After the addition of stromal mechanocytes to cultures of splenic cells with antigen it was found that stromal mechanocytes of bone-marrow origin have a marked inhibitory action on AFC formation in cultures, if added during the first 48 h of culture, but had no significant effect if added later during culture.

Stromal mechanocytes of different origin, when added to cultures of nonadherent cells, exhibit an action of the same character as during association with a complete population of splenic cells. Their action is unconnected with any change in the survival rate of the cells in culture or with allogeneic combination of stromal and lymphoid cells. The differences between the action of thymic and bone-marrow fibroblasts in the presence of a deficiency of A cells show that the latter (possibly on account of the presence of splenic stromal mechanocytes among them) make the spleen cells less sensitive to the effect of mechanocytes of other hematopoietic organs.

The fibroblasts exerted their complete effect on AFC production only in the case of adhesion to the surface of the culture vessel.

The radiosensitivity of the stromal mechanocytes is reflected in a D_0 value of the order of 200 R, and consequently, irradiation in doses of 5000 R depressed the proliferative powers of fibroblasts added to the culture practically completely [2]. Proliferation of stromal fibroblasts in culture is probably not essential for the realization of their action.

The results of this investigation are evidence that certain functions of the mechanocytes are connected with the microenvironment and are directed toward the selective repression of some and stimulation of other pathways of differentiation of lymphocytes in the organs of immunogenesis.

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PROPERTIES OF T CELLS SYNTHESIZING MACROPHAGE MIGRATION INHIBITION FACTOR IN THE H-2 SYSTEM

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UDC 612.112.2-06:612.112.94

It was shown by means of the indirect macrophage migration inhibition test in the H-2 system, using fractionated lymphocytes, that macrophage inhibition factor (MIF) is produced by T but not by B cells. Cells producing MIF are more sensitive to the action of anti-θ-antibodies than T-killer cells. MIF formation by lymph node cells was detected at earlier periods after immunization than the cytotoxic activity of these cells. The results obtained are evidence of differences between the subpopulation of T cells synthesizing MIF and cytotoxic T lymphocytes.

KEY WORDS: inhibition of migration of macrophages; cytotoxic effect; H-2 system; T and B lymphocytes.

The macrophage migration inhibition (MMI) test in vitro is nowadays recognized to be the analog of the hypersensitivity of delayed type (HDT) test in vivo [9]. This test in mice [19], hens [14], guinea pigs [8], rabbits [15], and man [17] is a T-dependent phenomenon. Meanwhile activated B cells [18] and even cultured

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(Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 6, pp. 701-704, June, 1978. Original article submitted July 18, 1977.

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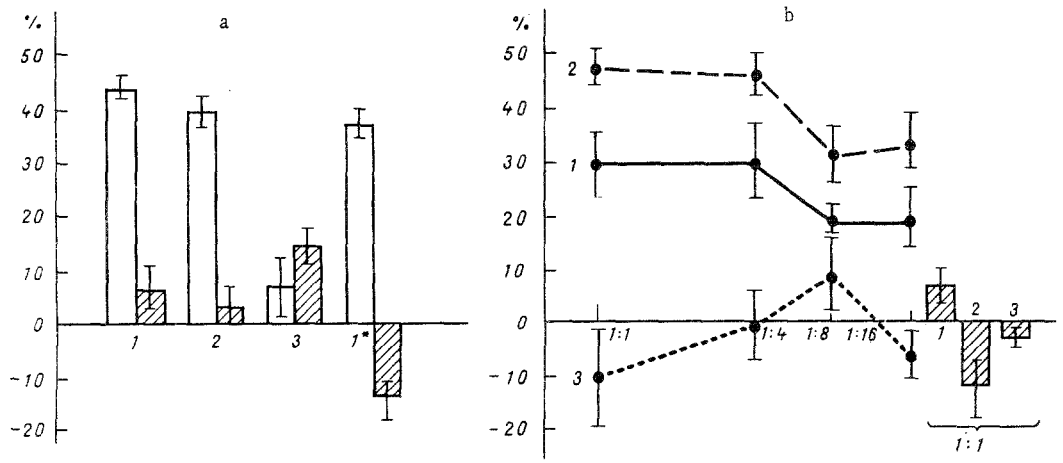

Fig. 1. MIF production by T and B fractions of immune lymphocytes for immune lymphocytes and target cells in the ratio of 1:1 (a) or different ratios shown along abscissa (b). 1) Original lymphocytes; 1*) the same, treated with Boyle's solution; 2) T fraction; 3) B fraction. Immune lymphocytes incubated with allogeneic target cells (unshaded columns and curves) or syngeneic target cells (shaded columns). Ordinate, MMI index (in %).

strains of nonlymphoid cells [20] produce a migration inhibition factor (MIF) in the S phase of the cell cycle. The properties of the T cells which synthesize MIF have so far received little study. Although MIF producers, like cytotoxic T-lymphocytes, are sensitive to anti-θ-serum [19], these T cells differ in the affinity and specificity of their receptors [5, 13], their attraction to lymphoid organs, and their cell diameter [19]. The effector cells of HDT have recently been shown to differ from cytotoxic lymphocytes in relation to Ly antigens [11].

