ROLE OF THE THYMUS IN REGULATION OF PRODUCTION OF MACROPHAGE MIGRATION INHIBITORY FACTOR IN MICE OF DIFFERENT GENOTYPES

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The effect of the thymus on production of macrophage migration inhibitory factor (MMIF) was studied in C57BL and CBA mice thymectomized at the age of 4-6 weeks. MMIF production in response to stimulation by tuberculin was estimated at the peak of the immune response on the 1st-21st days after the operation. This factor was found to be produced as early as the 1st day after thymectomy. MMIF production is absent in athymic nude mice. Thymectomy also stops the immune response in the early stages of its development. Spontaneous migration of macrophages also is modified in immune and nonimmune animals. These changes are more marked in C57BL mice.

KEY WORDS: thymus; thymectomy in the adult state; migration inhibitory factor; spontaneous migration; macrophage.

It has now been established that the thymus plays an important role in the development of immunological processes [3, 4, 7, 8]. However, its role in the regulation of production of mediators of cellular immunity has been inadequately studied. One such mediator is macrophage migration inhibitory factor (MMIF). This factor plays an important role in interaction of sensitized lymphocytes with macrophages in the effector stage of the immune response with a course resembling that of hypersensitivity of delayed type. There have been only isolated studies of the effect of thymectomy on MMIF production [5, 6].

In the investigation described below the role of the thymus in regulation of MMIF production under the influence of tuberculin was studied in mice of two lines differing with respect to the strong histocompatibility locus. It was shown previously that MMIF production in response to stimulation by BCG is activated more strongly in C57BL mice than in CBA mice [2].

EXPERIMENTAL METHOD

Experiments were carried out on C57BL ($H\text{-}2^b$) and CBA ($H\text{-}2^k$) mice aged 4-6 weeks and on nude and nu^+/nu^- mice. The nude mice were obtained from the Department of Pure-Line Animals, Institute of Biophysics, Ministry of Health of the USSR. The C57BL and CBA mice were thymectomized under hexobarbital anesthesia by the usual method [7]. The control group consisted of intact mice and mice undergoing a mock operation. The technique of the mock operation was the same as that of thymectomy, except that the thymus was not removed. On the 1st-21st days after the operation the mice were immunized intraperitoneally with Freund's complete adjuvant in a dose of 500 μg per mouse (0.5 ml). MMIF production was tested by the direct capillary macrophage migration inhibition test, using peritoneal exudate cells at the peak of the immune response (C57BL on the 3rd day, CBA on the 6th day after immunization [2]). Dry purified tuberculin (100 μg/ml) was used as the antigen. The results were assessed as the migration index (MI) and the percentage inhibition of migration (PIM):

$$MI = \frac{\text{Zone of migration of cells with antigen}}{\text{Zone of migration of cells without antigen}} \times 100\%;$$

$$PIM = 100\% - MI.$$

Spontaneous migration of peritoneal exudate cells from immunized and unimmunized mice, estimated by measuring the zone of migration of macrophages from a capillary tube in the absence of antigen, also was

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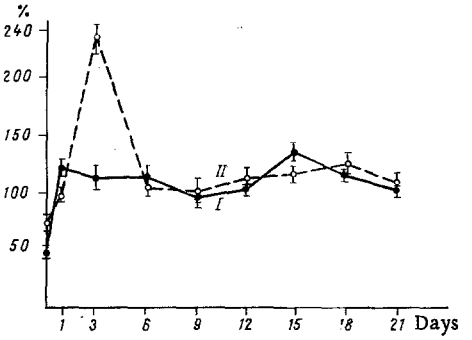

Fig. 1. MMIF production in C57BL (I) and CBA (II) mice at different times after thymectomy. Here and in Fig. 3: abscissa, time after thymectomy (in days); ordinate, MI (in %).

