NATURE OF IMMUNODEFICIENCY INDUCED BY INJECTION OF LECTINS AND CYCLOPHOSPHAMIDE

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It was shown previously [2] that consecutive injections of T-cell mitogens (lectin, LcA; concanavalin A, con A) and cyclophosphamide lead to inhibition of the T-dependent immune response (antibody production against sheep's red blood cells — SRBC, the formation of delayed-type hypersensitivity — DTH, and its suppressors), while preserving the immune response to thymus-independent antigen (lipolysaccharides — LPS).

The aim of the present investigation was to study the mechanisms of the observed anergy.

EXPERIMENTAL METHOD

Male (CBA × C57BL/6)F_1 mice weighing 18-20 g, obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR, were used as experimental animals.

Lentil lectin (CaA), generously provided by Dr. I. Hilgert, Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Prague, and concanavalin A (con A), obtained from Difco, were used as T mitogens. The LcA (1 mg) or con A (100 μg) was injected intravenously in 0.5 or 0.2 ml respectively of physiological saline.

Cyclophosphamide (CP), obtained from Saransk Medical Preparations Factory, was dissolved in sterile distilled water and injected intraperitoneally in a dose of 200 mg/kg 2 days after the lectin. The experimental animals were subjected to various tests 7 days after receiving CP.

The cytotoxic test was carried out by the usual method. Suspensions of splenocytes ($2 \cdot 10^7$/ml) from intact mice or mice pretreated with lectin and CP, were treated with monoclonal antibodies to Thy 1.2-antigen (clone F7D5) in a dilution of 1/50-1/500, or with rabbit serum against mouse Ig (dilution 1/4-1/8), with rabbit complement. Antibodies to Thy 1.2-antigen were provided by Dr. V. Holan (Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Prague) and the serum against mouse Ig by Dr. E. V. Sidorova.

After incubation for 45 min at 37°C the suspensions were stained with a mixture of trypan blue and eosin and the percentage of stained (dying) cells was determined in a Goryaev counting chamber.

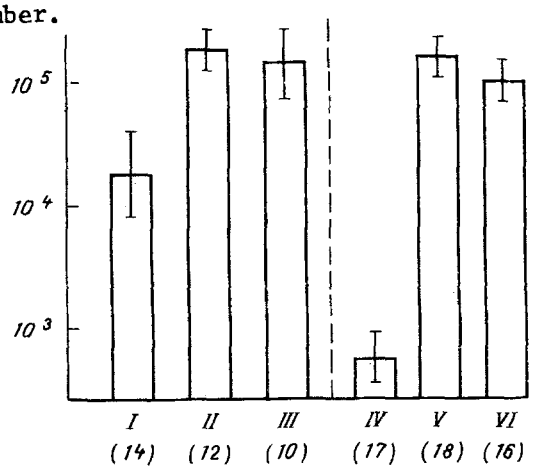

Fig. 1. Investigation of suppressor properties of splenocytes from mice receiving lectins and CP in an adoptive system. Abscissa, group of animals. Splenocytes of donors: I) con A + CP; II) con A + CP + N; III) N; IV) Lc + CP; V) LcA + CP + N: VI) N. Ordinate, number of AFC to SRBC on 8th day after transplantation of splenocytes of experimental animals into lethally irradiated syngenic recipients and stimulation twice by SRBC. Here and in Fig. 2, number of mice given in parentheses.

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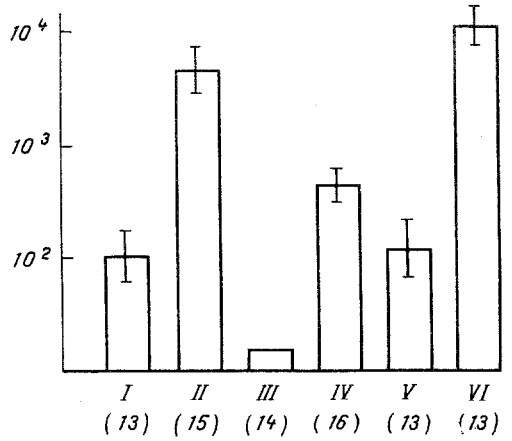

Fig. 2. Restoration by intact cells of ability of mouse splenocytes, pretreated with con A and CP, to give immune response. Abscissa, group of animals; ordinate, numnumber of AFC and SRBC on 8th day after transplantation of donors' cells into lethally irradiated syngeneic recipients and stimulation twice by SRBC.

