

INTERLINEAR DIFFERENCES OF ANTIBODY FORMATION IN INBRED MICE IMMUNIZED WITH ONE OR TWO ANTIGENS

R. V. Petrov, V. M. Man'ko,
and É. I. Panteleev (Moscow)

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The authors have previously shown that mice of different highly inbred lines produce different quantities of antibodies when immunized by a single dose of nonpathogenic leptospires. Two alternative lines of animals (C57B1 and C3H) were discovered, in which the difference in the intensity of agglutinin production was 15-20 times [2]. Other authors [3-6] have reported linear differences in antibody formation.

The presence of alternative lines of mice, reacting differently to the same antigen, made it possible to develop a method of immunomorphological analysis in normal conditions and in radiation sickness, enabling specific cell differences of antibody formation to be distinguished from nonspecific reactions to antigenic stimulation.

The object of the present investigation was to continue the study of this immunobiological phenomenon which had been discovered by using one (sheep's erythrocytes) or a combination of two heterologous antigens (Leptospira canicola and sheep's erythrocytes).

EXPERIMENTAL METHOD

The discovery of alternative lines of mice reacting differently to immunization by different antigens has considerably extended the possibilities of the method of immunomorphological analysis, has clarified the question of the degree of genetic determinants of antibody formation, and has provided a model for solving many problems in immunology and related fields.

The experiments of series I were carried out on highly inbred male mice weighing 18-28 g of lines CBA, C57B1/6J (from the "Stolbovaya" nursery of the Academy of Medical Sciences of the USSR) and mice of lines C3H/HeDiSn, C57B1/10ScSn, CC57BR, CC57W, and A (these lines were reared in the authors' laboratory). The animals were immunized by intraperitoneal injections of 1 ml of a 1% suspension of sheep's erythrocytes washed three times with physiological saline. On the 31st day, the mice were reimmunized. A third injection of erythrocytes was given to these animals 33 days after reimmunization.

Starting on the 3rd day, blood was systematically taken from the caudal vein of the experimental mice into glass capillary tubes. After the blood had clotted, the fibrin clot was extracted and one end of the capillary tube with the serum was sealed with plasticine. The capillary tubes with the serum were kept in the cold (3-7°) until used in the hemagglutination reaction.

The serum was titrated in a volume of 0.1 ml by twofold dilutions (1:2, 1:4, 1:8, and so on) for the presence of agglutinins against sheep's erythrocytes. As a rule, the agglutination reaction was carried out on the 2nd-3rd day after taking the blood.

The titers of antibodies were expressed as logarithms in accordance with the usual methods [1,8] and the results obtained were used for plotting graphs and for statistical analysis.

The experiments of series II were carried out on three highly inbred lines of mice CBA, C3H, and C57B1 from the "Stolbovaya" nursery of the Academy of Medical Sciences of the USSR, of which two were alternatives to leptospires (C57B1 and C3H) [2], and two, as the experiments of series I revealed, to sheep's erythrocytes (CBA and C57B1). The animals were immunized intraperitoneally with two heterologous antigens (sheep's erythrocytes by the same method as in series I) and leptospires (L. canicola strain "Ramenka rat") in a dose of 0.2 ml (approximately 50-60 leptospires per field of vision of themicroscope). The sera obtained were divided into two equal parts. One half was

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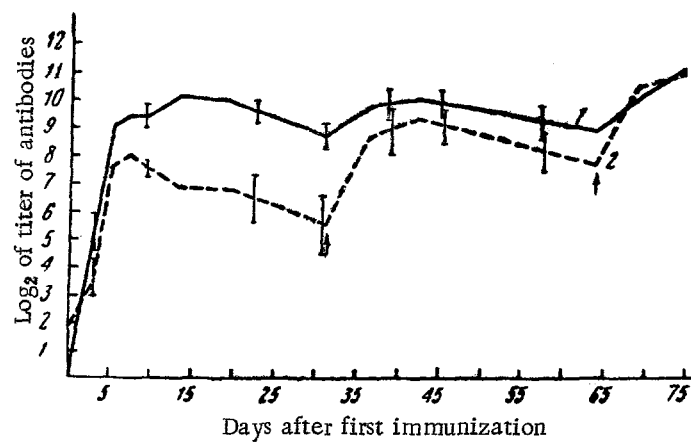


Fig. 1. Titers of agglutinins against sheep's erythrocytes in mice of alternative lines CBA and C57B1 at various times after immunization (mean data for 10 animals of each line). 1) CBA line; 2) C57B1 line. The arrows indicate the first, second, and third immunization.

