EFFECT OF VARIOUS FACTORS ON THE FORMATION OF IMMUNOLOGICAL MEMORY

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Experiments on mice in which sheep's red cells were used as antigen showed that the height of the secondary immune response of spleen cells in situ or in adoptive transfer is inversely proportional to the dose of red cells used for primary immunization. Cyclophosphamide, injected into the animals simultaneously with the antigen, stimulates the immune response of the mice to the second injection of red cells, but this effect is observed only when comparatively large doses of antigen are used for priming. The mechanisms of this phenomenon are discussed.

KEY WORDS: *immunological memory; antibodies; cyclophosphamide.*

Immunological memory is a phenomenon whose mechanisms have already been studied on many occasions. One of the most important aspects from the theoretical and practical points of view is that of the factors responsible for maximal development of ability to give a secondary immune response. The size of the first dose of antigen injected is particularly important. Many workers have reported reciprocal relationships between the quantity of antigen used for sensitizing (priming) and the height of the secondary immune response [4, 6, 10, 12]. However, the causes of this reciprocal relationship are still largely unexplained. On the one hand, it could be due to the regulatory function of antibodies formed after priming; on the other hand, it could be the result of qualitative or quantitative changes in the population of immunocompetent cells under the influence of antigen [2, 7, 12, 13].

The object of the present investigation was to determine whether differences in the level of the secondary immune response after sensitization by different doses of antigen are determined by serum factors (antibodies) or by the state of the population of immunocompetent cells.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice weighing 20-26 g. Sheep's red cells (SRBC) injected intravenously twice at intervals of 7 days, were used as the antigen. The dose of antigen for primary immunization varied; the dose at the second injection was 10^6 SRBC. The height of the secondary immune response was assessed by the number of 19S antibody-forming cells (AFC), determined by the local hemolysis in agar method [8], in the spleen of mice on the 4th day after the second injection of antigen.

The secondary immune response also was studied by the adoptive transfer method. A suspension of mouse spleen cells prepared in a glass homogenizer and washed off with medium No. 199 was injected intravenously together with SRBC (10^6) in a dose of $50 \cdot 10^6$ nucleated cells into syngeneic recipients. These recipients had previously been irradiated in the Stebel'-3A apparatus in a dose of 900 R (4 h before transfer) or they had been given an intraperitoneal injection of 200 mg/kg cyclophosphamide (CP), 3-4 h before transfer. The number of AFC in the spleen of the recipient mice was counted by the method mentioned above 5 days later. The results of special experiments showed that the transplanted cells gave an identical response in the recipients prepared by the two different ways.

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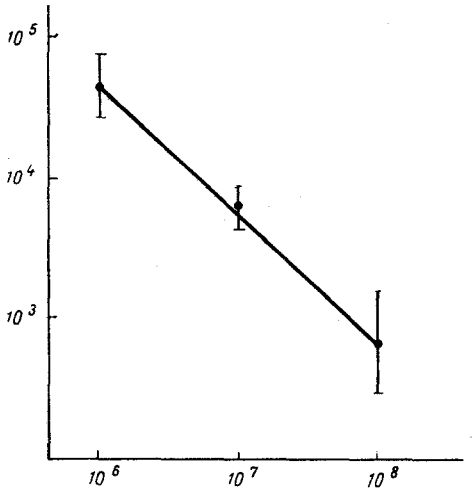

Fig. 1. Secondary immune response of mice previously immunized with various doses of SRBC. Abscissa, dose of SRBC injected at primary immunization; ordinate, here and in Figs. 2 and 3, number of 19S AFC in spleen.

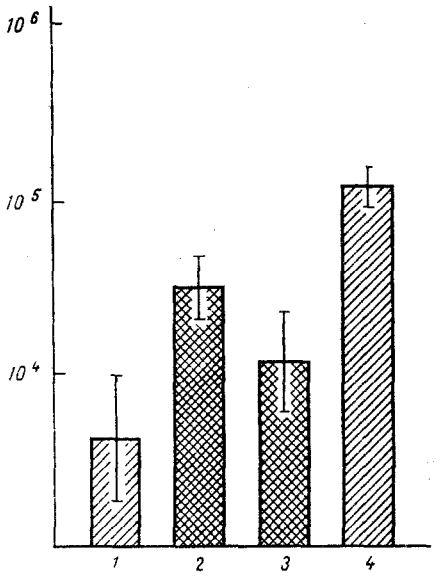

Fig. 2. Immune response of adoptively transferred spleen cells of intact and previously immunized mice. 1, 2) Primary immune response to 10^6 and 10^8 SRBC; 3, 4) secondary immune response of spleen cells primed with 10^8 and 10^6 SRBC.

TABLE 1. Effect of Serum Taken from Mice 7 Days after Immunization with Different Doses of SRBC on Secondary Immune Response

Serum	Number of AFC in spleen of recipient mice
Normal (control)	38 890 (23 440–48 640) $n=10$
Immune, taken from mice receiving:	
10^8 SRBC	32 580 (11 690–90 780) $n=3$
10^7 SRBC	34 120 (24 830±46 880) $n=11$
10^6 SRBC	61 580 (46 130–82 040) $n=14$

Legend. n) Number of animals.