The object of this investigation was to study MIF formation by fractionated T and B lymphocytes and to compare the sensitivity of MIF producers and cytotoxic lymphocytes to anti-θ-serum and also the kinetics of their formation after immunization in the H-2 system.

EXPERIMENTAL METHOD

Mice of strains C57BL/10Sn and B10.D2 were immunized by single injection of allogeneic sarcoma cells (5×10^7 cells per mouse), namely sarcoma SaI, growing in strain A, and sarcoma MKh11, growing in strain C57BL/10 respectively [2]. The ability of lymph node cells of these mice (b anti-a or d anti-b) to produce MIF and to induce a cytotoxic effect (CE) on incubation with the corresponding target cells was determined 8 days later. MIF synthesis was determined by the indirect MMI test; the supernatant obtained after incubation of immune (normal in the control) lymphocytes with spleen cells of the donor's (syngeneic in the control) strain was tested for its ability to inhibit migration of macrophages of CC57Br mice from microcapillary tubes placed in wells of Microtest II No. 3040 microdisks (Falcon Plastics, USA) [5, 6]. CE of the lymphocytes was determined in the microtest for destruction of ^{51}Cr-labeled peritoneal macrophages [4]. The lymphocytes were fractionated by the method described in [12], the adequacy of which in the H-2 system was verified previously [3]. Antibodies in the AKR anti-C3H anti-θ-serum obtained by the method described in [16] were determined by the two-stage cytotoxic test in vitro [1]. The reaction of the anti-θ-serum with thymocytes of C3H mice in a titer of 1:64 (batch I of anti-θ-serum) and in a titer of 1:256 (batch II of anti-θ-serum) was suppressed by adsorption with brain, but not bone marrow, of C3H mice. To eliminate T cells, 2×10^7 lymphocytes were incubated in 1 ml of anti-θ-serum (or normal AKR mouse serum in the control) for 30 min at 20°C and, after centrifugation, were reincubated for 1 h at 37°C in 1 ml of guinea pig complement, selected for its nontoxicity, diluted 1:2. The lymphocytes were washed 3 times and equalized for the number of living cells. Under these conditions the anti-θ-serum in a dilution of 1:3 killed about 30% of the lymph node cells.

EXPERIMENTAL RESULTS

As Fig. 1 shows, cells of the T fraction, separated from B-cells, produced MIF equally to (Fig. 1a) or even rather more actively than (Fig. 1b) the original lymphocytes. The MMI test for the original lymphocytes and cells of the T fraction was highly specific. Under the same conditions the B lymphocytes did not produce MIF. Treatment of the original lymphocytes with Boyle's solution [12], essential for lysis of the erythrocytes

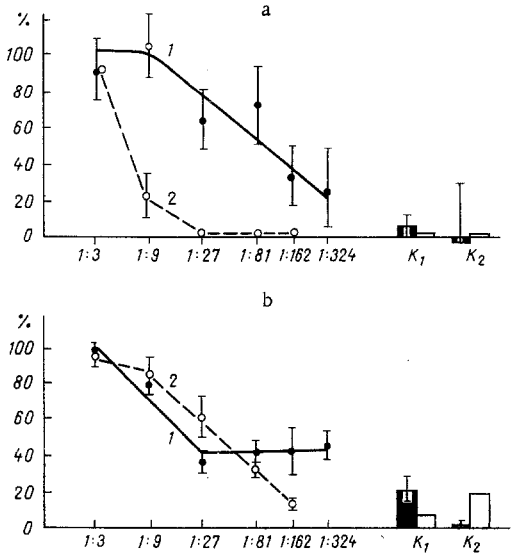

Fig. 2. Action of anti-θ-serum of batch I (a) and batch II (b) on MIF synthesis (1) and CE (2). K_1 and K_2) Controls; K_1) treatment with anti-θ-serum (1:3) + inactivated complement; K_2) treatment with normal mouse serum + active complement. Black columns – MMI index, unshaded columns – CE. Points show mean values of 3 or 4 experiments. Abscissa, dilutions of anti-θ-serum; ordinate, decrease in MMI index and CE (in %).