To determine the suppressor activity of the splenocytes of the experimental mice, the adoptive transfer method was used. For this purpose spleen cells of mice receiving lectins and CP ($5 \cdot 10^7$) and intact mice ($1 \cdot 10^7$), either mixed together or separately, were injected intravenously together with $2 \cdot 10^6$ SRBC into syngeneic recipients, irradiated in a dose of 9.5 Gy. On the 4th day they were given an intraperitoneal injection of $5 \cdot 10^8$ SRBC, and on the 8th day their antibody-forming cell (AFC) level was determined by Jerne's method of local hemolysis in gel.

The cooperative test was carried out by the method in [12]. Splenocytes of mice pretreated with lectin and CP ($1 \cdot 10^7$) and thymocytes ($5 \cdot 10^7$) and bone marrow cells ($1 \cdot 10^7$) of intact mice were injected, either separately or mixed together, intravenously into syngeneic mice irradiated in a dose of 9.5 Gy, together with $2 \cdot 10^6$ SRBC. After 4 days the recipients were given a test dose of SRBC ($5 \cdot 10^8$), and on the 8th day the number of AFC to SRBC in their spleen was determined by Jerne's method.

Statistical Analysis. The geometric mean (M_g) and its confidence intervals were calculated by Student's test at the $p < 0.05$ level.

EXPERIMENTAL RESULTS

Lectins are known to participate in the formation of antigen-nonspecific suppressors [4, 6, 7, 10]. At certain times after CP, suppressor cells also are activated [11, 13]. It was accordingly necessary to study the possible role of suppressor mechanisms in the form of anergy being studied. For this purpose splenocytes of experimental animals were studied in an adoptive system.

The results are given in Fig. 1. They show that splenocytes of animals pretreated with con A and CP or with LcA and CP (groups I and IV) give a sharply reduced response to SRBC compared with cells from intact donors (groups III and VI). However, on addition of splenocytes of mice pretreated with lectins and CP to spleen cells from intact donors (groups II and V) no reduction of the immune response of the latter was observed. These data are evidence of the absence of suppressor cells in mice receiving lectin and CP. Suppressor factor also was absent from the blood serum of these mice (data not given).

In the experiments of series II functional activity of T and B cells of mice receiving con A and CP was investigated in a cooperative test. The results are given in Fig. 2. As Fig. 2 shows, splenocytes of donors pretreated with con A and CP, thymocytes, and bone marrow cells (BM; groups I, III, and V) give a sharply reduced immune response compared with the positive control (group VI) — a mixture of thymocytes and BM cells of intact donors, giving a cooperative effect in response to SRBC. On mixing of splenocytes of animals receiving con A and CP and BM cells beforehand, some increase was observed in the immune response (group IV), due evidently to simple summation of the response of these cells. A marked cooperative effect was observed on the addition of thymocytes of intact donors to splenocytes pretreated with con A and CP (group III). In this case the level of the immune response rose up to that in the group with normal cooperation between intact T and B cells (group VI).

Thus normal B cells are present in the spleen of mice receiving con A and CP, but functionally helper T cells are absent.

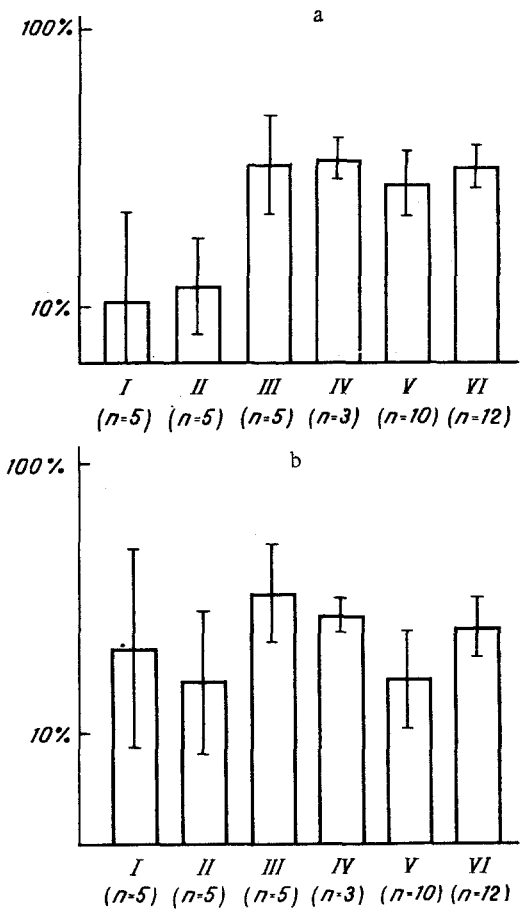

Fig. 3. Determination of number of T and B cells in spleen of mice receiving lectins and CP. Abscissa, group. Pretreatment of animals: I) with con A + CP; II) LcA + CP; III) con A; IV) LcA; V) CP; VI) control. Ordinate, percentage of stained (dying) cells after treatment with: a) monoclonal antibodies to Thy 1.2-antigen; b) rabbit serum against mouse Ig.

