# ANTIBODY FORMING FUNCTION OF THE REGENERATING SPLEEN

G. V. Kharlova, N. A. Kraskina, and V. I. Levenson

UDC 612.411:612.6.03/.017.1

An important factor in determining the extent of recovery of a regenerating organ is the study of its function. Among the many functions ascribed to the spleen, the only one at present capable of precise interpretation is the ability of this organ to produce specific antibodies in response to the intravenous injection of an antigen [7, 10]. This process can be estimated strictly quantitatively and can be characterized by the amount of antibodies formed by unit weight of spleen tissue.

As earlier investigations [3, 4, 8, 9] have shown, the intravenous injection of purified typhoid Vi antigen into mice leads to intensive formation of Vi antibodies, 90-95% of which are formed by the spleen.

The spleen of mice and rats is capable of regeneration [5, 6]. In the course of regeneration the Malpighian corpuscles increase in number of hypertrophy slightly. The principle changes in cell composition during regeneration occur on the first days after operation, with the appearance of new cell forms (transitional cells) and increased proliferation of reticulum cells, to be followed by increased proliferation of the cells of the lymphoid, myeloid, and erythroid series. The normal cell composition of the regenerating spleen is restored after 2 weeks.

The object of this investigation was to study the ability of the regenerating mouse spleen to form Vi antibodies.

## EXPERIMENTAL

Experiments were conducted on 300 inbred CC57BR mice. Through an incision in the left lumbar region 70-80% of the spleen tissue was taken from the animals, the wound was sutured in layers, and 30 days after the operation the mice were given an intravenous injection of 1 μg Vi antigen from Salmonella typhi [1]. The index of splenic function was the accumulation of Vi antibodies in the serum, determined at various times after immunization by the passive hemagglutination reaction [2].

The antibody-forming function of the spleen was also studied by transplantation of spleen cells of donor mice immunized with Vi antigen intraperitoneally into intact recipient mice. The transplantation technique was described earlier [3, 4].

## EXPERIMENTAL RESULTS

In all the experiments regeneration of the spleen was observed in each animal. The uniformity of the reaction to operative interference was probably attributable to the genetic homogeneity of the inbred mice.

Thirty days after operation the mean weight of the resected spleen was 100 mg or 69% of the weight of the control, intact spleen (145 mg).

The functional activity of the regenerating spleen was studied in two types of experiments. In the experiments of type I, 70% of the spleen tissue was removed from the mice, and 1 μg Vi antigen was injected intravenously 30 days after operation. The criterion of function of the regenerating spleen was the accumulation of Vi antibodies in the splenectomized mice.

The dynamics of the titer of Vi antibodies in the serum of the intact and splenectomized mice is shown in Fig. 1. The curves are of the same type, but the curve of antibody accumulation in the

Institute of Experimental Biology, Academy of Medical Sciences of the USSR; Moscow Research Institute of Epidemiology and Microbiology (Presented by Active Member of the Academy of Medical Sciences of the USSR N. A. Kraevskii). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 63, No. 6, pp. 74-77, June, 1967. Original article submitted December 13, 1965.

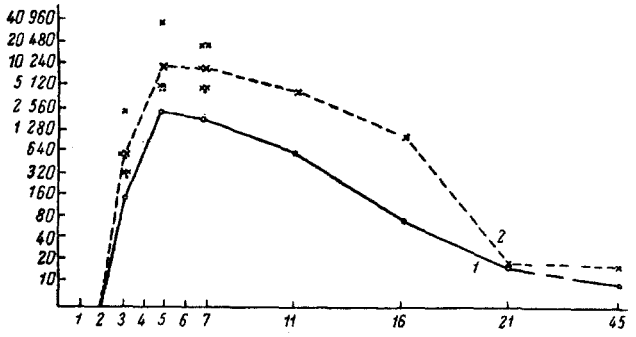


Fig. 1. Titer of Vi antibodies in the serum of splenectomized (1) and control (2) mice immunized with Vi antigen. Abscissa) Days; ordinate) titer of Vi antibodies.

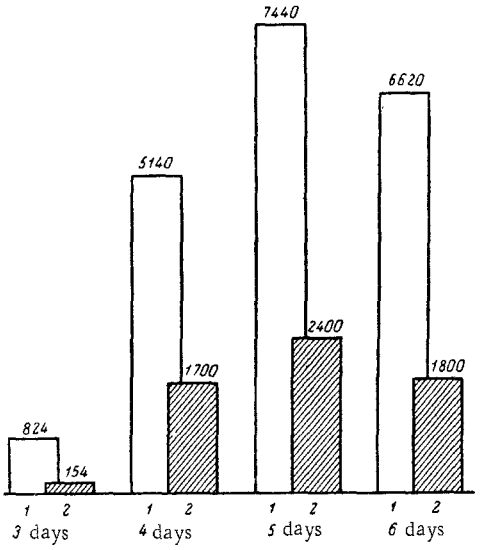


Fig. 2. Production of Vi antibodies in splenectomized (2) and control (1) mice at various times after immunization. Antibody production per diem is shown (in conventional units).

splenectomized animals lies below the control curve at all points. The difference between the curves is statistically significant.

