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INDUCTION OF T CELLS PRODUCING MACROPHAGE MIGRATION INHIBITION FACTOR BY MUTANT H-2 ANTIGENS *IN VIVO*

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KEY WORDS: macrophage migration inhibition factor; mutant H-2 antigens.

The main function of the immune system is to protect the organism against all that is biologically foreign, whether entering from outside or arising inside the organism as a result of a change in its own cells produced by a virus, carcinogen, or mutation [1]. Mutant lines of Hz1 ($H\text{-}2^{bm1}$) and M505 ($H\text{-}2^{bm3}$) mice differ from the original C57BL/6 ($H\text{-}2^{d}$) line by replacement of one or two amino acid residues in the molecule of the transplantation antigen [8], and the reaction of C57BL/6 lymphocytes to Hz1 ($H\text{-}2^{bm1}$) and M505 cells can be used as a model for the detection of weak changes in the organism's own antigens at different stages of carcinogenesis.

It was shown previously that mutant bm1 and bm3 antigens differ serologically only slightly [8], but they effectively induce antigen-specific proliferation of T cells and generation of T killer cells in mixed lymphocyte culture (MLC) *in vitro* [8, 9]. The authors have shown that by the second or third day, i.e., before proliferation develops and T killer cells appear, T cells producing macrophage migration inhibition factor (MIF) are formed in MLC of mutant and the original lines [6].

In the investigation described below, a technique devised by the authors themselves was used to induce MIF producers in the H-2 system by intravenous immunization [4] and to study the ability of MIF-producing T cells to respond to mutant antigens $H\text{-}2^{bm1}$ and $H\text{-}2^{bm3}$ by immunization *in vivo*.

EXPERIMENTAL METHOD

The genetic characteristics of inbred lines of mice used in the investigation are indicated in Table 1. C57BL/6 mice (abbreviated to B6, $H\text{-}2^{b}$) were immunized by a single intra-

TABLE 1. Genetic Characteristics of Lines of Mice Used for Immunization *in Vivo* against Mutant and Normal Transplantation Antigens

Line of mice	Abbreviated name of line	H-2 haplotype	Regions of H-2			Genetic basis of line
			K	I	D	
C57BL/6	B6	b	b	b	b	B6
B6.C-H(z1)Y	Hz1	bm1	bm1	b	b	B6
B6.M505Y	M505	bm3	bm3	b	b	B6
B10.D2	D2	d	d	d	d	B10

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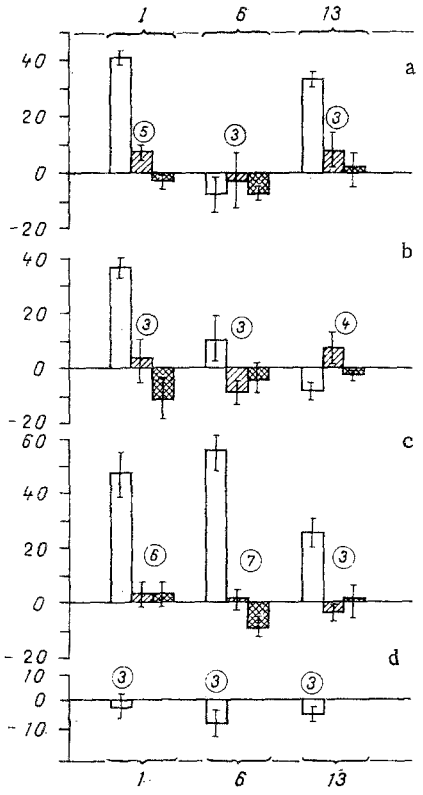

Fig. 1

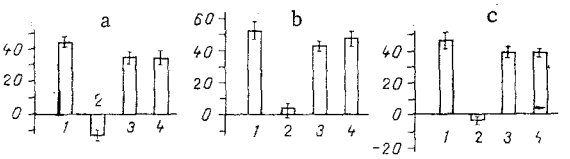

Fig. 2

Fig. 1. Reaction of MIF-producing T cells to mutant antigens and alloantigens at different times after intravenous immunization. a) Immunization with B6 anti-Hz1 (K^b anti-K^{bm1}); b) immunization with B6 anti-M505 (K^b anti-K^{bm3}); c) immunization with B6 anti-D2 (b anti-d, differences present between antigens of genetic basis); d) immunization with B6 anti-B6 (b anti-b, syngeneic). Reaction of immune lymphocytes to allogeneic cells of immunizing line (unshaded columns) or to syngeneic cells (obliquely shaded columns) is shown. Cross-hatched columns show reaction of nonimmune B6 lymphocytes to Hz1 (a), M505 (b), and D2 (c) (reaction in primary mixed lymphocyte culture *in vitro*). Columns with short vertical lines indicate M ± m for number of pure-line animals tested (circled number above columns). Abscissa, time after immunization (days); ordinate, MMI (%).