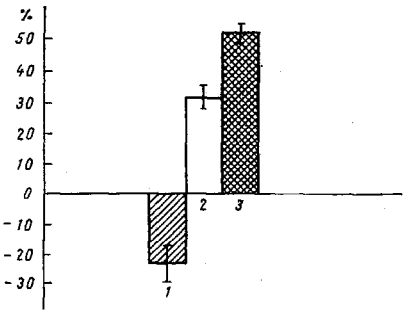

Fig. 2. MMIF production in nu/nu (1), nu^+/nu^- (2), and C57BL (3) mice. Ordinate, PIM. Abscissa, time after thymectomy (in days).

studied on the 1st-21st days. To obtain peritoneal exudate cells, 2 ml of 2% peptone solution was injected intraperitoneally 48 h before the experiment. The cell suspension was prepared by the usual method [1]. The results were calculated by the equation:

$$MI = \frac{\text{Zone of migration in thymectomized mice}}{\text{Zone of migration in intact mice}} \times 100\%.$$

To study the effect of the thymus on development of the immune response to tuberculin, C57BL mice were thymectomized on the 1st, 2nd, and 3rd days after immunization with Freund's adjuvant. MMIF production also was studied in athymic nu/nu mice and mice heterozygous for the nu gene. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The dynamics of MMIF production in the early stages after thymectomy in the adult state is illustrated in Fig. 1. Thymectomy completely abolished MMIF production by sensitized peritoneal exudate lymphocytes from mice of both lines throughout the period of investigation. Furthermore, on the addition of antigen, stimulation of macrophage migration was observed and was especially marked in CBA mice on the 3rd day after thymectomy (MI = 239.3 ± 5.54%). In mice undergoing the mock operation, MMIF production was undisturbed (MI = 46.6 ± 2.4%). Nude mice likewise did not produce MMIF in this system (PIM = −23.5 ± 6.2), whereas in nu^+/nu^- mice MMIF production was preserved (PIM = 32.8 ± 4.31) (Fig. 2).

The fact will be noted that thymectomy on animals in the adult state stopped the immune response in the early stages of development. If the mice were thymectomized on the 1st day after immunization, MMIF production was completely abolished (PIM = 34.2 ± 1.7), whereas on the 3rd day after immunization it was virtually indistinguishable from the control (PIM = 50.7 ± 1.64). In mice undergoing the mock operation, MMIF production still continued in this system (PIM = 53.4 ± 2.4).

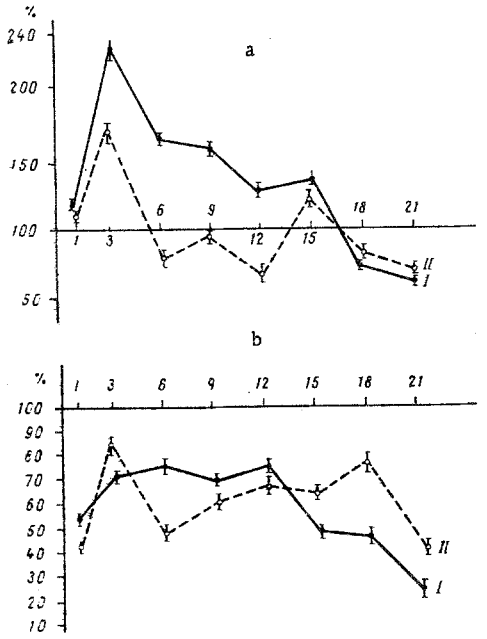

Fig. 3. Spontaneous macrophage migration in C57BL (I) and CBA (II) mice, immunized with BCG (a) or not immunized (b), at various times after thymectomy.

Thymectomy, it is important to note, disturbed the function not only of cells producing MMIF, but also of cells of the macrophage series. Moreover, in animals immunized with BCG and in unimmunized animals different effects were observed. The results for spontaneous macrophage migration in immunized mice are given in Fig. 3a. Migration activity of nonthymectomized mice is taken as 100%. Whereas immunization of intact mice approximately doubled the spontaneous migration of peritoneal exudate macrophages, this stimulating effect was not found in thymectomized mice. Macrophagal activity according to this index did not reach the control level at any time during the investigation. Spontaneous migration in unimmunized mice is shown in Fig. 3b. On the 1st day thymectomy caused little change in the ability of the macrophages to migrate, but after the 3rd day marked activation was observed, followed by a gradual decline. Starting from the 18th day this index was 22.4 ± 1.4% below the control level in the CBA mice and 30.7 ± 0.74% lower in the C57BL mice. On the 21st day this inhibition became more marked (34.6 ± 0.6 and 41.9 ± 0.4%, respectively).

