INDUCTION OF T CELLS PRODUCING MACROPHAGE MIGRATION INHIBITION FACTOR BY PRODUCTS OF THE K, I, AND D REGIONS OF THE H-2 COMPLEX IN MICE

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Products of the chief histocompatibility complex (H-2 in mice) play a very important role in regulating the specificity of the immune response. Most subpopulations of T lymphocytes recognize foreign antigens present in the form of a complex with products of single regions of H-2. However, ability to recognize K, D, and I molecules in the induction phase, and genetic restrictions for K, I, or D regions in the effector phase of the immune response are not identical for different populations of T cells: T killers and T suppressors, T helpers and T effectors of delayed-type hypersensitivity (DTH) [1]. Although ability to react to products of single regions of the H-2 complex is a fundamental characteristic of subpopulations of T lymphocytes, it has not yet been studied in the case of T cells producing macrophage migration inhibition factor (MIF). The writers showed previously that in the course of the immune response MIF production in the H-2 system is achieved by different populations of T lymphocytes: early and late MIF producers, differing in expression of Lyt-antigens, sensitivity to cyclophosphamide [4], and the response to mutant H-2 and minor H-antigen [6, 7]. Early MIF producers can be detected as early as on the first day after intravenous alloimmunization, whereas activity of late MIF producers can be detected in the second week or later [5, 6].

The aim of this investigation was to study the possibility of inducing an immune response of early and late MIF-producing T cells after intravenous immunization with allogeneic spleen cells, carrying differences in the K, I, or D regions of the H-2 complex.

EXPERIMENTAL METHOD

The genetic characteristics of the inbred lines of mice used in the work are given in Table 1 [12]. The chosen pairs of lines differed in relation to individual regions of the H-2 complex: K (A.AL and A.TL), I (A.TL and A.TH), or D (R107 or B10). The animals were immunized by a single intravenous injection of an irradiated (15 Gy) suspension (90 × 10^6 cells in 0.8 ml of Hanks' solution per mouse) of allogeneic normal spleen cells by the method developed by the writers previously [5]. On the 1st, 6th, or 13th days after immunization, spleen suspensions were obtained from the immune (normal in the control) animals and mixed in the ratio of 10:1 with spleen cells of the immunizing line (with syngeneic cells in the control).

TABLE 1. Genetic Characteristics of Lines of Mice Used in the Work

Line	Region of H-2 complex			Genetic basis of line
	K	I	D	
A.AL	k	k	d	A
A.TL	s	k	d	A
A.TL	s	k	d	A
A.TH	s	s	d	A
B10.D2 (R107) (abbreviated to R107)	b	b	d	B10
C57BL/10Sn (abbreviated to B10)	b	b	b	B10

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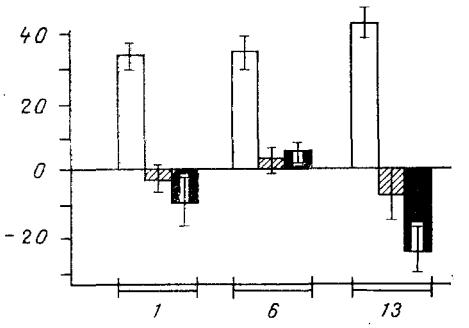

Fig. 1. Response of MIF-producing T cells to products of K region of H-2. Abscissa, time after immunization (in days); ordinate MMII (in %, M ± m). Unshaded columns — response of immune A.AL anti-A.TL cells to cells of immunizing line (A.TL); shaded columns — response of immune A.AL anti-A.TL cells to syngeneic cells (A.AL); columns shaded black — response of normal A.AL cells to A.TL cells (in primary mixed lymphocyte culture).

The cell mixtures were incubated at 37°C in an atmosphere of 5% CO_2 for 18 h in a final concentration of 2.75×10^6 cells/ml in a volume of 2 ml in wells of 24-well plastic plates (Linbro, USA) in medium RPMI-1640 (Flow Laboratories, England) with additives [6]. The culture fluids were separated from the cells by centrifugation (800g, 20 min) and tested for their ability to inhibit migration of normal peritoneal macrophages from C57BL/6 mice from glass capillary tubes (Behringwerke, West Germany) on the bottom of 96-well No. 3040 plastic plates (Falcon Plastics, USA), in the micromodification of the macrophage migration inhibition test (MMIT) [2]. Activity of MIF in the test culture fluids was determined as the macrophage migration inhibition index (MMII), by the equation:

$$\text{MMII} = \left(1 - \frac{\text{mean area of migration in experiment}}{\text{mean area of migration in control}}\right) \times 100\%.$$

The T-cell nature of MIF production after intravenous immunization was demonstrated by the writers previously [5, 6].

