4. V. S. Lusenko, I. P. Maevskaya, and L. Ya. Oleinik, in: Physiology, Biochemistry, and Pathology of the Endocrine System [in Russian], No. 3, Kiev (1973), p. 56.
5. S. G. Mamontov and L. I. Ivanova, Byull. Éksp. Biol. Med., No. 6, 740 (1976).
6. S. G. Mamontov, L. I. Ivanova, and V. B. Zakharov, Byull. Éksp. Biol. Med., No. 4, 97 (1974).
7. V. B. Okulov and S. A. Ketlinskii, Tsitologiya, No. 11, 1294 (1957).
8. P. V. Sergeev, R. D. Seifulla, and A. I. Maiskii, Molecular Aspects of the Action of Steroid Hormones [in Russian], Moscow (1971).
9. W. Hondius Boldingh and E. B. Laurence, Eur. J. Biochem., 5, 191 (1968).
10. O. H. Iversen, Arch. Pathol. Anat. Abt. B, Zellpathol., 28, 271 (1978).
11. E. B. Laurence and E. Randers Hansen, Arch. Pathol. Anat. Abt. B, Zellpathol., 9, 271 (1971).
12. S. Rothberg and B. C. Arp, J. Invest. Derm., 64, 245 (1975).

KINETICS OF PRODUCTION OF MACROPHAGE MIGRATION INHIBITION FACTOR IN MIXED CULTURES OF LYMPHOCYTES AND TUMOR CELLS

D. D. Kharkevich and A. P. Suslov

UDC 616-006-097.2-02:616.155.32/33-097.5]-092.4

KEY WORDS: natural producers of macrophage migration inhibition factor; mixed cultures of lymphocytes and tumor cells.

The continuous renewal of most tissues of the multicellular organism, taking place through intensive cell division, is responsible for the high frequency of appearance of mutant cells, proliferation of each of which may lead to disturbance of vitally important functions of different organs or to the development of a malignant tumor [6]. The diagnosis and timely elimination of foreign cells and supervision of genetic homeostasis of the organism are functions of the immune system. The concept of immunologic surveillance was formulated by Burnet in the mid 1960s [6], but the nature of the effector cells of immunologic surveillance has not yet been precisely established. The most likely candidates for the role of these cells, capable of reacting to a foreign antigen without previous immunization, are the natural killers (NK cells) [8] and natural producers of macrophage migration inhibition factor (MIF) [12], lymphocytes potentially capable of activating the antitumor resistance of macrophages [13]. The writers showed previously that T cells which are producers of MIF can distinguish between mutant H-2 antigens and original H-2 antigens during development of an immune response *in vivo* [3] and *in vitro* [5].

The object of the present investigation was to study the ability of nonimmune MIF producers to react to antigens of allogeneic and syngeneic tumors during the first days of mixed culture of lymphocytes and tumor cells (MCLT) *in vitro*. The preliminary results on recognition of syngeneic tumor antigens were published previously [2].

EXPERIMENTAL METHOD

Spleen cells of normal mice of lines C57BL/6, abbreviated to B6 (Haplotype $H\text{-}2^b$); C57BL/10Sn, abbreviated to B10 ($H\text{-}2^b$), DBA/2 ($H\text{-}2^d$), and CBA ($H\text{-}2^k$), were used as reacting cells in MCLT. The stimulating cells were tumor cells irradiated in a dose of 5000-10,000 rads, obtained from sarcoma MCh-11, T-lymphoma EL-4, and mastocytoma P-815, maintained in the writers' laboratory by regular passages in mice of lines B10, B6 and DBA/2 respectively. Reacting cells numbering 5×10^6 were mixed with 1×10^5 stimulating cells and incubated in a final volume of 2 ml of medium RPMI-1640 (from Flow Labs, England) with the addition of 10% embryonic calf serum (from Gibco, England), 2 mM L-glutamine (from Flow Labs), 0.005 M HEPES buffer, 5×10^{-5} M 2-mercaptoethanol, and antibiotics. Lymphocytes of syngeneic spleens,

Laboratory of Immunochemistry and Diagnosis of Tumors, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 2, pp. 53-55, February, 1982. Original article submitted July 3, 1981.

