CHANGES IN THE NUMBER OF ROSETTE-FORMING CELLS IN THE SPLEEN OF MICE TOLERANT TO SHEEP RED CELLS

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As a result of the induction of tolerance by injection of a large dose of sheep red cells and cyclophosphamide, the number of rosette-forming cells (RFCs) in the mouse spleen fell sharply but it recovered again after 14 days. Injection of cyclophosphamide alone had no effect on the relative number of RFCs. After a test injection of red cells the number of RFCs, like the number of plaque-forming cells, increased only slightly in the tolerant animals. A marked immune response for both indices was observed in the control animals (intact mice and mice receiving cyclophosphamide alone). The nature of RFCs in animals tolerant to sheep red cells is discussed.

In recent years reports have been published that the lymphoid tissue of intact and immune animals contains antigen-binding cells detectable by a method based on the immune adhesion of red cells to them (rosette-forming cells, RFCs). Rosette formation has been shown to take place because of receptors specific to this antigen on the surface of lymphoid cells [6, 9, 15]. The dynamics of the number of RFCs in the course of the immune response and their connection with the production of serum antibodies have been studied [7, 17, 20]. There is information in the literature on the character of changes in the number of RFCs in various forms of tolerance [4, 10, 12, 18]. However, few facts are available on tolerance induced by immunodepressants [8].

The object of this investigation was to study the RFC population quantitatively after the production of immunological tolerance in mice to sheep red cells by combined injections of antigen and cyclophosphamide (CP).

EXPERIMENTAL METHOD

Experiments were carried out on $(CBA \times C57BL/6)F_1$ hybrid mice and noninbred albino mice with a mean weight of 18-23 g. Tolerance to sheep red cells was produced by the scheme described earlier [2] by intraperitoneal injection of 6.2×10^9 sheep red cells followed by (43-45 h later) intraperitoneal injection of CP in a dose of 200 mg/kg. Intact mice and mice receiving CP or sheep red cells alone in the same doses were used as the control. The test injection of sheep red cells was given intravenously in a dose of 5×10^8 cells 2 weeks after the induction of tolerance.

The number of 19S plaque-forming cells (PFCs) was determined by the local hemolysis in gel test by Jerne's method [11].

The number of RFCs was determined by the method of Zaalberg [20] in the modification of Shearer and Cudkowicz [17]. A suspension of spleen cells made up in medium No. 199 was washed twice with the same medium during gentle centrifuging (1000 rpm, 5 min) and the concentration was adjusted to 2×10^7 cells/ml. The suspension of sheep red cells was washed 3 times in physiological saline (3000 rpm, 5-10 min) and then resuspended in medium No. 199 to a concentration of 5×10^7 cells/ml. A mixture of equal

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volumes of suspensions (0.5 ml of each) was incubated for 1 h 15 min at 37° C and, after careful resuspension, the number of rosettes was counted under the microscope in a Goryaev's chamber. Cells with 5 or more adherent (compactly bound) red cells on their surface were considered to be rosette-forming. For each mouse at least four chambers were counted and the number of RFCs then calculated in 10^6 nucleated cells and in the spleen as a whole.

Hemolysins and hemagglutinins in the blood were determined in the usual way by double serial dilutions.

EXPERIMENTAL RESULTS

Two series of experiments were carried out on 150 hybrid and 200 noninbred mice. In both series the state of immunological reactivity to sheep red cells was investigated after treatment of the animals by the scheme described above at various times before and after test injection of the antigen. The number of 19S-PFCs and -RFCs was determined in parallel tests in the spleen of the experimental and control animals.

The results of determination of the number of RFCs in the hybrid mice on the 7th and 14th days after the induction of tolerance are given in Table 1. Clearly the number of RFCs, expressed both in 10^6 nucleated cells and in the whole spleen, was much lower by the 7th day in the tolerant mice than in the intact animals and also in the animals receiving an injection of CP ($P < 0.0001$). In the animals receiving CP alone a marked decrease in the number of RFCs compared with the intact mice was observed only when calculated for the whole spleen, because of a sharp decrease in the total number of nucleated cells in the spleen as a result of the action of CP.

Tests on the 14th day showed that at the time of the test injection of antigen the number of RFCs in the tolerant animals was back to the level recorded in the intact mice. Some increase in the number of RFCs in the spleen of the tolerant animals and of the animals receiving CP could be connected with active proliferation of the lymphoid tissue of the spleen taking place by that time.

The production of 19S-PFCs on both the 7th and 14 days of the investigation was very low in the animals of all groups. Titers of hemagglutinins and hemolysins in the blood serum of the tolerant animals did not differ significantly from those in the intact mice (from below 1/10 to 1/20). The number of RFCs and PFCs in the spleen of the animals receiving 6.2×10^9 sheep red cells intraperitoneally was 1-2 orders of magnitude higher than in the mice of all the other groups.

The results of the study of the dynamics of the RFC and PFC populations in the spleen of the hybrid mice at different times after test injection of sheep red cells are given in Fig. 1. This shows that the immune response of the tolerant mice was significantly lower for all indices than the reaction of animals receiving CP alone. On the 6th-10th day the number of RFCs in the animals previously receiving CP was close to the level in the control, immunized mice, whereas in the tolerant animals the number of RFCs was within the limits of the original level or slightly higher.

Hemolysins were absent from the blood sera of the tolerant animals (titer below 1/10 compared with 1/640-1/1280 in the immunized controls), and the hemagglutinin titers were sharply reduced (mean 1/40 and 1/1280, respectively). Comparison of the dynamics of the change in serum hemagglutinin titers with the dynamics of the change in the number of RFCs showed definite correlation between them in the animals of all groups.