EXPERIMENTAL RESULTS

The response of MIF producers of line A.AL to cells of line A.TL when the reacting and stimulating cells differed only with respect to the K region of the H-2 complex (Table 1), is illustrated in Fig. 1. The response of the MIF producers was apparent as early as during the first day and it was still present both on the 6th and on the 13th day after immunization, whereas the response in the syngeneic control was absent at all times of investigation.

Data obtained during reciprocal immunization of mice of lines A.TL and A.TH, differing only with respect to the I region of H-2, are given in Fig. 2. In this case the response of the MIF producers was manifested at all times of the investigation after immunization; in the case of immunization with both A.TL anti-A.TH and A.TH anti-A.TL, the response increased from the first to the 13th days.

Results obtained by reciprocal immunization of mice of lines R107 and B10, differing with respect to the D region of the H-2 complex, are given in Fig. 3. As Fig. 3 shows, differences with respect to the D region of H-2 did not induce any significant response of MIF producers both on the first and on the 6th and 13th days after reciprocal immunization.

The response of immune cells to syngeneic cells, and also that of normal cells to the corresponding antigen, it will be noted, was absent in all cases studied (Fig. 1-3).

Thus both early and late MIF-producing T cells responded during immunization with irradiated spleen cells *in vivo* to products of the K and I regions of the H-2 complex, whereas products of the D region virtually did not stimulate MIF production. Similar results were ob-

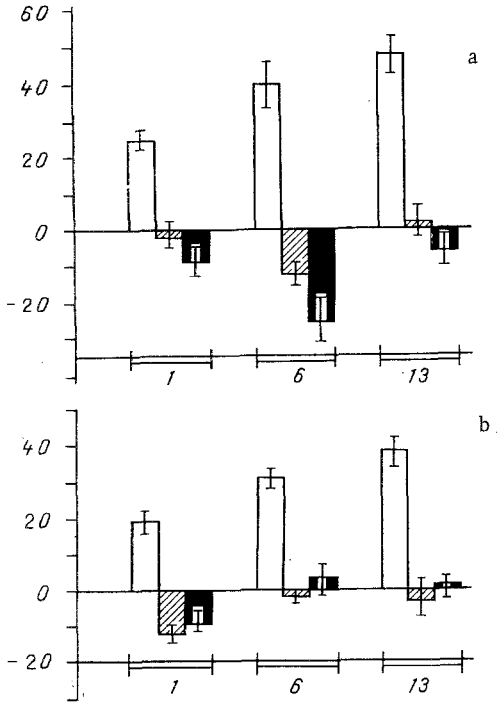

Fig. 2

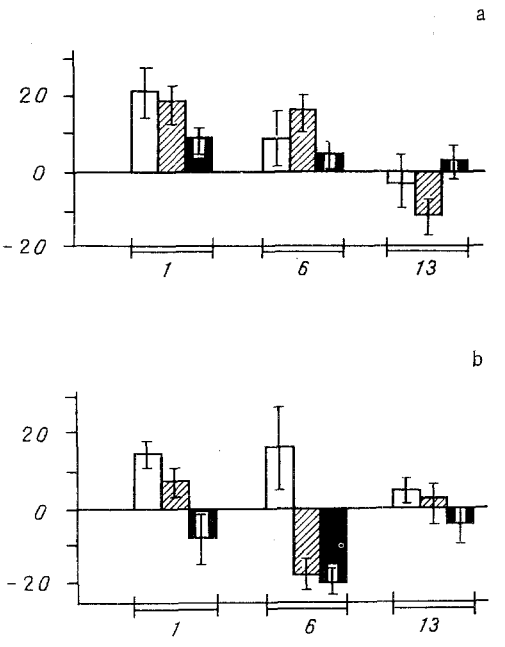

Fig. 3

Fig. 2. Response of MIF-producing T cells to products of I region of H-2 complex. Unshaded columns — response of immune cells: a) A.TL anti-A.TH to A.TH cells, b) A.TH anti-A.TL to A.TL cells; obliquely shaded columns — response of immune cells: a) A.TL anti-A.TH to syngeneic A.TL cells, b) A.TH anti-A.TL to A.TH; black columns — response of normal cells to corresponding antigen: a) A.TL to A.TH, b) A.TH to A.TL. Remainder of legend as to Fig. 1.

