REACTION OF IMMUNOLOGICALLY TOLERANT MICE TO THE POLYCLONAL STIMULATOR SALMOZAN

T. K. Kondrat'eva, L. N. Fontalin,
M. A. Tumanyan, I. A. Kondrat'eva,
N. G. Sinilova, and T. K. Novikova

UDC 615.276.4.105.46.076.9

KEY WORDS: immunologic tolerance; cyclophosphamide; B lymphocytes; polyclonal stimulators; salmozan.

The mechanisms of immunologic tolerance to thymus-dependent and thymus-independent antigens, obtained with the aid of cyclophosphamide (CP), have been discussed for several years. In particular, the problem of damage to antigen-specific B cells in this form of tolerance is not yet clear [4, 7, 8, 12, 19]. It was shown previously that the immunostimulator salmozan activates B lymphocytes of intact mice nonspecifically (polyclonally) [1, 5, 9].

The aim of this investigation was to study the response of antigen-specific B cells of tolerant mice to salmozan.

EXPERIMENTAL METHOD

Hybrid male (CBA × C57BL/6)F_1 mice weighing 18-20 g were obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR. Immunologic tolerance was induced by injecting $6.2 \cdot 10^9$ sheep's red blood cells (SRBC) intraperitoneally into mice, and two days later this was followed by intraperitoneal injection of 200 mg/kg of CP (from Saransk Medical Preparations Factory). After 7-8 days the experimental or control mice (receiving CP alone or nothing) were given an injection of SRBC ($5 \cdot 10^8$ cells, intravenously) or of E. coli lipopolysaccharide (LPS, from Difco, USA), in a dose of 50-100 μg, intravenously, or of salmozan (Sal), a polysaccharide of the somatic O-antigen of Salmonella typhi Ty_2, in a dose of 100 μg intraperitoneally. Sal was isolated by the aqueous phenol method followed by careful hydrolysis with acetic acid [2]. Sal contained neither lipids nor protein. Preliminary experiments verified the absence of serologically determinable cross-reaction between Sal, LPS, and SRBC. In some experiments the experimental mice were injected initially with SRBC, and with Sal 24 h later. Three days after injection of Sal or LPS or 4 days after injection of SRBC the number of antibody-forming cells (AFC) against SRBC in the spleen was counted in the animals by Jerne's method. In parallel tests the number of AFC was determined in animals not receiving the test injection of antigen or of the polyclonal activator. The results were subjected to statistical analysis with calculation of the geometric mean M_g and its confidence interval at the $P < 0.05$ level.

Some experiments were performed on B mice. For this purpose adult mice were thymectomized and irradiated 2-3 weeks later in a dose of 8.5 Gy on the EKU-50 (^{60}Co) apparatus with a dose rate of 0.34 Gy/min. Each mouse received an injection of 10^6-$2 \cdot 10^6$ embryonic liver cells from syngeneic donors 1-3 h after irradiation. The B mice obtained in this way were used in the experiments 4-6 weeks later.

EXPERIMENTAL RESULTS

The results of the experiments of series I are summarized in Table 1. Injection of Sal caused the number of AFC against SRBC to be increased by 20-100 times both in mice not treated beforehand in any way (group No. 6) and in mice receiving preliminary CP (No. 5). This nonspecific polyclonal response was 20-30 times

N. F. Gamaleya Research 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 P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 6, pp. 714-716, June, 1985. Original article submitted June 11, 1984.

0007-4888/85/9906-0772$09.50

TABLE 1. Response of Tolerant Mice to Nonspecific (Sal) and Specific (SRBC) Stimuli

Group No.	Preliminary injections	Test injection	Number of mice	Number of AFC per 10^6 cells	Number of AFC per spleen
1	SRBC + CP	—	16	⩽0,28 (<0,6)	⩽62 (<129)
2	CP	—	17	⩽0,15 (<0,22)	⩽42 (<65)
3	—	—	12	⩽0,13 (<0,18)	⩽23 (<30)
4	SRBC + CP	Sal	24	2,2 (1,4—3,4)	555 (351—876)
5	CP	Sal	18	6,2 (4,4—8,9)	1 763 (1 155—2 689)
6	—	Sal	19	6,0 (3,3—10,9)	1 836 (1 211—2 784)
7	SRBC + CP	SRBC	15	⩽0,26 (0,41)	<147 (<317)
8	CP	SRBC	18	124 (84—181)	37 827 (26 160—54 747)
9	—	SRBC	12	225 (179—283)	60 752 (46 725—79 150)

Legend. Data shown as geometric mean M_g and confidence intervals.

