be noted that the decrease in the differences took place in every case on account of a marked increase in the level of the immune response of mice of the lines giving a low response and a relatively small increase in the level of antibodies in lines giving a high response.

In another series of experiments the dynamics of antibody formation was investigated in mice of the extreme lines DBA/2 and C3H. As Fig. 1 shows, the highest antibody titer was obtained in mice of the low-response line DBA/2 to begin with (20th-25th day) and then it began to fall gradually. In the high-response C3H line of mice the antibody titers were higher on the 20th, 25th, and 30th day after the first immunization. The antibody titers before the 15th day in both lines of mice were 1:32 or less, i.e., lower than the initial dilution of the serum. All comparison based on differences between the lines must therefore be made after the 20th day, just as in the first experiment, the differences between lines were reduced in the secondary response.

Differences between the extreme lines of mice C3H and DBA/2 during immunization with this particular toxoid are the greatest of all which the writers have been able to obtain with the aid of many different antigens. In this case after the primary response the difference amounted to 32 times, compared with only 8-10 times for sheep's red cells and 15-20 times for leptospires [3]. Considering the greater differences between the extreme lines in antibody titers when this particular antigen is used, it can be hoped that it will prove useful during the further investigation of the mechanisms of genetic control of the immune response.

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