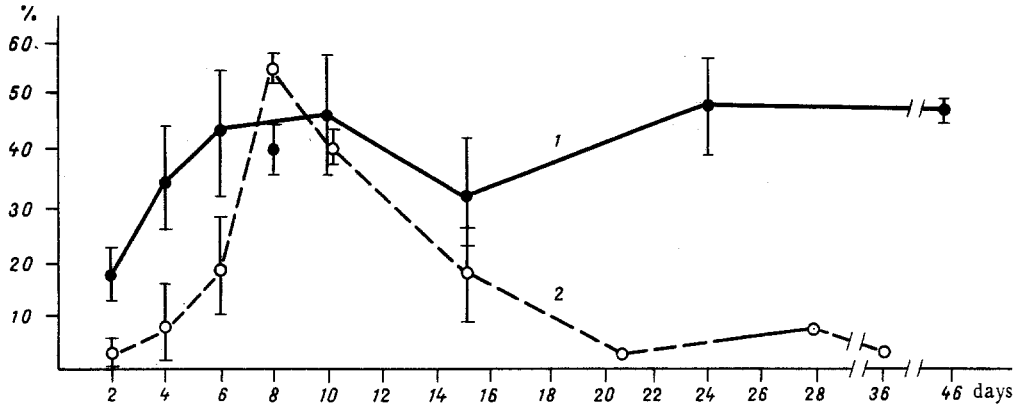

Fig. 3. MIF production and CE at different times after immunization. Points show mean values from 3 or 4 experiments. Abscissa, days after immunization; ordinate, MMI index (1) and CE (2).

to obtain the B fraction, did not affect the ability of the lymphocytes to produce MIF. Differences between the activity of the T and B fractions still remained when the lymphocytes and target cells were present in different proportions (Fig. 1b).

Treatment of the immune lymphocytes with 1:3 dilutions of anti-θ-serum showed that MIF synthesis was inhibited by higher dilutions (1:324-1:486) than CE (1:9 for anti-θ-serum of batch I and 1:162-1:243 for batch II). It follows from Fig. 2 that the character of dependence of inhibition of MIF synthesis on the titer of anti-θ-serum differed for batches I and II of that serum: in the first case a gradual decrease in the effect of the anti-θ-serum was observed, whereas in the second case there was a sharp drop in the sensitivity of the MIF producers after the first 2 dilutions, whereas the action of the subsequent dilutions of anti-θ-serum remained at the same level (about 40% suppression).

The study of the kinetics of CE and of MIF production showed that activity of the MIF producers appeared as early as on the 2nd day, reached a maximum after 5-6 days, and lasted at least for 1.5 months. CE on the other hand did not appear until the 6th day, reached a maximum on the 8th day, and then fell to zero in the course of a week (Fig. 3).

The MIF producers were found to be more sensitive to anti-θ-antibodies than the killers when both batches of anti-θ-serum were used. The difference between the results for anti-θ-serum of the different batches may be due either to differences in the content of anti-θ-antibodies of different classes (or subclasses), to the action of which the MIF producers differ in their sensitivity, or to a difference in the content of other, not anti-θ-antibodies (for example, anti-Ly-antibodies), to which the MIF producers are sensitive. The second hypothesis seems less likely: absorption of anti-θ-serum by C3H mouse brain completely abolished the blocking action of this serum on MIF synthesis for all dilutions tested.

In this investigation, by contrast with the results obtained by Harrington [10], MIF synthesis was discovered not only after disappearance of the CE, but also before its appearance. The disagreement between these findings can be explained by differences in the methods and in the target cells used: spleen cells in this investigation, tumor cells in that of Harrington [10].

The differences in the kinetics of formation of MIF producers and killers and in their sensitivity to the action of anti-θ-serum, together with other features distinguishing them [5, 13, 19], is evidence that T cells producing MIF are not identical and, possibly, are less mature than T cells responsible for CE. This hypothesis is in agreement with earlier observations showing the lower avidity and the less selective specificity of the receptors of the MIF producers than of the killers [5]. These differences are evidently unconnected with differences in the sensitivity of the MMI and cytotoxicity tests, for depending on the conditions of the experiments described above, either of these reactions was discovered in the absence of the other.

A combination of several technical approaches, based on differences in the properties of the MIF producers and killers may enable these subpopulations of T cells to be isolated for a closer analysis of their properties and roles in the immune response against transplantation and tumor antigens.

The authors are grateful to N. N. Medvedev for providing the mice, to G. N. Drizlikh for useful advice, and to G. N. Vornakova for expert technical help. The investigation was partly subsidized by the World Health Organization.

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