PSEUDOTOLERANCE OBSERVED IN ADULT MICE AFTER REPEATED INJECTIONS OF RAT ERYTHROCYTES

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Immunological areactivity of CC57Br mice was studied after 2 injections of rat erythrocytes. This phenomenon arises only in response to injection of high enough doses of the antigen. The areactivity was shown to be due to extracellular factors (conjecturally antibodies), whereas the lymphocytes of areactive animals when cultivated in vivo exhibit not only normal, but even enhanced immunological potency.

There is a tendency nowadays in the literature to include under the common term of "immunological tolerance" a number of phenomena the distinguishing feature of which is specific areactivity to a particular antigen. It is thereby forgotten that, according to Medawar's definition [5], true immunological tolerance is due to a lesion of the central component of immunological processes, namely the specific reactivity of the lymphocyte population. Careful analysis of phenomena resembling tolerance is thus required in order to establish their true nature.

Among states resembling tolerance, the immunologically specific areactivity of mice of certain lines after double immunization with rat erythrocytes has been described [2].

The object of the present investigation was to study the connection between this phenomenon, on the one hand, and true immunological tolerance and Rowley's phenomenon [6], on the other hand.

EXPERIMENTAL METHOD

Adult CC57Br mice were immunized intravenously with erythrocytes from sheep or from August rats. The animals were sacrificed 4 days later and the number of antibody-forming cells (AFCs) in the spleen determined by the method of local hemolysis in gel [3]. As complement, dried guinea pig complement in a dilution of 1 : 5 was used in the reaction with sheep's erythrocytes and rabbit serum (fresh or kept at −20°C), diluted 1 : 2.5, in the reaction with rat erythrocytes. In some experiments the method of cultivation of spleen cells in vivo [4] in mice irradiated in a dose of 600 R was used. The dose of spleen cells injected was 1×10^8 nucleated cells, injected intravenously. The test dose of antigen was 5×10^8 rat erythrocytes (intravenous injection). The number of AFCs in the recipients' spleen was determined after 5 days.

The results were subjected to statistical analysis: the geometric mean was calculated for the number of AFCs for each experimental group, and its confidence limits (at $P < 0.05$) and the significance of the differences between the results obtained in the comparable groups were determined.

EXPERIMENTAL RESULTS

As Table 1 shows, the immune response of the mice immunized twice with rat erythrocytes was significantly reduced by comparison with the primary response. The difference was observed for all intervals

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TABLE 1. Immune Response of Mice Immunized with 5×10^8 Rat or Sheep Erythrocytes

Number of injections of antigen (in days)	Interval between injections of antigen (in days)	Number of AFCs in spleen after immunization with:	
		rat erythrocytes	sheep erythrocytes
	7	5 521 (3 020—10 001) [12]	64 860 (40 090—105 000) [7]
2	14	8 974 (4 864—16 290) [5]	231 700 (177 800—302 000) [5]
	30	16 900 (8 570—32 340) [17]	—
1	—	69 020 (52 120—91 410) [28]	97 270 (73 790—128 200) [22]

Note: Here and in Table 1, mean values are given together with confidence limits (in parentheses) with a probability of $P < 0.05$; the number in square brackets below is the number of mice in the group.

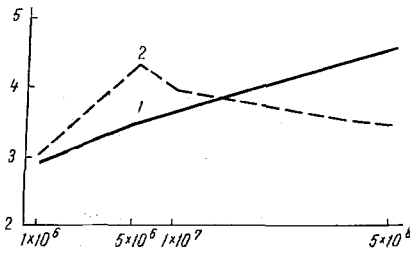

Fig. 1. Number of AFCs in mouse spleen on 4th day after single (1) and repeated (2) injections of various doses of rat erythrocytes. Abscissa, sessional dose of rat erythrocytes; ordinate, number of AFCs (logarithmic scale).

between injections of the antigen tested, but it was most marked when the interval was 7 days. After two injections of sheep's erythrocytes, on the other hand, the immune response was either indistinguishable from the primary response or it was significantly increased.