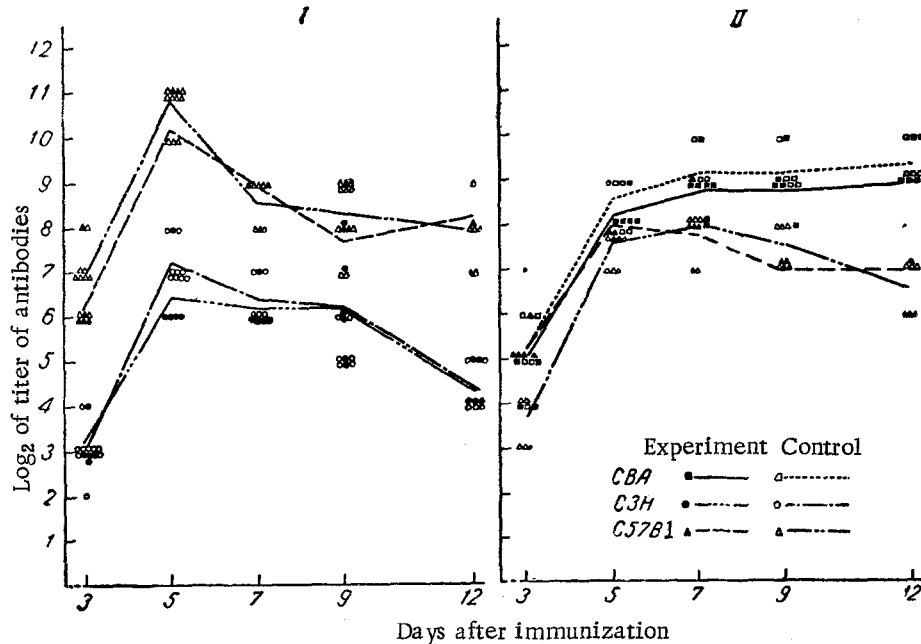


Fig. 2. Titers of agglutinins against leptospire and sheep's erythrocytes in mice of different lines immunized with one or two antigens: I) titers of agglutinins against leptospire after immunization of mice with leptospire (control) and after simultaneous immunization of mice with leptospire and sheep's erythrocytes (experiment); II) titers of agglutinins against sheep's erythrocytes after immunization of mice with sheep's erythrocytes (control) and after simultaneous immunization of mice with leptospire and sheep's erythrocytes (experiment).

titrated as in the experiment of series I and the other for the presence of agglutinins against leptospire. The method of reading the reaction of agglutination of leptospire was described previously [2].

EXPERIMENTAL RESULTS

It is clear from Fig. 1 and from the table that agglutinins against sheep's erythrocytes began to appear 2-3 days after immunization, and reached their maximal concentration on the 5th-7th days. In the intact mice of line C56B1, natural antibodies against sheep's erythrocytes were sometimes present in the serum in low titers (1:2-1:4),

Titers of Agglutinins in Blood of Mice of Different Lines after Immunization with Sheep's Erythrocytes*

| Line of mice | No. of mice | No. of inbreeding animals | Mean titers of antibodies (\log_2) at various times after immunization | | | | | | |
|-----------------------|-------------|---------------------------|--|-------------------------|-------------------------|-------------------------|-------------------------|----------------------------|----------------------------|
| | | | 3 days | 5 days | 9 days | 22 days | 31 days | 38 (7) † days | 42 (11) ‡ days |
| CBA ³ | | 5 | 5.2±0.37 (4.17÷6.23) | 8.6±0.24 (7.93÷9.27) | 9.2±0.2 (8.53÷9.87) | 9.0±0.44 (7.8÷10.2) | 9.2±0.2 (8.53÷9.87) | 11.6±0.24 (10.93÷12.27) | 11.0±0.32 (10.11÷11.89) |
| C3H/HeDiSn | 54 | 5 | 5.6±0.24 (4.93÷6.27) | 7.6±0.24 (6.93÷8.27) | 8.4±0.24 (7.73÷9.07) | 8.0±0.32 (7.11÷8.89) | 9.0±0.32 (8.11÷9.87) | 11.2±0.2 (10.64÷11.76) | 10.6±0.24 (9.93÷11.27) |
| C57B1/10ScSn | 74 | 4 | 4.5±0.64 (2.47÷6.53) | 8.5±0.29 (7.58÷9.42) | 7.5±0.58 (5.66÷9.34) | 6.8±0.75 (4.4÷9.2) | 6.3±0.47 (4.81÷7.79) | 10.5±0.29 (9.58÷11.42) | 9.8±0.12 (9.42÷10.18) |
| C57B1/6J ³ | | 5 | 4.2±0.2 (3.64÷4.76) | 7.6±0.39 (6.52÷8.68) | 7.8±0.2 (7.24÷8.36) | 6.2±0.2 (5.64÷6.76) | 6.2±0.58 (4.59÷7.81) | 10.4±0.39 (9.32÷11.48) | 10.4±0.24 (9.73÷11.07) |
| CC57BR | 49 | 5 | 5.4±0.39 (4.3÷6.48) | 8.2±0.2 (7.64÷8.76) | 7.6±0.39 (6.52÷8.68) | 7.8±0.37 (6.77÷8.83) | 7.0±0.32 (6.11÷7.89) | 10.2±0.37 (9.17÷11.23) | 10.2±0.39 (9.12÷11.28) |
| A/Mvf | 104 | 5 | 6.8±0.49 (5.44÷8.16) | 8.0±0 (8.0÷8.0) | 7.8±0.37 (6.78÷8.72) | 7.4±0.24 (6.74÷8.07) | 7.4±0.24 (6.74÷8.07) | 11.4±0.24 (10.73÷12.07) | 10.4±0.24 (9.73÷11.07) |

