MECHANISMS OF POLYCLONAL TOLERANCE INDUCED BY LIPOPOLYSACCHARIDE AND CYCLOPHOSPHAMIDE

T. K. Kondrat'eva, L. N. Fontalin, N. V. Mikheeva, and V. Holan

UDC 612.017.4.014.46:/615.276.4+615.277.3

KEY WORDS: immunologic tolerance; polyclonal stimulators; cyclophosphamide; lipopolysaccharide.

The writers showed previously that successive injections of polyclonal stimulators [lipopolysaccharide (LPS), salmosan] and of cyclophosphamide (CP) induce a unique kind of polyclonal tolerance [3]. Inhibition of the polyclonal response to various nonspecific stimulators and also significant weakening of antibody formation in response both to the corresponding LPS and to an unrelated antigen (sheep's red blood cells — SRBC), are observed under these circumstances. No such effect has been observed in animals receiving LPS only or CP only. The areactivity was not connected with the presence of suppressor cells or of suppressor factors in the serum of the experimental animals [1, 3].

The aim of this investigation was to continue the study of the mechanisms and cellular substrate of polyclonal tolerance.

EXPERIMENTAL METHOD

(CBA × C57BL/6)F_1 male hybrid mice weighing 18-20 g, obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR, were used in the experiments.

LPS from *Serratia marcescens* (LPS_{sm}, from Difco, USA) and LPS from *Brucella abortus* (LPS_{ba}), isolated and presented by Dr. V. E. Malikov, were used as the polyclonal stimulators. SRBC were used as the antigen.

Polyclonal tolerance was induced by intravenous injection of LPS in a dose of 50 μg, followed 2 days later by intraperitoneal injection of CP in a dose of 200 mg/kg. To determine the duration of polyclonal tolerance, at various times after treatment with the tolerogen, the mice were given an intravenous injection of 50 μg of LPS_{ba}, and the number of antibody-forming cells (AFC) to SRBC was determined 3 days later by Jerne's method.

The cytotoxic test was set up by the usual method. Splenocytes of the experimental mice were used as targets. The cell suspension was freed from erythrocytes and dead cells beforehand. The cell suspension was treated with monoclonal antibodies to Thy-1.2 (clone F7D5) or with rabbit antiserum to mouse IgG (generously provided by E. V. Sidorova) with rabbit complement. After incubation for 45 min at 37°C the suspension was stained with a mixture of solutions of trypan blue and eosin and the percentage of stained (dead) cells was counted in a Goryaev counting chamber.

The cooperative test was set up by the method described previously [12]. For this purpose, 10^7 bone marrow cells, 5×10^7 thymocytes, and 10^7 splenocytes from intact or polyclonally tolerant donors were injected intravenously into lethally irradiated (dose 9 Gy) recipients. At the same time, 2×10^6 SRBC per mouse were added to the cell suspension. After 4 days 5×10^8 SRBC were injected intraperitoneally into the experimental mice and the number of AFC to SRBC was determined 4 days later by Jerne's method.

The delayed-type hypersensitivity (DTH) test was carried out by the methods in [3, 9]. Mice subjected to tolerogenic treatment and control (intact or receiving CP only) animals were each given an intravenous injection of 0.5×10^6 SRBC; 4 days later, 10^8 SRBC were injected into a hind foot pad, and physiological saline was injected into the opposite limb. After 24 h

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. N. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 10, pp. 483-485, October, 1987. Original article submitted November 27, 1986.