Similar changes in all indices were observed in the experiments on noninbred mice. The quantitative differences between the experimental and control groups were a little less marked in the noninbred mice, possibly on account of the lower level of immunological tolerance which they developed.

The results thus demonstrate that after a test injection of antigen the number of RFCs and PFCs and the hemagglutinin and hemolysin titers in the tolerant animals were sharply reduced by comparison with mice of the control groups. The absence of any increase in the number of RFCs in the tolerant animals after a test injection of antigen also was observed by the study of other models of tolerance [12, 18].

It is more difficult to interpret the quantitative changes in the RFC population arising as a result of the induction of tolerance in the animals (before the test injection of antigen). According to some observations [12], the number of RFCs in animals in which tolerance was induced in the early postnatal period subsequently was indistinguishable from normal. Investigations by other workers show that at short

TABLE 1. Number of Rosette-Forming Cells in Spleen of (CBA × C57BL/6)F_1 Mice at Various Times after Induction of Tolerance*

Group of mice	7th day				14th day			
	per 10^6 nucleated cells	P	per spleen	P	per 10^6 nucleated cells	P	per spleen	P
1 (Intact)†	225 (188—270) n=24	—	47 640 (38 370—59 160) n=24	—	—	—	—	—
2 Receiving CP	173 (124—242) n=15	P_2—P_1>0,05	8 770 (5 998—12 820) n=15	P_2—P_1<0,0001	167 (104—267) n=10	P_2—P_1>0,05	53 460 (31 120—91 830) n=10	P_2—P_1>0,05
3 (Tolerant)	43 (27—68) n=13	P_3—P_2<0,0001	2 399 (1 545—3 720) n=13	P_3—P_2<0,0001	201 (132—308) n=10	P_3—P_2>0,05	53 460 (37 070—77 090) n=10	—
4 Receiving 6.2 × 10^9 sheep red cells	895 (575—1393) n=4	P_4—P_1<0,0001	291 700 (167 900—507 000) n=4	P_4—P_1<0,0001	1396 (1222—1596) n=9	P_4—P_1<0,0001	389 000 (311 900—485 300) n=9	P_4—P_1<0,0001

*Geometric mean values and confidence limits at $P < 0.05$ given in this table.

†Combined results obtained by the investigation of RFCs in intact mice on the 7th and 14th days given. n) Number of animals.

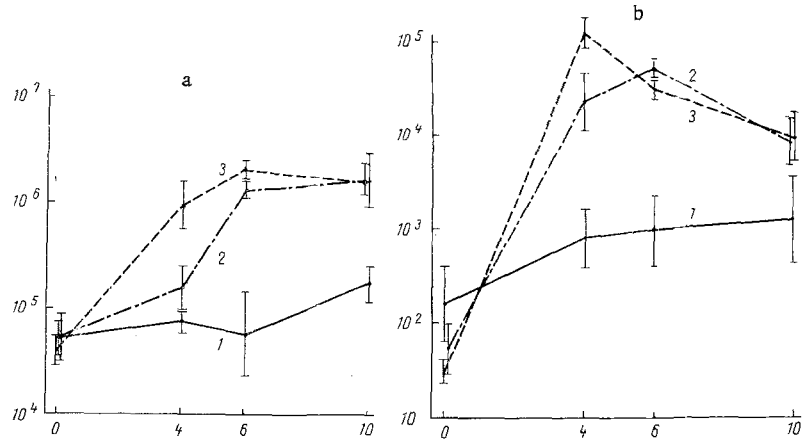

Fig. 1. Dynamics of changes in number of RFCs (a) and PFCs (b) in hybrid mice tolerant to sheep red cells, after test injection of antigen: 1) tolerant mice; 2) animals receiving CP only; 3) intact immunized mice. Abscissa, day of investigation; ordinate, number of RFCs (a) and of PFCs (b).

times after the induction of tolerance in adult animals the number of RFCs in the spleen is significantly lowered [4, 8]. In the present experiments the number of RFCs was significantly lower on the 7th day after the induction of tolerance, but was back to normal by the 14th day. Injection of CP alone did not affect the relative (expressed per 10^6 cells) number of RFCs, in agreement with the observations of Bach and Dardenne [5].

The decrease in the RFC level in the early stages after the induction of tolerance accords with the view that during the induction of tolerance lymphocytes with specific affinity for the particular antigen concerned are eliminated [3, 16]. It is more difficult to explain the restoration of the number of RFCs that takes place later. It is clear from the results described that the animals still remained tolerant at these times. Even 3-4 weeks after the induction of tolerance the specific immunological reactivity of the lymphocyte population was significantly lowered [1].

Despite the restoration of the original RFC level, the inability of the animals to give a complete immune response demonstrates the qualitatively different character of the RFC population in the tolerant animals. Under normal conditions the RFCs include cells of both thymus and bone marrow origin, whose role differs significantly in the formation of the immune response [6, 9, 15]. In the later stages of tolerance a deficiency of competent T-cells can be observed, despite the restoration of the normal number of precursors of antibody-forming cells [13, 14, 19]. The restoration of the total number of RFCs in the present experiments may perhaps reflect this particular phase of tolerance. This hypothesis, of course, requires experimental confirmation.

A certain proportion of the RFC population is also known to consist of cells actively secreting antibodies [6, 7, 12, 17]. The writers have previously pointed out that "incomplete" antibodies, maintaining immunological reactivity against the particular antigen concerned, are found in the blood of animals in the late stages of tolerance [1]. Most probably, therefore, some of the RFCs in tolerant animals are responsible for the production of these antibodies.

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