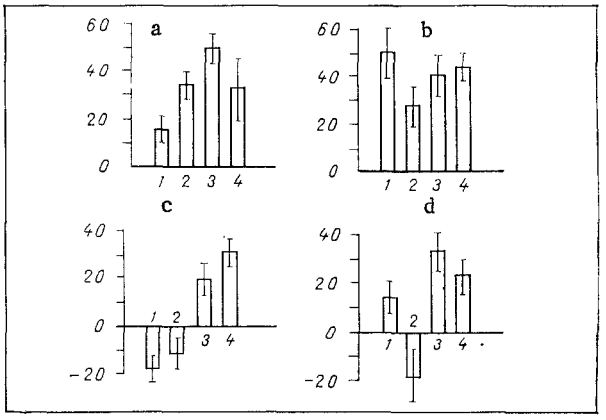

Fig. 1

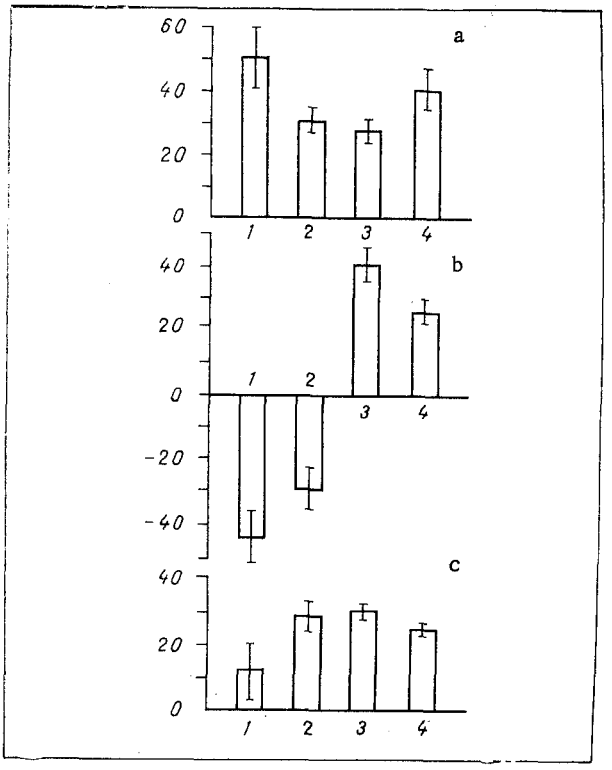

Fig. -

Fig. 1. Kinetics of MIF production in mixed culture of spleen cells and allogeneic tumor cells. a) B10 + EL-4, b) CBA + EL-4, c) B6 + MCh-11, d) CBA + MCh-11. Abscissa, age of MCLT (in days); ordinate, MII (in %). Short vertical lines indicate standard error of four determinations.

Fig. 2. Kinetics of MIF production in mixed culture of spleen cells and syngeneic tumor cells. a) B6 + EL-4, b) B10 + MCh-11, c) DBA/2 + P-815. Remainder of legend as to Fig. 1.

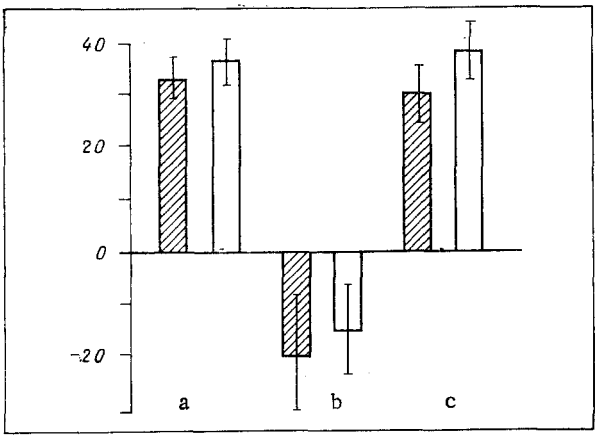

Fig. 3. Sensitivity of MIF producers reacting to syngeneic tumor antigens and H-2 alloantigens to anti-Thy-1,2 antibodies. a) Untreated B6 spleen cells; b) B6 spleen cells treated with normal mouse serum and complement. Shaded columns represent reaction to EL-4 stimulator cells, unshaded columns represent reaction to allogeneic D2 spleen cells. Ordinate, MII (in %) with standard error of four determinations.