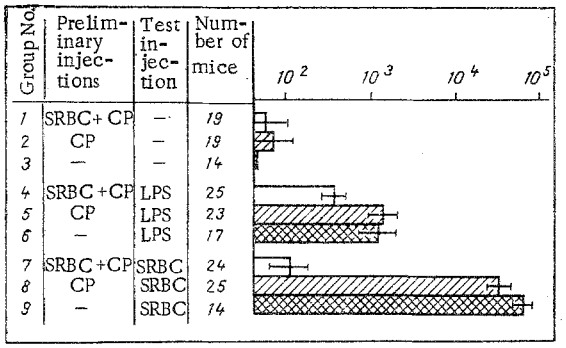

Fig. 1. Response of tolerant mice to E. coli LPS. Horizontal axis here and in Figs. 2 and 3: number of AFC to SRBC in spleen.

weaker than the immune response to the specific immune stimulus (Nos. 8 and 9). Preliminary injection of SRBC and CP induced tolerance to the test injection of SRBC (No. 7). The response of the tolerant mice to Sal is the most interesting (No. 4). It will be clear from Table 1 that Sal induced a significant (by one order of magnitude) increase in the number of AFC to SRBC in tolerant mice. However, the response of tolerant mice to Sal was 3 times weaker than the response of control animals receiving CP alone or nothing at all beforehand (No. 4 compared with Nos. 5 and 6).

Similar patterns also were observed in the experiments of series II in which another polyclonal stimulator, namely LPS, was used instead of Sal (Fig. 1).

Tolerance to the experimental test system thus extends partially also to the ability of lymphocytes of the corresponding specificity (anti-SRBC) to respond to nonspecific stimuli, although this areactivity is weaker than that to the specific antigen.

It was important to discover whether this weakened reactivity to the polyclonal stimulator was due to damage to the corresponding B cells or whether it was mediated by regulatory T cells. To study this problem experiments were carried out on B mice (Fig. 2).

When injected into control B mice (intact or receiving CP beforehand), Sal caused an increase in the number of AFC against SRBC in the spleen (Fig. 2). The polyclonal effect of Sal is thus thymus-independent. In-

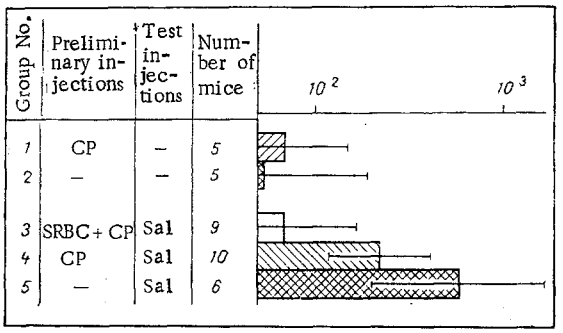

Fig. 2. Response of tolerant B mice to salmozan.

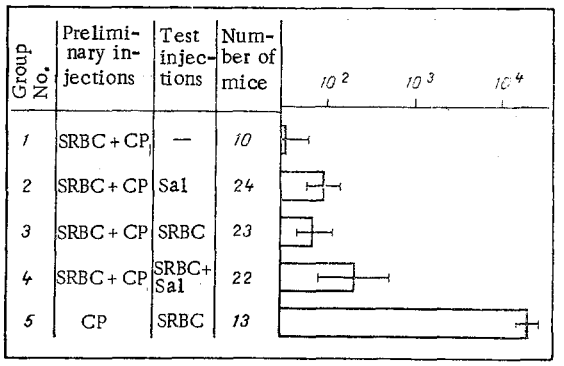

Fig. 3. Response of tolerant mice to successive injections of antigen and salmozan.

jection of Sal into tolerant B mice either gave no effect whatever, or the effect was much weaker than when Sal was injected into the control B mice ($t = 2.7$; $P < 0.05$). Areactivity of the tolerant mice to Sal was thus not mediated by regulatory T cells.

In the experiments of series III the effectiveness of consecutive injections of SRBC and Sal into tolerant mice was studied (Fig. 3). The effect of consecutive injections of SRBC and Sal into tolerant animals did not differ significantly from the effect of Sal alone. This effect was two orders of magnitude weaker than the immune response of the control animals. Thus no loss of tolerance was observed as a result of consecutive injections of the antigen and polyclonal stimulator.

The experimental results agree with data obtained previously [3, 7] on the mixed character of this form of tolerance. The weaker ability of B cells of tolerant animals (compared with the control) to respond to polyclonal stimulators of antibody production against the tolerogen is evidence of their injury in the course of induction of tolerance. This deficiency of the response of antigen-specific B cells to Sal cannot be due to the absence of regeneration of membrane immunoglobulins, as has been claimed [12], since the targets for polyclonal action of microbial polysaccharides and lipopolysaccharides are cell receptors of a different (nonimmunogolobulin) nature [10, 14, 16]. Weakened ability to respond to polyclonal stimulation also has been observed in certain other forms of B-cell tolerance [11, 17, 18, 20].

Nevertheless, in our own experiments tolerant mice reacted (although less strongly than the controls) to polyclonal activators; this response, moreover, was sometimes stronger than the response to the specific antigenic stimulus (Table 1, Fig. 1). This may be explained on the basis of previous data showing the absence of specific T helpers [3, 6, 7, 13, 15], essential for the response to thymus-dependent antigen, but not for the response to polyclonal activators, in tolerant animals.