The quantitative relationships between the immune responses to primary and secondary injection of rat erythrocytes depended on the dose of antigen given on each occasion (Fig. 1). If the sessional doses of antigen were 1×10^6 erythrocytes the immune response was of low intensity both to the first and to the second immunization at an interval of 1 month. A single injection of 5×10^6 erythrocytes led to a state of increased immunological reactivity in the mice: an immune response of secondary type was obtained to revaccination with the same dose after 7-30 days. With a further increase in the sessional dose of antigen to 1×10^7 erythrocytes the difference between the primary and secondary responses disappeared again. Finally, with the largest dose (5×10^8) the immune response to a second injection of antigen was much weaker than that to a single injection. The intensity of the primary immune response rose steadily with an increase in the dose of antigen.

On analysis of the results it was postulated that the character of the response to the second injection, if the doses of rat erythrocytes were not too small, was determined by two factors: sensitization of the lymphocytes by the antigen and the blood level of circulating antibodies after the first immunization. Depending on the relative importance of these two factors, either an increase or a weakened immune response was observed to the second injection of antigen. To verify this hypothesis, the immunological reactivity of spleen cell populations was investigated in mice immunized with 5×10^8 rat erythrocytes and cultivated in vivo in irradiated recipients. Some of the recipients also were immunized 7 days before irradiation with 5×10^8 rat erythrocytes. The results of this series of experiments (Table 2) indicate that the immunological reactivity of the spleen cells of mice immunized 7 days before cultivation (group 2) was statistically significantly higher than the reactivity of the spleen cells of the intact mice (group 4). This increased immunological reactivity of the cell population of previously immunized mice did not reveal itself in previously immunized recipients (group 1). The immune response of the spleen cells of the intact mice also was sharply reduced in immunized recipients (group 3).

Hence, in mice immunized with 5×10^8 rat erythrocytes a factor with a powerful depressant action on the immune response of both intact and sensitized lymphocytes is present. This factor is radioresistant

TABLE 2. Immunological Reactivity of Spleen Cell Populations of Intact and Sensitized Mice Cultivated in Vivo in Irradiated Intact and Previously Immunized Recipients

Group No.	Donors	Recipients	Number of AFCs; in spleen
1	Immune	Immune	146 (51—416) [8]
2	"	Intact	9 705 (5 559—16 940) [7]
3	Intact	Immune	47 (15—150) [8]
4	"	Intact	2 489 (1 574—3 936) [8]
5	—	"	<18 [6]
6	Intact	—	51 290 (12 360—211 800) [7]
7	Immune	—	802 (230—2 799) [6]

Note: Test dose of antigen was injected into all recipients and also into mice of groups 6 and 7.

and evidently consists of antibodies circulating in the animals' blood after the first immunization. The ability of the antibodies to weaken the immune response as by a feedback mechanism has been described previously in other models [1, 8, 9]. The recovery of the immunological reactivity of the cells after their transfer into an unimmunized recipient, observed in the present experiments, does not agree with Rowley's hypothesis [7] that these antibodies act directly on the cell and do not block antigen, as most workers believe.

The phenomenon under investigation is only outwardly similar to immunological tolerance: immunodepression is connected, not with the inhibition of specific reactivity of the population of immunocompetent cells (on the contrary, their immunological reactivity was increased), but with extracellular factors (conjecturally antibodies). It is known [2] that this phenomenon is observed only in some lines of mice. Presumably antibodies of CC57Br mice possess well-marked depressant properties relative to this antigen.

LITERATURE CITED

1. V. I. Levenson and E. V. Chernokhvostova, Zh. Mikrobiol., No. 4, 30 (1967).
2. O. V. Rokhlin et al., Genetika, No. 7, 71 (1966).
3. N. K. Jerne and J. Nordin, Science, 140, 405 (1963).
4. T. Makinodan et al., in: Mechanisms of Antibody Formation, Prague (1960), p. 182.
5. P. B. Medawar, in: Mechanisms of Immunological Tolerance, Prague (1962), p. 17.
6. D. A. Rowley and F. W. Fitch, J. Exp. Med., 120, 986 (1964).
7. D. A. Rowley, J. Exp. Med., 121, 683 (1965).
8. R. J. W. Ryder and R. S. Schwartz, J. Immunol., 103, 970 (1969).
9. T. Tada and K. Okumura, J. Immunol., 106, 1002 (1971).