The results were subjected to statistical analysis by Student's t test (calculation of the geometric mean and confidence intervals at $P < 0.05$).

EXPERIMENTAL RESULTS

The results of the experiments of series I showed that with an increase in the dose of antigen at the first injection the immune response to the second injection of SRBC diminished progressively (Fig. 1). With the doses of antigen used, the number of AFC formed as the result of the secondary immune response was an inverse linear function of the dose of SRBC.

The titers of antibodies (hemagglutinins) formed in the mice after primary immunization and detectable at the time of the second injection of antigen (7th day) were 1:40-1:160, 1:80-1:160, and 1:320 following immunization with 10^6, 10^7, and 10^8 SRBC respectively. The effect of these antisera on the level of the secondary immune response was studied in the ex-

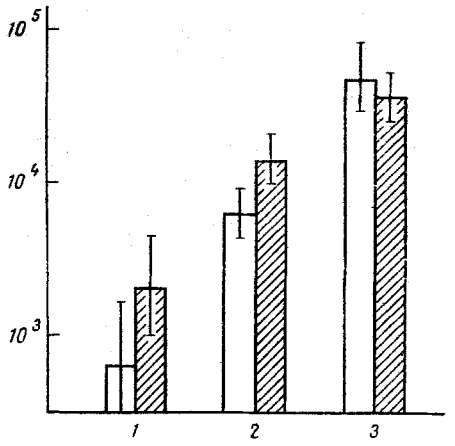

Fig. 3. Effect of CP on capacity for secondary immune response. 1, 2, 3) Secondary immune response of mice receiving 10^8, 10^7, and 10^6 SRBC at primary immunization. Unshaded columns — response of mice receiving only SRBC at primary immunization, shaded columns — response of mice receiving CP plus SRBC at primary immunization.

periments of series II. For this purpose, mice immunized twice with 10^6 SRBC at an interval of 1 week received an injection of 0.35-0.5 ml of the test sera 24 h before the second injection of antigen. The results of determination of the number of AFC in the spleen of these animals are given in Table 1.

Table 1 shows that the blood serum of mice immunized with 10^7 and 10^8 SRBC has no effect on the secondary immune response, whereas the serum of animals receiving 10^6 SRBC has a stimulating action, slight but significant in degree, compared with the control (injection of normal serum).

To rule out any possible effect of circulating antibodies on the secondary immune response the reactivity of spleen cells of mice sensitized with different doses of antigen was studied in adoptive transfer in the experiments of series III. The results are given in Fig. 2.

The number of AFC formed in the spleen of the recipient mice was considerably (by an order of magnitude) less than after transfer of cells from mice immunized primarily with 10^8 SRBC, compared with the effect observed by transplantation of cells from animals receiving 10^6 SRBC (Fig. 2). The secondary response after priming with a relatively high dose of antigen (10^8 SRBC) also was lower than the primary response to the same dose.

The immunodepressive agent CP, if injected in doses completely suppressing the primary immune response, is known to have only a relatively weak effect on the development of immunological 19S memory if it is injected on the day of sensitization with the optimal dose (10^6 SRBC) of antigen [1]. In the experiments of series IV the effect of CP on formation of the ability to give a secondary immune response when different doses of red cells were used for sensitization was investigated. For this purpose, 3-4 h before the injection of antigen, the mice were given an intraperitoneal injection of CP in a dose of 100 mg/kg. The mice were given 10^6 SRBC 7 days later. The results of determination of the secondary immune response are given in Fig. 3.

In the case of injection of comparatively large doses of antigen (10^7 and 10^8 SRBC) for primary immunization, administration of CP facilitated the development of immunological memory (Fig. 3): The response of the animals to the second injection of antigen was doubled and trebled (although it still remained depressed). If, however, a small dose (10^6 SRBC) was given for sensitization, an accompanying injection of CP had no effect.

It can be concluded from the results of these investigations that weakening of the formation of immunological memory in the system of cells producing 19S antibodies, if large doses of antigen are used for priming, is due not to feedback mechanisms affected through circulating antibodies [2, 13], but to a change in the state of the population of immunocompetent cells. Evidence in support of this conclusion is given by the similarity between the results of the study of the secondary immune response in situ and in culture in vivo, as well as by the absence of any effect of specific antisera on the realization of immunological memory. It can accordingly be postulated that the stimulating action of CP is not due to suppression of antibody production in response to the first injection of relatively high doses of antigen. Such an effect may be due to a factor formed in the body under the influence of CP, which stimulates immunogenesis [11]. Another possibility is that CP inhibits the production

of suppressor cells formed as the result of the injection of SRBC in sufficiently high doses [3, 14, 15]. It has been suggested, in particular [5, 9], that this mechanism lies at the basis of the potentiating action of CP during the development of hypersensitivity of delayed type. Finally, it can tentatively be suggested that CP changes the ratio between the number of primed T and B cells, thus ensuring their more efficient cooperation in the secondary immune response. The experimental verification of these hypotheses will shed light on certain aspects of the mechanisms of formation and realization of immunological memory.

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