In the experiments of series III the number of T and B cells was determined in the spleen of the experimental mice, using antibodies to Thy 1.2-antigen and serum against mouse Ig. The results are shown in Fig. 3. Data on the cytotoxic action of antibodies to Thy 1.2-antigen on splenocytes of the experimental animals are given in Fig. 3a, which shows that preliminary injection of lectins and CP (groups I, II) leads to a significant fall in the level of Thy-1^+ T cells compared with animals receiving CP only (group V), LcA or con A only (groups III and IV), or intact animals (group VI; $p < 0.05$). On treatment of splenocytes of the experimental mice with serum against mouse Ig (Fig. 3b) the number of Ig^+ B cells in mice receiving lectins and CP (groups I and II) did not fall below that in the control animals (group III). The very small decrease in the number of B cells in mice receiving CP (groups I, II, and V) compared with animals receiving lectins only or with intact animals, is evidently connected with increased sensitivity of the B cells to the action of CP [5, 9].

This investigation thus showed that, first, functionally active helper T cells are absent (while B cells are normal) in mice receiving a T-cell mitogen (LcA or con A) and CP consecutively; second, the total number of Thy-1^+-cells in the spleen is reduced (while the number of Ig^+-cells remains unchanged); third, suppressor cells, which may imitate the effects of anergy or deletion of T cells are absent.

All these effects were observed only when the action of the T-mitogen was combined with that of CP.

The results are in good agreement with those of a previous investigation [2], in which it was shown that the immune response only to thymus-dependent (SRBC), but not to thymus-independent (LPS) antigens is suppressed in mice receiving a T mitogen together with CP.

It remains unclear how the effects of functional anergy of the T cells or their direct deletion can be explained. However, the total loss of Thy-1^+-cells in the spleen of the experimental animals is in favor more of the second hypothesis. We know [3, 9] that lymphocytes are most sensitive to CP when in the cell cycle. It is therefore very probable that the T-cell immunodeficiency which we observed is due to selective elimination by cyclophosphamide (or more correctly, by its breakdown products [3, 9]) of T cells whose proliferation

was induced by lectins. Indirect proof of this point of view is given by the B-cell immunity, observed by the writers previously [1, 8], resulting from combined administration of B-mitogens (LPS) and CP. It is to be hoped that selective damage to individual T-cell subpopulations may be observed by the use of a similar principle, by choosing mitogens with a more selective action in combination with CP.

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ROLE OF THE DOPAMINERGIC SYSTEM IN THE STIMULATING EFFECT OF MURAMYL DIPEPTIDE ON THE IMMUNE RESPONSE

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The action of biologically active peptides on immune resistance of the body is currently under intensive study. These compounds include the mycobacterial peptidoglycan muramyl dipeptide (MDP). Besides its adjuvant properties, MDP can also activate macrophages [14] and helper T cells [12], and in high doses, it can include suppressor T cells [11]. MDP may also have an influence not only on the immune system, but also on the CNS, as is shown by the pyrogenic and somnogenic effects of this compound [7, 8].

Despite the extensive study of the biological activity of MDP the mechanism of its stimulating action and the role of the CNS in this process are not yet clear. The aim of the present investigation was to study this problem.

EXPERIMENTAL METHOD

Experiments were carried out on 140 male CBA mice weighing 23 g and aged 3-4 months. The mice were immunized with a suspension of sheep's red blood cells (SRBC) in a single dose of $5 \cdot 10^6$ cells. The magnitude of the immune response was determined by counting the number of rosette-forming cells (RFC) on the 5th day after immunization [4]. MDP (N-acetyl-muramyl-L-alanyl-D-isoglutamine; from Spofa, Czechoslovakia) was injected intraperitoneally together with the antigen in a dose of 1 or 5 mg/kg, and haloperidol (Gedeon Richter, Hungary) was injected intraperitoneally in a dose of 1 mg/kg twice a day for 2 days. The first injection was given 30 min before immunization.

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