Thymectomy, it will be noted, inhibited MMIF production equally in the mice of both lines, but marked stimulation of macrophage migration was observed on the 3rd day in CBA mice on the addition of antigen (MI = 239 ± 5.5%), whereas in C57BL mice stimulation was weaker and was observed on the 1st day (120.9 ± 2.3%). Spontaneous migration of macrophages showed sharper changes in C57BL mice, especially on the 18th-21st days. Evidently the differences between the two lines could be due to differences in the ability of their T lymphocytes to synthesize MMIF or to differences in their number. It can tentatively be suggested that changes in spontaneous migration depend on differences in functional activity of cells of the macrophagal series. Thymic factors can probably regulate the activity not only of lymphocytes, but also of other cells.

The results are evidence that production of MMIF depends on the thymus, as is also confirmed by the absence of MMIF production in nude mice. In these animals the cell population responsible for MMIF production may perhaps be absent. The consequences of thymectomy in the adult state can provisionally be explained by a sharp fall in the thymosin level in the peripheral blood after removal of the thymus [9]. Possibly as a result of this, nonspecific T_1-suppressors, inhibiting the immune response, accumulate. Thymectomy evidently does not affect the effector cells, for it stops the immune response only in its early stages, but not in the later stages. Removal of the thymus may perhaps also lead to a redistribution of cells or may cause the cells to lose their ability to recognize the antigen. At the present time, however, other mechanisms cannot be ruled out as an explanation of this phenomenon.

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EFFECT OF SPONTANEOUS LOSS OF TOLERANCE ON T AND B LYMPHOCYTE FUNCTION IN MICE

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Functional activity of spleen and thymus cells of mice tolerant to sheep's red blood cells was studied 1 and 4 days after induction of tolerance. Tolerance was obtained with the aid of cyclophosphamide. Complete restoration of the immunocompetence of the thymus cells was found after 4 weeks. The functional activity of splenic T and B lymphocytes also was partly restored 4 weeks after induction of tolerance. Preliminary thymectomy weakened but did not prevent complete restoration of competence of splenic T cells. No T suppressors were found in the thymus and spleen of the tolerant animals.

KEY WORDS: immunologic tolerance; T and B lymphocytes; T suppressors; thymectomy.

In the absence of an antigen immunologic tolerance is spontaneously lost with the passage of time. In some forms of tolerance which has received the most study, function of the B cells is restored first, whereas the helper function of the T cells remains inhibited for several months [8]. Meanwhile, in tolerance induced by thymus-dependent antigens with the aid of cyclophosphamide (CP), partial recovery of the immunoreactivity of the lymphocytes in situ or in an adopted system was observed within 3 or 4 weeks [5, 7].

The object of the present investigation was to study the functional status of the B cells, T helpers, and T suppressors of the spleen and thymus in the stages of formation and partial loss of tolerance to sheep's red blood cells (SRBC), induced with the aid of CP. Because of the contradictory nature of data in the literature on the effect of thymectomy on formation and loss of tolerance [2, 7, 9, 12-14] it was decided to study the function of T and B lymphocytes in thymectomized animals subjected to the above-mentioned tolerogenic treatment.

EXPERIMENTAL METHOD

Male CBA and (CBA × C57BL/6)F_1 mice weighing 18-22 g were used. Tolerance to SRBC was induced by intraperitoneal injection of SRBC ($6.2 \cdot 10^9$) and CP (200 mg/kg). Control animals either received CP alone or were untreated. Tolerance was investigated 1 and 4 days after injection of CP. Thymectomy was performed on the animals 2 weeks before induction of tolerance. The donors' cells were injected intravenously into syngeneic recipients previously irradiated in a dose of 950 R (separately or in various combinations), in doses of $5 \cdot 10^7$ thymus and spleen cells of the experimental mice, $1 \cdot 10^7$ to $5 \cdot 10^7$ intact bone marrow cells, and $1 \cdot 10^7$ intact spleen cells. Simultaneously with the cells the animals received $2 \cdot 10^6$ SRBC, and 4 days later a

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