EFFECT OF REPEATED IMMUNIZATION OF RATS ON THEIR REACTIVITY TO SHEEP'S ERYTHROCYTES

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Frequent intravenous injections of sheep's erythrocytes reduce the immunoreactivity of rats. With an increase in the number of injections of antigen the degree of inhibition of the immune response is intensified. By cultivating lymphocytes in vivo in irradiated recipients it was shown that the reactivity of the cells themselves is not thereby reduced. The results suggest that circulating antibodies are the cause of the observed immunodepression.

A lowering of the immunoreactivity of animals after frequent repeated injections of antigen has been demonstrated in several experimental models [4, 6, 7]. However, the reasons for the observed immunodepression have not been adequately investigated. It is not clear what lies at the basis of the phenomenon: depression of reactivity of the lymphocyte population or the immunodepressive action of an extracellular factor. In other words, it has to be decided whether the phenomenon is a type of immunological tolerance or a manifestation of self-regulation of the immune response.

The object of this investigation was to assess the immunoreactivity of the lymphocytes of repeatedly immunized rats.

EXPERIMENTAL METHOD

Sheep's erythrocytes were injected intravenously into adult August rats and noninbred albino rats repeatedly (up to 11 times) at intervals of 2-3 days in one of the following doses: 1×10^6, 1×10^9, or 5×10^9 cells. Two days after the end of this course of injections of antigen the rats received an intravenous injection of 1×10^9 sheep's erythrocytes. From 3 to 4 days later the number of antibody-forming cells (AFC) was determined in the spleen and in the submandibular lymph glands by the method of local hemolysis in gel [2]. In some experiments, 24 h before immunization (1×10^9 sheep's erythrocytes, intravenously) or 48 h thereafter, 1 ml of antiserum was injected into the rats. The antiserum was obtained from rats on the 2nd day after the last of 3 intravenous injections of 5×10^9 sheep's erythrocytes, at intervals of 2 days. The antiserum contained hemolysins in a titer of 1:10,240 and hemagglutinins in a titer of 1:5,120. The serum of rats immunized with a single intravenous injection of 10 μg Salmonella typhi O-antigen was used as the control.

The immunoreactivity of cells from the spleen and submandibular lymph glands was tested by cultivation in vivo in recipient rats irradiated in a dose of 650 R [3]. From 2 to 2.5 h after irradiation the recipient rats received an intravenous injection of 5×10^7 nucleated cells from the spleen or submandibular lymph glands, and 1.5-2 h later the same recipients received an intravenous injection of 1×10^9 sheep's erythrocytes. In some experiments, 1 h before transplantation of the cells, the recipient rats received an injection of antibodies (1 ml antiserum, intravenously). The number of AFC in the spleen of the recipient rats was determined 5 days later by the method of local hemolysis in gel.

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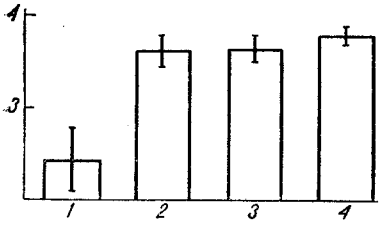

Fig. 1

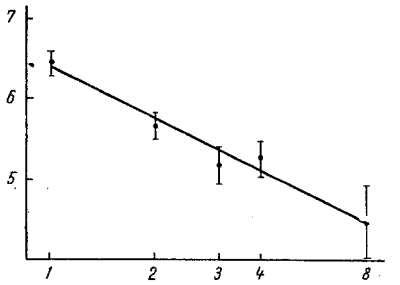

Fig. 2

Fig. 1. Effect of antibodies on immunoreactivity of rats to sheep's erythrocytes: 1) antibodies injected 24 h before immunization; 2) antibodies injected 48 h after immunization; 3) antibodies against Salmonella typhi O-antigen injected 24 h before immunization with sheep's erythrocytes; 4) control. Ordinate: log of number of AFC in rats' spleen on 4th day after injection of antigen.

Fig. 2. Effect of number of injections of antigen on immune response of rats. Abscissa, number of intravenous injections of 5×10^9 sheep's erythrocytes (logarithmic scale) given at intervals of 2 days; ordinate, log of number of AFC in rats' spleen on 4th day after last injection of 5×10^9 sheep's erythrocytes.

