ROLE OF SPLEEN CELLS IN REGULATION OF PRODUCTION OF MACROPHAGE MIGRATION-INHIBITING FACTOR IN MICE

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Increasing attention is nowadays being paid to the role of the spleen and its connection with other immunocompetent organs in the regulation of many immunologic processes. Data in the literature on this subject are few in number and contradictory in nature. For instance, Viallat et al. [10] showed that spleen cells after thymectomy in mice in the adult state have a marked stimulating effect on the course of the mixed lymphocyte culture reaction, although other workers [4, 5] found the appearance of suppressor activity after thymectomy in the same reaction and in response to mitogens. However, the question of regulation of lymphokine production has so far received little study.

The object of this investigation was to study the role of the spleen in regulation of production of migration-inhibiting factor (MIF) in mice.

EXPERIMENTAL METHOD

Experiments were carried out on C57BL inbred mice and (CBA × C57BL)F_1 hybrids aged 4-6 weeks. To assess MIF production mice were immunized intraperitoneally with killed BCG vaccine in Freund's complete adjuvant in a dose of 500 μg per mouse (0.5 ml). At the maximum of the immune response (3rd day in C57BL mice and 5th day in the hybrids) peritoneal exudate cells were obtained and MIF production was estimated by the direct capillary test [2]. Dry purified tuberculin, added to the cells in a dose of 100 μg/ml, was used as the antigen. The results were estimated by the equation:

$$\text{Percentage inhibition of migration (PIM)} = 100\% - \left(\frac{\text{Weight of zone of migration with antigen}}{\text{Weight of zone of migration without antigen}} \cdot 100\%\right).$$

Thymectomy and splenectomy were performed by appropriate methods [7, 8]. MIF production was studied after removal of the spleen alone, and also after removal of the spleen at different times before and after thymectomy in the adult state. Animals undergoing mock operations and intact animals served as the control.

In addition, on the 3rd day after thymectomy spleen cells were obtained from C57BL mice and mixed with peritoneal exudate cells obtained on the 3rd day after immunization of syngeneic mice with BCG. The ratio between the numbers of spleen and peritoneal exudate cells were 1:1, 1:2, 1:3, and 1:5. MIF production was assessed after incubation for 17-24 h.

Later the spleen cells were divided into subpopulations adherent and nonadherent to glass [1].

EXPERIMENTAL RESULTS

The writers showed previously [3] that thymectomy in the adult state abolished MIF production with effect from the first day after the operation. By contrast, removal of the spleen alone did not affect lymphokine production by peritoneal exudate cells. In a group of C57BL mice PIM after splenectomy was 52.6 ± 2.005, in intact animals it was 53.7 ± 0.9263,

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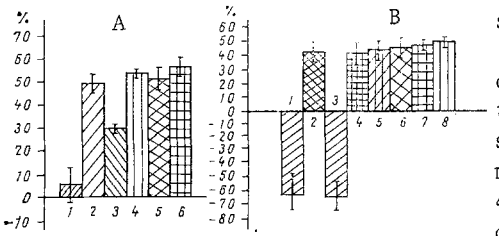

Fig. 1. Effect of thymectomy and splenectomy in the adult state on MIF production of C57BL (a) and (CBA × C57BL)F_1 (b) mice. A: 1) 3rd day after thymectomy, 2) splenectomy on 3rd day after thymectomy, 3) thymectomy on 3rd day after splenectomy, 4) intact mice, 5) splenectomy, 6) mock splenectomy; B: 1) 1st day after thymectomy, 2) splenectomy on 1st day after thymectomy, 3) 3rd day after thymectomy, 4) splenectomy on 3rd day after thymectomy, 5) thymectomy on 3rd day after splenectomy, 6) splenectomy, 7) mock splenectomy, 8) intact mice. Here and in Figs. 2 and 3, ordinate — PIM.

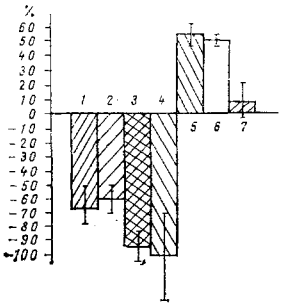

Fig. 2

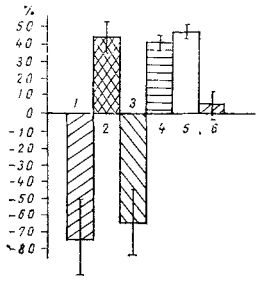

Fig. 3

Fig. 2. Effect of spleen cells from C57BL mice thymectomized in the adult state on MIF production by syngeneic peritoneal exudate cells. 1) Suspension of spleen cells and peritoneal exudate cells in ratio 1:1; 2) in ratio 1:2; 3) in ratio 1:3; 4) in ratio 1:5; 5) mixture of intact spleen and peritoneal exudate cells in ratio 1:1; 6) intact cells; 7) 3rd day after thymectomy.