TABLE 1. Abolition of MIF Production in Syngeneic MCLT and Allogeneic Mixed Lymphocyte Culture by Irradiation of Reacting Lymphocytes and Absence of MIF Activity in Mixture of Supernatants of Monocultures (M ± m)

Stimulating cells*	Period of incubation, days	Reacting cells: B6 intact A	B6 intact B	B6 irradiated A	DBA/2 intact A	DBA/2 intact B	DBA/2 irradiated A	B10 intact A	B10 intact B
B10·D2	1	40±4†	—	1±2	—	—	—	—	—
	2	31±2		—3±1					
	3	32±3		—1±2					
EL-4	1	36±2	—4±4	4±3	—	—	—	—	—
	2	29±7	—2±2	—9±2					
	3	26±2	—2±3	—5±3					
P-815	1	—	—	—	12±11	—3±3	4±3	—	—
	2				27±7	—1±2	0±3		
	3				29±3	0±4	—2±2		
	4				24±2	1±3	2±4		
MX-11	1	—	—	—	—	—	—	—44±5	1±3
	2							—27±6	12±4
	3							39±5	0±4
	4							24±4	—11±2

*Stimulating D2 spleen cells and tumor cells were irradiated in all cases in a dose of 5000-10,000 rads.
†MII calculated relative to syngeneic control (mean of three determinations with standard error).
Legend. A) MII in culture fluids of mixed cultures of lymphocytes and tumor cells; B) MII in mixture of culture fluids from monocultures of reacting and stimulating cells.

irradiated in the same dose and used in the same concentration as tumor cells, were used in the control and stimulating cells. Reacting cells in the positive control and in some experiments were mixed with allogeneic stimulators of line B10·D2 (abbreviated to D2; H-2^d). Incubation was carried out in 24-well plastic plates (from Linbro, No. 76-033-05, England) at 37°C in an atmosphere of 5% CO_2.

From the 1st through the 4th day of incubation the contents of the individual wells were removed, the cells were separated by centrifugation at 1000g for 30 min, and the resulting supernatants were tested on peritoneal macrophages of normal B6 mice in a micromodification of the macrophage migration inhibition test [4]. The results were assessed as the macrophage migration inhibition index (MII, %), calculated by the equation: $MII = (a-b)/a \times 100\%$, where a and b are the mean weight of the projection of the migration zone on paper in the syngeneic control and experiment respectively. B6 spleen cells were treated with anti-Thy-1,2 serum (from Searle Diagnostic, England) and complement (from Cedarlane, Canada) by the method described previously [1].

EXPERIMENTAL RESULTS

The kinetics of the reaction of MIF production in response to stimulation by allogeneic (Fig. 1) and syngeneic (Fig. 2) tumor cells from the 1st through the 4th day of MCLT is illustrated in Figs. 1 and 2.

Significant MIF production was discovered in MCLP of syngeneic or allogeneic lymphocytes with EL-4 cells as early as on the 1st or 2nd day of incubation (Fig. 1a, b; Fig. 2a). The reaction developed later in MCLT of syngeneic or allogeneic lymphocytes with MCh-11 or P-815 cells — on the 2nd (P-815) or 3rd (MCh-11) days of culture (Fig. 1c, d; Fig. 2b, c); in the presence of MCh-11 cells, moreover, both allogeneic and syngeneic spleen cells reacted by production of MIF. Culture fluids from mixed cultures of lymphocytes irradiated in a dose of 5000 rads with irradiated tumor cells, and also mixtures of culture fluids from spleen cells and tumor cells incubated separately, did not exhibit MIF activity (Table 1). Comparison of the kinetics of appearance of MIF activity for syngeneic and allogeneic MCLT revealed no significant differences.