Fig. 2. Sensitivity of B6 anti-Hz1 (a) and B6 anti-D2 (b, c) MIF producers to treatment with anti-Thy-1.2 serum with complement on 1st (a, b) and 6th (c) days after immunization. Reacting cells were untreated (1) or treated: with anti-Thy-1.2 serum with complement (2), with normal mouse serum and complement (3), and with anti-Thy-1.2 serum with inactivated complement (4). Ordinate, MMI (%).

venous injection of irradiated (1500 rads) suspension ($90 \cdot 10^6$ cells in 0.8 ml of Hanks' solution per mouse) of normal spleen cells from mice of mutant lines Hz1 and M505, of allogeneic line B10.D2 (abbreviated to D2, H-2^d) in the positive control, and syngeneic line B6 in the negative control. On the 1st, 6th, or 13th days a suspension of spleen cells was obtained from the immune (in the control, from normal) animals and mixed in the ratio of 10:1 with spleen cells of the immunizing line (in the control, with syngeneic cells). The mixture of cells was incubated at 37°C in an atmosphere of 5% CO_2 for 18 h in a final concentration of $2.75 \cdot 10^6$ ml in a volume of 2 ml, in wells of a 24-well plastic plate (Linbro, USA) in medium RPMI-1640 (from Flow Laboratories, Great Britain), with the following additions: 10% embryonic calf serum (from Gibco, Great Britain), 2 mM L-glutamine (Flow Laboratories), 0.005 M HEPES buffer, and antibiotics penicillin and streptomycin, in a concentration of 100 U/ml each. The culture fluids were separated from the cells by centrifugation (800g, 20 min) and tested for ability to inhibit migration of normal peritoneal macrophages of B6 mice from glass capillary tubes (from Behring, West Germany) along the bottom of 96-well plastic plates (from Falcon, USA) in a micromodification of the macrophage migration inhibition test [2]. Activity of MIF in the culture fluids was estimated by means of the macrophage migration inhibition index (MMI), determined by the equation:

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

Lymphocytes were treated with anti-Thy-1.2 serum and complement by the method recommended by the firm "Cedarlane" (Canada) in two stages: $2 \cdot 10^7$ cells were incubated in 1 ml of anti-Thy-1.2 serum (from Searle Diagnostics, USA), diluted tenfold (in the control, with normal mouse serum) for 45 min at 20°C, and after centrifugation (250g, 7 min) the cells were incubated in 1 ml complement (from Cedarlane), diluted tenfold (in the control, with complement inactivated by heating to 56°C for 30 min).

EXPERIMENTAL RESULTS

As will be clear from Fig. 1, MIF producers can react to mutant antigens and alloantigens as early as 24 h after immunization. On the 6th day after immunization the reaction to both mutant lines disappears, whereas that to alloantigens remains. On the 12th-13th day after immunization differences appeared in the reaction to mutant lines: The reaction to M505 was absent as before, whereas that to H-1 reappeared. The reaction to allogeneic D2 cells was reduced by half by the 13th day, although it still remained at a significant level ($P < 0.05$). In all versions of testing of activity of the MIF producers studied, incidentally, there was no reaction of B6 lymphocytes, immunized against Hz1, M505, D2, and B6 to syngeneic B6 cells, and no reaction of normal lymphocytes of B6 mice to Hz1, M505, and D2 cells, in both cases relative to the reaction of normal B6 lymphocytes to syngeneic cells (Fig. 1).

To study the nature of MIF-producing cells in the test system, spleen cells were treated with anti-Thy-1.2 serum with complement. It will be clear from Fig. 2 that treatment of this kind completely abolished the reaction of the MIF producers to mutant antigens and alloantigens on both the 1st day and 6th day after immunization, whereas treatment with normal mouse serum and complement, and also with anti-Thy-1.2 serum with inactivated complement, did not affect the reaction.