*The confidence intervals of the logarithms of the geometrical means were calculated by the method of I. P. Ashmarin and A. A. Vorob'ev with the value $P = 0.05$.

†The day after reimmunization is given in parentheses.

‡The mice were obtained from the "Stolbovaya" nursery of laboratory animals of the Academy of Medical Sciences of the USSR in June, 1963.

as other investigators have noted [4,9,10]. After the 9th day the titers of agglutinins in the mice of several lines began to fall or remained at the same level.

After primary and reimmunization, the sera of the mice of line CBA possessed the highest titers of antibodies against sheep's erythrocytes ($10.1 \log_2$) and the sera of the mice of line C57B1 had the lowest titers ($8.0 \log_2$ after the first immunization and $9.4 \log_2$ after the second immunization, see Fig. 1). In other experiments (repeated) this difference persisted ($9.2 \log_2$ after first immunization and $11.6 \log_2$ after second immunization for mice of line CBA and $7.8 \log_2$ and $10.4 \log_2$, respectively for mice of line C57B1, see table). These two lines were called alternatives in relation to this particular antigen.

The animals of the remaining lines occupied an intermediate position between these two lines of mice.

Reimmunization on the 31st day caused an increase in the antibody titers in the mice of all the lines. The antibody concentration was maximal also on the 5th-7th days and it remained at this level for approximately 10 days, after which the titers of hemagglutinins gradually fell.

After the third immunization, the titers of hemagglutinins rose again, but in contrast to the first and second immunization, the interlinear differences in antibody production disappeared. The dynamics of the accumulation of antibodies in the serum of the mice when sheep's erythrocytes were used as antigen differed slightly from the pattern in the place when leptospire were used as antigen [2]. For instance, after immunization of mice with leptospire, the interlinear differences disappeared after the second immunization, whereas in the case of immunization with sheep's erythrocytes, the interlinear differences did not disappear until after the 3rd immunization.

In the experiments of series II, the animals received simultaneous injections of two heterologous antigens (leptospire and sheep's erythrocytes). The intensity of antibody formation (the primary response) against the two antigens was then compared in mice immunized separately with only one of the above antigens (mice of lines CBA and C57B1 were immunized with sheep's erythrocytes, mice of lines C57B1 and C3H with leptospire) and in mice of these three lines immunized with the two antigens at the same time.

It is clear from Fig. 2 that the titers of the antibodies against each antigen, when injected together, remained at the same level as when immunization took place separately.

If the mice of the two alternative lines in relation to leptospire were immunized additionally by sheep's erythrocytes, the production of antibodies against leptospire did not fall by a significant degree, as was also the case with the production of antibodies against sheep's erythrocytes. The same picture was also seen in relation to alternative lines against sheep's erythrocytes. As before, the highest titers of antibodies against leptospire were observed in mice of line C57B1, and the lowest in mice of line C3H, while the highest titers of antibodies against sheep's erythrocytes were seen in mice of line CBA, and the lowest in mice of line C57B1. Line CBA in the first case and C3H in the second, which are not shown in Fig. 2, as previously occupied an intermediate position.

LITERATURE CITED

1. I. P. Ashmarin and A. A. Vorob'ev, Statistical Methods in Microbiological Investigation [in Russian], Leningrad (1962).
2. R. V. Petrov, V. M. Man'ko, and I. K. Egorov, DAN SSSR, 153, 3, 728 (1963).
3. I. Davidsohn and K. Stern, Cancer Res., 9, 426 (1949).
4. I. Davidsohn and K. Stern, J. Immunol., 72, 216 (1954).
5. J. K. Dineen, Nature, 202, 101 (1964).
6. M. A. Fink and V. A. Quinn, J. Immunol., 70, 61 (1953).
7. N. Gengozian and T. Makinodan, J. Immunol., 80, 189 (1958).
8. P. A. Gorer, Cancer Res., 7, 634 (1947).
9. K. Stern and I. Davidsohn, J. Immunol., 72, 209 (1954).