The titers of hemolysins and hemagglutinins were determined in the animals' blood by the usual method. The antibody titers were expressed in logarithms to base 2 and the initial dilution of the serum was 1:10. The experimental results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

A course of repeated intravenous injections of sheep's erythrocytes, in both high (5×10^9) and low (1×10^6) doses lowered the level of immunoreactivity of the rats below that of the control animals not previously exposed to experimental treatment of any kind. On the 4th day after intravenous injection of 1×10^9 sheep's erythrocytes, the number of AFC found in the spleen of the experimental rats was 96.2×10^3 (animals previously receiving several injections of 5×10^9 sheep's erythrocytes) and 472×10^3 (animals previously receiving several injections of 1×10^6 sheep's erythrocytes). Under the same conditions the immune response of the control animals was 1552×10^3 AFC. The decrease in the immune response of the experimental animals was statistically significant.

A possible reason for the observed decrease in immunoreactivity of the experimental rats could be antibodies circulating in the blood stream. Before the test injection hemolysins were found in the blood of rats repeatedly immunized with 5×10^9 or 1×10^6 sheep's erythrocytes in titers of 10.8 ± 0.4 and 9.8 ± 0.4 respectively. A special series of experiments showed that these antigens, if injected into intact rats 24 h before immunization with sheep's erythrocytes, sharply inhibited the immune response, judging from the number of AFC in the spleen (Fig. 1). The number of AFC in the spleen was reduced only slightly if the antibodies were injected 48 h after the antigen. The immune response of the rats of this group was of the same order as after injection of antiserum of another specificity.

The temporary decrease in immunoreactivity of the rats after a single injection of 5×10^9 sheep's erythrocytes (Fig. 2) was rather unexpected. With an increase in the number of preliminary injections of the antigen the level of immunoreactivity fell. Before a second injection of antigen given 2 days after the first, hemolysins circulated in the rats' blood in very low titers (1:40), although inhibition of the immune response was clear. The phenomenon observed was presumably connected with depression of the reactivity of the lymphocytes themselves. To test this hypothesis, the method of cultivating lymphocytes from rats of the various experimental groups in vivo in lethally irradiated recipient rats was used. Parallel with this, and in the same series of experiments, the hypothesis of "redislocation" of the immune response from the spleen to the distant lymph glands during repeated intravenous immunization was tested.

TABLE 1. Immunoreactivity of Spleen and Lymph Gland Cells of Rats of Different Experimental Groups in Situ and during Cultivation in Vivo

Rats	Source of cells	Reaction of donors		Reaction of recipients	
		No. of rats	Number of AFC in spleen or lymph glands* ($\times 10^3$)	No. of rats	Number of AFC in spleen ($\times 10^3$)
Intact	Reaction of recipients	11	135,5 (112,2—163,7)	16	14,0 (7,1—27,4)
	Lymph glands	8	2,1 (1,3—3,4)	10	6,3 (2,9—13,7)
Sensitized	Spleen	7	152,8 (118,0—197,7)	5	156,7 (61,9—396,3)
Repeatedly immunized	Spleen	14	1,8 (1,1—2,9)	17	26,0 (13,9—48,8)
	Lymph glands	9	0,6 (0,4—1,0)	9	9,4 (3,3—26,4)
Irradiated (control)	—	—	—	10	0,2 (0,1—0,4)

*Calculated per 5×10^7 nucleated cells

The results in Table 1 show that after repeated intravenous injections of antigen there was no change in the localization of the immune response: statistically significant ($P < 0.001$) inhibition of the immune response to intravenous injection of a test dose of 1×10^9 sheep's erythrocytes was observed both in the spleen and in the lymph glands of the donor rats. However, during cultivation of the cells in vivo the immune response of the spleen and lymph gland cells of the repeatedly immunized rats was actually stronger than the cell response of the intact donors. An even stronger immune response was observed if the donors of the cells were previously immunized by a single injection. The difference in the immunoreactivity of the spleen cell populations was statistically significant.

Special experiments showed that injection of antibodies into the irradiated recipients sharply suppressed the immune response of the cells cultivated in vivo and obtained from both the control and experimental rats.

It can be concluded from the results of this investigation that the areactivity arising after frequent intravenous injections of sheep's erythrocytes into rats is due to circulating antibodies formed as a result of the preceding injections of antigen. The results indicating that the lymphocytes of donors with a high antibody titer give a better immune response when tested in an intact irradiated recipient do not confirm the view that the antibodies act directly on the immunocompetent cell, leading to the induction of tolerance [1, 5].

The fact that immunoreactivity is lowered immediately after a single injection of antigen, when the titers of circulating antibodies are still low, requires further study. Depression of the immune response by means of antibodies, when present in concentrations which cannot be determined serologically, has been described in the literature [8].

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