The ability of Sal to potentiate the resistance of irradiated animals to the pathogenic microflora was demonstrated previously [5]. The results of the present investigation suggest that Sal may also abolish the undesirable side effects of certain antitumor preparations (including CP), by activating both antitumor immunity and also the resistance to infectious diseases, which often complicate chemotherapy, within certain limits.

LITERATURE CITED

1. S. K. Abazbekov and M. A. Tumanyan, Byull. Éksp. Biol. Med., No. 1, 58 (1977).
2. A. P. Duplishcheva, K. K. Ivanov, and N. G. Sinilova, Radiobiologiya, No. 2, 243 (1965).
3. I. A. Kondrat'eva, T. K. Novikova, and L. N. Fontalin, Byull. Éksp. Biol. Med., No. 9, 314 (1979).
4. T. K. Novikova, I. A. Kondrat'eva, and L. N. Fontalin, Byull. Éksp. Biol. Med., No. 1, 27 (1979).
5. M. A. Tumanyan, A. P. Duplishcheva, M. S. Gordeeva, et al., in: Advances in Immunology, ed. O. V. Baroyan [in Russian], Moscow (1977), pp. 113-119.
6. L. N. Fontalin, T. K. Novikova, I. A. Kondrat'eva, et al., Byull. Éksp. Biol. Med., No. 4, 445 (1976).
7. L. N. Fontalin and L. A. Pevnitskii, Immunological Tolerance [in Russian], Moscow (1978).
8. L. N. Fontalin and T. B. Prigozhina, Byull. Éksp. Biol. Med., No. 1, 33 (1979).
9. L. N. Fontalin, M. A. Tumanyan, T. K. Kondrat'eva, et al., Zh. Mikrobiol., No. 10, 68 (1983).
10. A. Coutinho, L. Forni, and T. Watanabe, Eur. J. Immunol., 8, 63 (1978).
11. E. Diener, U. E. Diener, C. A. Waters, et al., in: Cellular and Molecular Mechanisms of Immunologic Tolerance, T. Hraba and M. Hasek, eds., New York (1981), pp. 231-238.
12. J. G. Howard and F. L. Shand, Immunol. Rev., 43, 43 (1979).
13. A. Many and R. S. Schwartz, Proc. Soc. Exp. Biol. (New York), 133, 754 (1970).
14. F. Melchers, J. Andersson, W. Lernhardt, et al., Immunol. Rev., 52, 89 (1980).
15. J. F. A. P. Miller and G. F. Mitchell, J. Exp. Med., 131, 675 (1970).
16. G. Möller, Cold Spring Harbor Symp. Quant. Biol., 41, 217 (1977).
17. G. J. V. Nossal and B. L. Pike, J. Exp. Med., 148, 1161 (1978).
18. D. E. Parks and W. O. Weigle, J. Immunol., 124, 1230 (1980).
19. J. A. Remshaw, P. A. Bretscher, and C. R. Parish, Eur. J. Immunol., 7, 180 (1977).
20. D. W. Scott, M. Venkataraman, and J. J. Jandinsky, Immunol. Rev., 43, 241 (1979).

CYTOTOXIC ANTIBODIES AGAINST THYMOCYTE ANTIGENS IN RHEUMATIC FEVER AND OTHER DISEASES

T. A. Danilova, N. G. Guseva, and É. N. Kosmatova

UDC 616-002.77-092:612.112.94.017.4

KEY WORDS: cytotoxic antibodies; thymocytes; rheumatic fever; autoimmune process.

Cytotoxic antibodies against lymphocytes (LCA) are found in several autoimmune diseases: systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), systemic scleroderma (SSD), in certain virus infections, and also in normal blood donors [12, 15, 16]. LCA are often directed against HLA antigens and constitute the basis of HLA typing, but they may react with other antigens of T and B cells, including antigens of autologous lymphocytes [12, 16]. In most cases they are cold and belong to the IgM class, but antibodies reacting at 37°C, most frequently IgG, are found. There is evidence that in some diseases LCA take part in disturbing regulation of the immune response, for they can inactivate or eliminate suppressor T cells [10].

Cytotoxic antibodies against thumocyte antigens have received much less study. Spontaneously appearing thymocytotoxic antibodies are found in the sera of various lines of mice [13]. In man cytotoxic antibodies against mouse thymocytes have been found in patients with schizophrenia [3, 4]. In autoimmune diseases there have been only isolated studies of this type. Lavastida et al. [11] found antibodies against mouse thymocytes in SLE, whereas Steiner et al. [14] found them against human thymocytes in patients with RA. At the same time, it has

N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR. Institute of Rheumatology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 6, pp. 716-718, June, 1985. Original article submitted October 12, 1984.