The results thus showed that by the 1st day after immunization MIF-producing T cells can recognize mutant H-2 antigens. The authors showed previously that MIF-producing T cells can recognize mutant H-2^{bm1} and H-2^{bm3} antigens in MLC *in vitro* [6]. Taken together, these results obtained by the authors *in vivo* and *in vitro* indicate that among precursors of MIF producers there are clones which recognize mutant H-2 antigens modified by a point mutation, and that induction of an immune response of a subpopulation of MIF-producing T cells against these antigens is possible. In the early stages of the immune response against the original K^b antigen, when considerable cross reaction of MIF products to mutant K^{bm1} and K^{bm3} antigens is discovered [3], induction of clones recognizing these antigens evidently takes place, whereas in the course of the immune response, when the cross reaction disappears [3], mutant-recognizing MIF producers either fall under suppressor control or are eliminated. Disappearance of the reaction of MIF producers to mutant H-2 antigens in the course of the immune response correlates with replacement of markers of the MIF producer: of "early" with the Lyt-1^+2^+ phenotype, by "late" with the Lyt-1^+2^- phenotype [5]. The possibility cannot be ruled out that "late" MIF producers can be induced by mutant H-2 antigens far less readily than "early" producers.

The results indicate the existence of T lymphocytes capable of being activated in a very short time by mutant H-2 antigens, and on repeated contact with them, they secrete a factor which can greatly strengthen the protective properties of macrophages: MIF is inseparable from macrophage activation factor [7], and on that account accumulation of macrophages in the zone of reaction of the organism to a "foreign" factor and activation of their cytotoxicity are evidently mutually coupled functions of this T-cell mediator [7, 10]. Since mutant-recognizing MIF producers can be activated within 24 h, MIF production by immune T lymphocytes begins 6 h after contact with the antigen, and only 2-3 h is sufficient for activation of a large number of macrophages by this factor [7, 11], it can be postulated that MIF producers are among the key cells of immunologic surveillance, capable of mobilizing the protective properties of the organism quickly and effectively.

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PROTECTIVE ACTION OF DOUBLE-STRANDED INTERFERON-INDUCING COMPLEXES AGAINST EXPERIMENTAL ENCEPHALOMYOCARDITIS IN MICE

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Previous investigations demonstrated the interferon-inducing activity of poly(G):poly(C) and of double-stranded RNA (dsRNA) [1, 2, 5]. Different series of preparations were tested in a number of experimental virus infections, including tick-borne encephalitis, herpes, etc. [3, 6]. The experimental model of encephalomyocarditis in mice was found to be promising for the study of the antiviral action of interferon inducers. The presence of tRNA-like structures in the virus genome and sensitivity to the action of tRNA, as Stebbing et al. [7] showed, also presupposed their sensitivity to RNA-like interferon inducers.

This paper gives the results of a study of the antiviral activity of two interferon inducers, polyguacyl and phage dsRNA, in experimental encephalomyocarditis in mice.

EXPERIMENTAL METHOD

Mouse encephalomyocarditis (EMC) virus, belonging to the genus *Cardiovirus* of the family Picornaviridae, was maintained by passage in a culture of L-929 mouse fibroblasts and titrated *in vivo* in mice by intracerebral and intramuscular injection. Noninbred male albino mice weighing 10-12 g were used.

A complex of polyguanylic and polycytidylic acids (polyguacyl, batch 811117), synthesized in the Laboratory of Polymers (Leningrad Institute of Nuclear Physics, Academy of Sciences of the USSR), and dsRNA of amber-mutant phage, obtained in the Institute of Microbiology, Academy of Sciences of the Latvian SSR, were used as interferon inducers. Polyguacyl was obtained as a sterile aqueous solution in a concentration of 2 mg/ml, and dsRNA was obtained in lyophilized form. The interferon inducers were made up in the required concentration in distilled water or in physiological saline. Animals of the control group received water or physiological saline, respectively.

The interferon inducers were tested beforehand for interferon-inducing activity. The substanced were used in a dose of 5 mg/kg. For intracerebral injection this dose was contained in a volume of 0.03 ml; for intraperitoneal injection, 0.2 ml.

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