To explain the nature of the cells producing MIF on contact with syngeneic tumor cells, the reacting cells were treated with anti-Thy-1,2 serum and complement. As Fig. 3 shows the

initial reaction of B6 splenic lymphocytes to cells of syngeneic T-lymphoma EL-4 and to allogeneic D2 spleen cells (in the positive control) was completely abolished after treatment of the reacting cells with anti-Thy-1,2 serum and complement whereas treatment with normal mouse serum and complement did not abolish the reaction under the same conditions. Consequently, the reaction discovered is T-dependent.

The experiments thus showed that MIF producing T cells can react in the early stages of primary MCLT (1st-3rd days) by mediator production to cells of allogeneic and syngeneic tumors. The reaction of nonimmune MIF producers was discovered in the early stages of MCLT, before any reaction of primary killers to cells of syngeneic T-lymphoma EL-4 [9]. The reaction to tumor antigens studied in the present investigation is analogous to the reaction of normal rat spleen cells producing MIF on contact with cell lines expressing leukemia viruses or endogenous C-type viruses [14]. These cells were found to be resistant to treatment with bovine serum against rat thymocytes with guinea pig complement [14].

The present investigation showed that primary immunologic recognition of syngeneic tumor antigens by MIF producers in mice is due to T lymphocytes. It was shown previously that nonimmune MIF producers reacting to cells of allogeneic, recombinant, and mutant lines of mice in mixed lymphocyte culture and sensitive to treatment with anti-Thy-1,2 antibodies with complement [5, 10] and also to anti-Ly-antibodies in the response to alloantigens [12], indicate the T-cell nature of primary recognition of MIF producers in mice.

Several common properties of natural killers (NK cells) and natural MIF producers have now been discovered [14]. The reaction to syngeneic tumor cells discovered in the present investigation and due to nonimmune cells consists in production of mediator of cellular immunity, and it is evidently a manifestation of natural immunity. It has been suggested that the entry of a foreign antigen from outside or the appearance of a malignant tumor inside the organism stimulates natural MIF producers to liberate a lymphokine which is able to attract macrophages to the location of the antigen, to keep them there, and to activate them [11]. The biological impotance of this reaction has been demonstrated in investigations showing that metabolism is considerably intensified in macrophages under the influence of MIF, and they acquire increased bacteriostatic properties as well as the ability to destroy tumor cells highly effectively, without injuring normal cells [7, 13].

Natural MIF producers, like natural killers, are thus evidently effector cells which are responsible for the earliest operative defensive reactions of the organism in the course of antitumor immunologic surveillance.

LITERATURE CITED

1. B. D. Brondz, A. P. Suslov, and S. G. Egorova, Scand. J. Immunol., 8, 109 (1978).
2. A. P. Suslov, B. D. Brondz, A. V. Karaulov, et al., in: Advances in Comparative Leukemia Research, 1979, New York (1980), p. 319.
3. A. P. Suslov, S. Gering, D. D. Kharkevich, et al., Byull. Éksp. Biol. Med., No. 4, 437 (1980).
4. A. P. Suslov and A. D. Chernousov, Byull. Éksp. Biol. Med., No. 8, 236 (1979).
5. D. D. Kharkevich, A. P. Suslov, and Z. K. Blandova, Byull. Éksp. Biol. Med., No. 2, 191 (1981).
6. F. M. Burnet, Cellular Immunology [Russian translation], Moscow (1971).
7. J. R. David, Fed. Proc., 34, 1730 (1975).
8. R. B. Herberman and H. T. Holden, Adv. Cancer Res., 27, 305 (1978).
9. E. Kedar, E. Unger, and M. Schwartzbach, J. Immunol. Meth., 13, 1 (1976).
10. S. Landolfo, M. Giovarelli, and G. Forni, Transplantation, 27, 290 (1979).
11. D. S. Nelson, in: Immunobiology of Macrophage, New York (1976), p. 401.
12. W. Newman, S. Gordon, U. Hammerling, et al., J. Immunol., 120, 927 (1978).
13. W. F. Piessens, W. H. Churchill, and J. R. David, J. Immunol., 114, No. 1, 293 (1975).
14. J. M. Sharma, R. B. Herberman, J. Y. Djeu, et al., J. Immunol., 123, 222 (1979).