On the basis of the results shown in Fig. 1 the amount of antibodies formed by the mouse in a particular time interval was calculated with the aid of a formula allowing for the titer of antibodies in the serum and their natural rate of decay (Fig. 2).

It is clear from Fig. 2 that in the splenectomized mice the antibody production was only 25-30% of its level in the normal animals. Since in these experimental conditions Vi antibody production took place almost entirely in the spleen, it can be concluded that the regenerating spleen formed less Vi antibodies than the intact organ. This decrease in production was not connected with the smaller mass of the regenerating spleen, for calculations of the amount of antibodies formed per milligram of spleen tissue gave values of 155 units for the control and only 55 units for the experimental animals.

Consequently, the ability of the tissue of the regenerating spleen to form Vi antibodies was lower than that of normal spleen tissue by almost 67%. This conclusion was fully confirmed by the results of the experiments of type II, in which regenerating spleen tissue of immunized mice was transplanted into recipient mice.

Seventy percent of the spleen tissue was removed from the donor mice. Thirty days later these mice and also the intact (control) animals were immunized by the intravenous injection of 1 μg Vi antigen. Three days after immunization the regenerating spleen was extracted. Suspensions were made from the extracted spleens in Hanks's solution and injected intraperitoneally into intact recipient mice in a dose of 100 mg spleen tissue per recipient. The amount of Vi antibodies found in the blood serum of the recipients was used as index of the function of the regenerating spleen cells.

The results of four such experiments are summarized in the table. They show that the transplantation of tissue of both the normal and the regenerating spleen led to the appearance of Vi antibodies in the recipient's blood. However, the antibody titer was much higher in the recipients of normal spleen cells than in the mice receiving cells of the regenerating spleen. Calculation of the antibody production showed that the regenerating spleen tissue formed only one-eighth the amount of antibodies formed in the recipient mouse by normal spleen tissue.

Antibody Formation by Spleen Cells Transplanted into Recipient Mice

| Animals | Wt. of tissue transplanted (in mg) | Mean titer of Vi antibodies* in recipients' sera | Antibody production over 3 days | |
|---|---|---|---|---|
| | | | absolute | % of control |
| Recipients of regenerating spleen cells | 100 | 1:100 | 190 | 13 |
| Recipients of control spleen cells | 100 | 1:880 | 1530 | 100 |

*3 Days after transplantation of cells.

Both the adopted criteria thus showed that the functional activity of the regenerating spleen was considerably reduced when calculated per unit weight of tissue. In other words, restoration of the mass of the spleen tissue in the course of regeneration was not accompanied by the parallel recovery of the antibody-forming function.

Two possible explanations of this phenomenon may be suggested: 1) because of changes in the blood supply to the transplanted spleen, contact between the cells and the antigen injected into the blood stream was disturbed, thus interfering with the formation of a normally developed immunologic response; 2) as the result of regeneration,hypertrophy generations of cells unable to react to Vi antigen or to form Vi antibodies appear. This explanation does not imply any gross changes in the morphology of the spleen, for the number of cells per milligram of tissue and the cell composition of the regenerating spleen were very close to those of the normal organ. All that can be postulated is that the lymphoid cells arising as the result of proliferation in the course of the regeneration process differ in their immunologic properties from the lymphoid cells of the intact spleen. Further experimental investigations are needed to test the validity of these hypotheses.

## LITERATURE CITED


1. D. D. Efimov, Zh. Mikrobiol., No. 11, 115 (1961).
2. N. A. Kraskina and N. M. Gutorova, in the book: Immunology and Prophylaxis of Intestinal Infections [in Russian], Moscow (1962), p. 180.
3. N. A. Kraskina and V. I. Levenson, Byull. éksp. Biol., No. 1, 65 (1963).
4. V. I. Levenson and N. A. Kraskina, Byull. éksp. Biol., No. 12, 64 (1962).
5. L. D. Liozner and G. V. Kharlova, Byull. éksp. Biol., No. 4, 96 (1960).
6. L. D. Liozner and G. V. Kharlova et al., in the book: Processes of Regeneration and Cell Multiplication [in Russian], Moscow (1961), p. 17.
7. M. P. Pokrovskaya, V. I. Levenson, and N. A. Kraskina, in the book: Textbook of Microbiology, Clinical Features, and Epidemiology of Infectious Diseases,in Several Volumes [in Russian], Moscow, 3 (1964), p. 190.
8. M. P, Pokrovskaya et al., Zh. Mikrobiol., No. 8, 9 (1963).
9. M. P. Pokrovskaya et al., Zh. Mikrobiol., No. 9, 72 (1963).
10. W. H. Taliaferro and L. G. Taliaferro, J. infect. Dis., 90, p. 205, (1952).