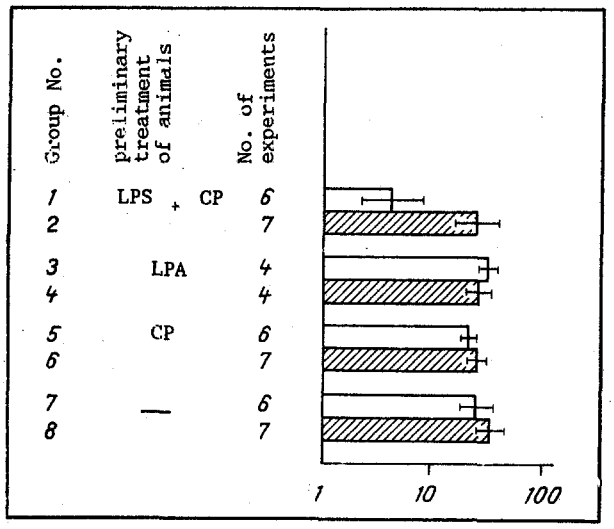

Fig. 1. Content of Thy-2.1$^+$- and Ig$^+$-cells in spleen of polyclonally tolerant mice. Horizontal axis — percentage of dead splenocytes after treatment with antibodies to mouse Ig (unshaded columns) and to Thy-1.2-antigen (shaded columns) in the presence of complement.

the thickness of both limbs was measured with a micrometer. The level of the DTH response was determined as the difference between the size of the edema in the experimental and control limbs.

EXPERIMENTAL RESULTS

In the experiments of series I the duration of polyclonal tolerance was studied. For this purpose, at different times after tolerogenic treatment (7, 14, and 21 days) the polyclonal response to LPS was estimated from the number of AFC to SRBC. Experimental mice were tested with LPS_{ba}, which differs from LPS_{sm} in both the lipid and the polysaccharide region [11]. Mice receiving CP only, and a reacting injection of LPS_{ba} were used as the controls. The experimental results showed that a significant difference between groups of mice subjected to tolerogenic treatment and receiving CP only was maintained for 2 weeks. By the 3rd week the level of the polyclonal response was the same in both groups of animals.

The next task in the work was to determine directly the levels and functional activity of T and B cells in the spleens of the polyclonally tolerant mice. In the experiments of series II the level of B cells (Ig$^+$) and T cells (Thy-1.2$^+$) in the spleen of the polyclonally tolerant animals was determined by the cytotoxic test. As will be clear from Fig. 1, neither LPS nor CP separately caused any significant change in the level of both Ig$^+$- and Thy-1.2$^+$-cells (groups Nos. 3-6) 7 days after injection of CP. Conversely, successive injection of LPS and CP caused a significant decrease in the number of Ig$^+$-cells (group No. 1), whereas the number of Thy-1.2$^+$-cells was unchanged. Thus in animals subjected to tolerogenic treatment with LPS and CP, a deficiency of B cells, carrying a cell membrane-associated immunoglobulin, was observed.

In the experiments of series III the functional activity of T and B cells of polyclonally tolerant animals was studied in the cooperative test. The ability of the splenocytes of the experimental animals to interact with bone marrow cells or thymocytes of intact donors in response to SRBC was investigated in syngeneic lethally irradiated recipients. Recipients receiving either bone marrow cells, or thymocytes, or splenocytes of intact or tolerant donors served as the control.

As Fig. 2 shows, during transfer of splenocytes of tolerant donors to lethally irradiated recipients, a greatly weakened immune response was observed to SRBC compared with that found on transfer of splenocytes from intact donors (groups Nos. 1 and 5). Addition of thymocytes of intact donors to splenocytes of tolerant donors gave no significant increase in the number of AFC to SRBC (group No. 3). Conversely, addition of bone marrow cells to splenocytes of tolerant mice led to restoration of the immune responses to the level of the response of splenocytes from intact donors (groups Nos. 2 and 5).

