# PRIMARY AND SECONDARY IMMUNE RESPONSE IN ANIMALS AFTER SPONTANEOUS LOSS OF TOLERANCE

K. A. Kazaryan, M. V. Tat'yan,
S. D. Kirakosyan, and L. N. Fontalin

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The primary and secondary immune response was studied in mice in which tolerance was induced by injection of sheep's erythrocytes and cyclophosphamide (CP). In the early stages (from 1 to 8 weeks) after induction the mice were immunized with sheep's erythrocytes either in a single dose of $5 \cdot 10^8$ cells or in two doses each of $1 \cdot 10^6$ cells. Both methods of immunization gave equal results in the control animals. In the experimental animals the process of formation and (or) realization of immunological memory was impaired to a greater degree and recovered more slowly than ability to give a primary response.
KEY WORDS: immunological tolerance; cyclophosphamide; immunological memory.

As a result of the combined administration of sheep's erythrocytes and the immunodepressant cyclophosphamide (CP) to mice a state of partial immunological tolerance arises, and in the course of time it disappears spontaneously [4, 5, 10]. The dynamics of the loss of tolerance is usually judged by the reaction of the animals to a single injection of an optimal dose of antigen, but this gives only incomplete information about this process.

The object of this investigation was to compare two indices of recovering immunoreactivity: 1) the capacity for the formation and realization of immunological memory and 2) ability to give a primary immune response.

## EXPERIMENTAL METHOD

Experiments were carried out on (CBA × C57BL/6)$F_1$ mice weighing 20-22 g. Tolerance was induced by intraperitoneal injection of $6.2 \times 10^9$ sheep's erythrocytes followed (after 45-48 h) by injection of CP in a dose of 200 mg/kg [4]. At various times after the induction of tolerance some of the experimental and control animals (receiving either CP only or nothing at all) $5 \cdot 10^8$ sheep's erythrocytes were injected intravenously and 4 days later the number of antibody-forming cells (AFC) in the spleen was counted by Jerne's method [11]. Another group of animals received an intravenous injection of $1 \cdot 10^6$ sheep's erythrocytes; 7 days later the injection was repeated, and after a further 4 days the number of AFC in the spleen was counted.

## EXPERIMENTAL RESULTS

The immunoreactivity of the animals receiving CP only was almost completely restored as early as after 2 weeks The results of the investigation of the control animals at all times (1-8 weeks after injection of CP) are therefore pooled in Fig. 1. As Fig. 1 shows, after two injections of $1 \cdot 10^6$ sheep's erythrocytes into intact mice a rather more intensive immune response was observed than after a single injection of $5 \cdot 10^8$ sheep's erythrocytes. Mice receiving CP previously reacted to both methods of immunization absolutely identically. After preliminary injection of CP the reactivity of the animals to a single injection of $5 \cdot 10^8$ sheep's erythrocytes was unchanged and their response to two injections of $1 \cdot 10^6$ sheep's erythrocytes was reduced very slightly.

Laboratory of the Molecular Bases of Immunogenesis, Institute of Experimental Biology, Academy of Sciences of the Armenian SSR. Laboratory of Immunological Tolerance, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 6, pp. 716-718, June, 1977. Original article submitted November 10, 1976.