Fig. 3. Action *in vitro* of adherent and nonadherent spleen cells on 3rd day after thymectomy in adult state on MIF production by syngeneic peritoneal exudate cells in ratio 1:1. 1) Adherent spleen cells + intact peritoneal exudate cells; 2) nonadherent spleen cells + intact peritoneal exudate cells; 3) adherent spleen cells + peritoneal exudate cells from thymectomized mice; 4) nonadherent spleen cells + peritoneal exudate cells of thymectomized mice; 5) intact cells; 6) 3rd day after thymectomy.

and in animals undergoing the mock operation 56.8 ± 1.01 ($P > 0.1$ and $0.02 > P < 0.05$, respectively. In the group of hybrids after splenectomy PIM was 43.9 ± 2.1, compared with 46.6 ± 1.8 in intact animals and 46.3 ± 0.92 in animals undergoing the mock operation ($P > 0.1$ in both cases; Fig. 1).

However, splenectomy in mice after preliminary thymectomy led to recovery of the ability of immune peritoneal exudate cells to synthesize MIF on both the 1st and 3rd days after thymectomy: PIM was 41.7 ± 1.6 and 41.3 ± 1.7, respectively (Fig. 1B). In the control, PIM was 46.6 ± 1.8 in intact mice ($P < 0.05$) and 43.9 ± 2.1 in splenectomized mice ($P < 0.001$; Fig. 1B). If the spleen was removed first, and then the thymus, MIF production was preserved, although depressed somewhat: PIM was 42.4 ± 1.8. Removal of the spleen thus prevents interruption of MIF production.

On the addition of spleen cells from thymectomized mice, MIF production was sharply inhibited (Fig. 2). A similar effect was obtained when the cells were used in all ratios. The effect of spleen cells when the ratios between the cells were 1:1 and 1:2 was identical (PIM

−65.02 ± 5.5 and −61.5 ± 6.0; $P > 0.1$), but differed significantly ($P < 0.05$) from its value when the ratios were 1:3 and 1:5 (PIM −97.8 ± 4.8 and −100.3 ± 14.2), when the effect was equal ($P > 0.1$). On addition of antigen, instead of inhibition an effect of stimulation of migration of the cells from the capillary tube was observed. It was more marked at ratios of 1:3 and 1:5, i.e., it was not connected with a simple increase in the concentrations of spleen cells, which themselves migrate much more intensively than peritoneal exudate cells.

Fractionation of spleen cells from thymectomized mice into subpopulations adherent and nonadherent to glass showed that the effect of inhibition of MIF production is linked with the adherent cell subpopulation. Nonadherent cells not only did not inhibit MIF production (PIM was 45.4 ± 2.526), but actually restored it when added to peritoneal exudate cells from thymectomized mice (PIM = 41.7 ± 3.706). Adherent cells, however, completely abolish MIF production (PIM was −76.05 ± 11.362; Fig. 3).

These data can be explained on the basis of information in the literature that thymectomy in the adult state leads to accumulation of nonspecific T_1-suppressor cells in the spleen during the first 4-5 weeks after thymectomy, on account of absence of thymic humoral factor and disturbance of differentiation of the cells into mature T_2-lymphocytes [11]. This view is also indirectly confirmed by the work of Fox et al. [6], who found that cultures enriched with suppressors abolish MIF production. On exhaustion of the suppressor cells in these cultures no such effect was observed. The existing data are evidence that T_1-cells are adherent to glass [11], but this does not completely rule out the role of macrophages, for they also adhere to glass and can exert a suppressive effect [9].

The results described above thus indicate that the spleen participates directly in regulation of production of MIF, one of the mediators of cellular immunity, by peritoneal exudate cells. Thymectomy in the adult state causes the spleen cells to acquire the ability to inhibit MIF production by immune peritoneal exudate cells of intact syngeneic mice. Removal of the spleen by itself does not affect MIF production, but its removal both before and after thymectomy in the adult state restores lymphokine production, when absent in thymectomized mice. The effect of inhibition of MIF production is connected with the adherent subpopulation of spleen cells. The effect is reversible and removal of the adherent cells restores MIF production.

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