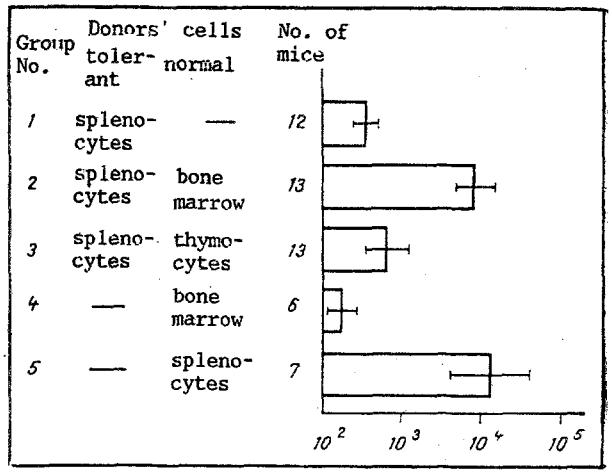

Fig. 2. Investigation of splenocytes of polyclonally tolerant mice in the cooperative test. Horizontal axis — number of AFC to SRBC 8 days after transplantation of donors' cells into lethally irradiated recipients.

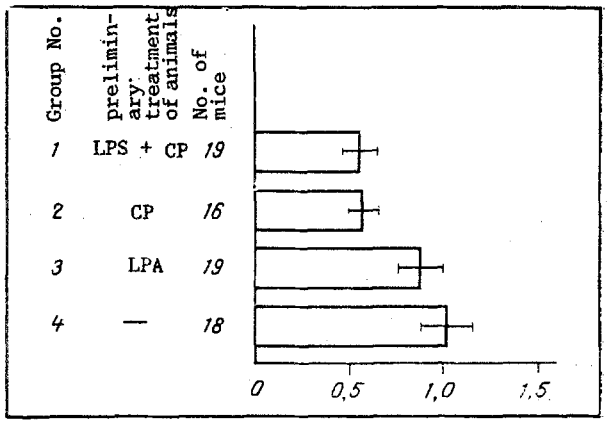

Fig. 3. DTH reaction to SRBC in polyclonally tolerant animals. Horizontal axis — degree of edema of the limb after reacting injection of antigen (in mm).

Thus in the last series of experiments coincident results were obtained, indicating a deficiency of immunologically active B cells in polyclonally tolerant animals. Neither T helper-cell activity nor the total number of T cells was changed in the experimental animals.

In the experiments of series IV the process of formation of the DTH reaction was investigated in polyclonally tolerant mice. SRBC were used as the antigen. It will be clear from Fig. 3 that injection of CP caused a certain fall in the level of DTH to SRBC (group No. 2) compared with the response in animals receiving only LPS beforehand (group No. 3) or in intact mice (group No. 4). The DTH response in animals subjected to complete tolerogenic treatment (LPS + CP, group No. 1) was indistinguishable from that of mice receiving CP only. Thus polyclonal tolerance did not change the formation of DTH effectors to "foreign" antigen.

The results thus show that consecutive injections of LPS and CP induce a state of nonspecific areactivity, which lasts at least 2 weeks. This form of areactivity is due to a general deficiency of B cells. Immunoreactivity of the T cells to foreign antigens and their total number are unaffected under these circumstances.

We know that CP, in large doses, damages B cells [6], but this effect is short-lasting. In fact, in the present experiments the number of Ig^+-cells in the spleen of animals receiving CP 7 days previously did not differ significantly from the control (Fig. 1). The B-cell immunodeficiency arising as a result of consecutive injection of LPS and CP cannot therefore be explained by the direct injurious action of CP on resting B cells. No such effect was observed likewise after administration of LPS without CP (Fig. 1).

The B-cell immunodeficiency arising as a result of consecutive injections of LPS and CP can be explained by a combination of two facts: the mitogenic action of LPS on B cells [5] and selective sensitivity of rapidly dividing lymphoid cells, such as lymphoma cells, to CP [6]. CP evidently selectively eliminates B cells which have become involved in proliferation by LPS. A similar mechanism is postulated for specific immunologic tolerance induced by consecutive injections of an antigen and CP [2, 8, 13]. The difference is that the majority of antigens stimulate a very small part of the total lymphocyte pool, whereas no fewer than 40% of B cells are sensitive to the mitogenic action of LPS [8, 13]. It is this which determines the nonspecific character of polyclonal tolerance and the general deficiency of B cells.

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