Fig. 3. Response of MIF-production T cells to products of D region of H-2 complex. Unshaded columns — response of immune cells: a) R107 anti-B10 to B10 cells, b) B10 anti-R107 to R107; obliquely shaded columns — response of immune R107 anti-B10 cells (a) and B10 anti-R107 (b) to syngeneic cells: R107 (a) and B10 (b); black columns — response of normal cells: a) R107 to B10, b) B10 to R107. Remainder of legend as in Fig. 1.

tained for effectors of DTH *in vivo* [11], confirming the connection between the DTH reaction *in vivo* and the phenomenon of macrophage migration inhibition *in vitro*, established previously by classical studies [8, 9]. The absence of response to products of the D region of H-2, noted above, is evidently connected with the fact that region H-2K, as many investigations have shown, is immunologically stronger than the H-2D region [12]. For instance, it has been demonstrated that the intensity of the graft versus host reactions depends to a greater degree on differences for the H-2K region than on differences for H-2D; in the case of differences for H-2K, graft rejection and antibody formation in higher titers take place much faster than in the case of differences for H-2D [12].

However, experimental models both *in vivo* and *in vitro*, on which it was possible to detect a response of MIF producers to products of the D region of H-2, have been described in the literature. For instance, by contrast with results for MIF producers obtained after intravenous immunization *in vivo* and in the DTH test *in vivo*, MIF producers induced in primary mixed lymphocyte culture *in vitro* respond both to the K and I regions and to the D region of H-2 [10].

Previously, during immunization with allogeneic tumor cells against the whole $H\text{-}2^b$ haplotype *in vivo*, we obtained a response of MIF producers during subsequent contact *in vitro* with cells carrying only the D^b-region of the $H\text{-}2^b$ immunizing complex. It can be postulated that the response to the immunologically "weaker" H-2D region depended to a greater degree than H-2K on the features of regulation of the immune response of the MIF producers, and could be controlled both by suppressors acting directly on MIF producers, and by cells producing a factor with activity opposite to that of MIF.

Considering data obtained by ourselves and other workers [6, 7, 10] it can be concluded that MIF producers possess the universal property of responding to transplantation antigens, for they can be induced during immunization *in vivo* and *in vitro* by strong, weak, and also mutant histocompatibility antigens.

LITERATURE CITED

1. B. Z. Brondz and O. V. Rokhlin, Molecular and Cellular Bases of Immunologic Recognition [in Russian], Moscow (1978).
2. A. P. Suslov and A. D. Chernousov, Byull. Éksp. Biol. Med., No. 8, 236 (1979).
3. A. P. Suslov, S. Gering, D. D. Kharkevich, et al., Tsitologiya, No. 4, 437 (1980).
4. A. P. Suslov, D. D. Kharkevich, B. D. Brondz, et al., Tsitologiya, No. 9, 1110 (1982).
5. A. P. Suslov, D. D. Kharkevich, and A. V. Karaulov, Immunologiya, No. 1, 49 (1983).
6. D. D. Kharkevich, A. P. Suslov, and Z. K. Blandova, Byull. Éksp. Biol. Med., No. 4, 443 (1984).
7. D. D. Kharkevich and A. P. Suslov, Byull. Éksp. Biol. Med., in Press.
8. B. R. Bloom, Adv. Immunol., 13, 101 (1971).
9. J. R. David, Proc. Natl. Acad. Sci. USA, 56, 72 (1966).
10. S. Landolfo, F. Marcucci, M. Giovarelli, et al., Immunogenetics, 9, 245 (1979).
11. F. I. Smith and J. F. A. P. Miller, J. Exp. Med., 150, 965 (1979).
12. C. D. Snell, J. Dausset, and S. Nathenson, Histocompatibility, New York (1976).