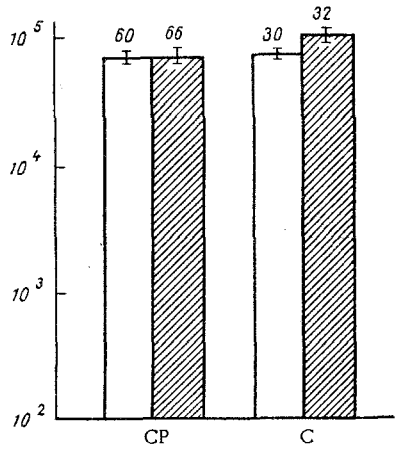


Fig. 1

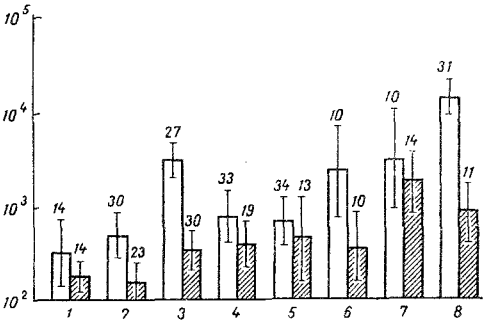


Fig. 2

Fig. 1. Comparative effectiveness of double immunization with $1 \cdot 10^6$ sheep's erythrocytes and a single immunization with $5 \cdot 10^8$ sheep's erythrocytes. Ordinate, number of AFC in spleen 4 days after last injection of antigen. CP) Animals previously (1-8 weeks beforehand) receiving CP, 200 mg/kg; C) control animals. Unshaded columns represent immune response to single injection of $5 \cdot 10^8$ sheep's erythrocytes, shaded columns represent response to two injections each of $1 \cdot 10^6$ sheep's erythrocytes. Numbers above columns give number of mice.

Fig. 2. Dynamics of recovery of immunoreactivity judged from differences between indices after induction of tolerance. Ordinate, number of AFC in spleen 4 days after last injection of antigen; abscissa, stages of induction of tolerance (in weeks). Unshaded columns show immune response to single injection of $5 \cdot 10^8$ sheep's erythrocytes. Shaded columns show response to two injections of $1 \cdot 10^6$ sheep's erythrocytes. Numbers above columns indicate number of mice.

The immune response of the experimental animals is illustrated in Fig. 2. As Fig. 2 shows, the recovery of immunological reactivity took place irregularly. The first clear signs of recovery were observed 3 weeks after the induction of tolerance. During the next 2-3 weeks this process ceased or even went into reverse, to become reactivated at the latest times of observation (6-8 weeks after induction of tolerance). However, even after 8 weeks the immunoreactivity of the experimental animals remained weaker than that of the controls by both methods of immunization (Figs. 1 and 2).

The experimental animals gave a weaker response to two injections of $1 \cdot 10^6$ than to a single injection of $5 \cdot 10^8$ sheep's erythrocytes (Fig. 2). This difference was statistically significant in tests carried out 2, 3, 6, and 8 weeks after induction of tolerance, but at other times it amounted to no more than a tendency. The significance of this difference is increased by the fact that in the control animals, on the other hand, two injections of $1 \cdot 10^6$ sheep's erythrocytes proved more effective (Fig. 1). It should also be remembered that the second injection of $1 \cdot 10^6$ sheep's erythrocytes was given 1 week later than in the mice receiving a single injection of $5 \cdot 10^8$ sheep's erythrocytes, i.e., at a time when immunoreactivity could be expected to have recovered more completely.

The results thus indicate that the ability of partially tolerant animals, at the stage of gradual loss of tolerance, to form and realize immunological memory in response to injection of small doses of antigen is much more severely impaired than their ability to give a primary response to injection of a larger dose of antigen. Several explanations of this fact may be suggested. Immunological memory formed in response to injection of a small dose of antigen is known to be more T-dependent than the immune response to a massive injection of the same antigen. Meanwhile, when tolerance is induced with CP mainly T lymphocytes are damaged [1, 3, 5, 12, 13]. It is also known that small doses of antigen selectively stimulate cell clones whose receptors have increased affinity for that particular antigen [6, 9, 14, 15], whereas in tolerant animals the most avid clones are absent or suppressed [7, 8, 14]. Finally, the possibility cannot be ruled out that the formation and realiza-

tion of immunological memory when small doses of antigen are used are more vulnerable to the action of blocking antibodies which appear in the animals 3-4 weeks after the induction of tolerance [2].

All the factors indicated above may be responsible for the selective impairment of the formation and (or) realization of immunological memory in partially tolerant animals compared with the primary response. The wave-like course of recovery of immunoreactivity in the tolerant animals, observed previously, is interesting. The important point is that the secondary fall of the curve of recovery of reactivity occurs at the fourth to fifth week, i.e., it coincides with the times of appearance of blocking antibodies. This suggests that blocking antibodies may play the role of a factor delaying recovery of immunoreactivity and modifying the dynamics of the process.

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