PRODUCTION OF MACROPHAGE MIGRATION INHIBITION FACTOR BY T-CELLS REACTING TO MUTANT H-2 ANTIGENS IN MIXED LYMPHOCYTE CULTURE

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Antigens of the principal histocompatibility complex (H-2 in mice), modified by viruses, haptens, and other foreign antigens, act as markers of their "altered self" and serve as signals for triggering mechanisms of immunologic surveillance [2]. Mutation changes in H-2 antigens also are recognized by the immune system as their "altered self," and mutant antigens of the H-2K^b allele (K$^{bm^1}$ and K$^{bm^3}$) [8] induce only T-cells but not serologic reactions [10]. T-cells producing migration inhibition factor (MIF), immune to antigens of the original H-2^b haplotype, distinguish mutant H-2 antigens from normal, by not reacting to antigens of mutants H-2$^{bm^1}$ and H-2$^{bm^3}$ [5]. However, it is not yet clear whether an immune response of MIF producers can be induced directly against mutant antigens themselves.

The object of this investigation was to study the possibility of inducing MIF producers in a unidirectional mixed lymphocyte culture (MLC) during the reciprocal reaction of nonimmune T-cells of the original and mutant lines of mice.

EXPERIMENTAL METHOD

Spleen cells from individual intact C57BL/6Y mice [abbreviated to B6(H-2^b)] or of mutant lines B6.C-H(z1)/Y [abbreviated to Hz1(H-2$^{bm^1}$)] and B6.M505/Y [abbreviated M505(H-2$^{bm^3}$)] were used as reacting lymphocytes in MLC. They were mixed in the ratio of 1:1 with irradiated (1500 rads) allogeneic stimulating spleen cells from B10.D2 [abbreviated to D2(H-2^d)] and Af-(H-2^a) mice (positive control), with syngeneic cells (negative control), and with mouse spleen cells differing from the reacting lymphocytes with respect to the mutant antigen H-2.

Mixtures of cells were incubated in a concentration of $5 \cdot 10^6$ cells/ml in RPMI-1640 medium with the addition of 10% embryonic calf serum, 2 mM L-glutamine, 0.005 M HEPES, $5 \cdot 10^{-5}$ M 2-mercaptoethanol, and antibiotics. Incubation was carried out in 24-well plastic plates (Flow Labs, Great Britain) at 37°C in an atmosphere of 5% CO_2. The contents of the cultures were centrifuged from the second through the fifth day of culture at 1000g for 30 min and the supernatants obtained were tested for ability to inhibit migration of peritoneal macrophages from nonimmune B6 mice. The macrophage migration inhibition test was set up in a simplified micromodification [6] of the capillary method. The results were expressed as the macrophage migration inhibition index (MMI):

$$\text{MMI} = \frac{a-b}{a} \cdot 100\%,$$

where a and b are the mean weights of the zone of migration in the syngeneic control and experimental respectively. Treatment of the B6 spleen cells with anti-Thy-1,2 serum (Searle Diagnostic, Great Britain) with guinea pig complement was carried out by the method described previously [3].

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TABLE 1. MIF Production in Mixed Lymphocyte Culture during Reaction to Mutant and Allogeneic H-2 Antigens*

Stimulating cells†	Responding cells		
	C57BL/6Y ($H\text{-}2^b$)	B6.C-H-(z1)/Y ($H\text{-}2^{bm1}$)	B6.M505/Y ($H\text{-}2^{bm3}$)
B10.D2 ($H\text{-}2^d$)	44±3	37±3	31±7
Af ($H\text{-}2^a$)	39±3	—	—
C57BL/6Y ($H\text{-}2^b$)	0	51±7	37±7
B6.C-H (z1)/Y ($H\text{-}2^{bm1}$)	35±10	0	40±6
B6.M505/Y ($H\text{-}2^{bm3}$)	31±6	40±1	0
F_1 (Hz 1×M505) ($H\text{-}2^{bm1}$/$H\text{-}2^{bm3}$)	31±8	—	—

*Data in Table 1 shown in the form of MMI (in percent) with standard error. MMI calculated relative to syngeneic control.
†Line and haplotype (in parentheses) of cells shown.

EXPERIMENTAL RESULTS

The results are summarized in Table 1; the maximal reaction of MIF production with different combinations of allogeneic and mutant cells was assessed from the second to the fifth day of MLC. MIF production was detected in MLC in all combinations of reacting and stimulating cells. The magnitude of this reaction did not differ significantly when cells of mutant and ordinary allogeneic lines were used as reacting or stimulating lymphocytes. It will be clear from Table 1 that B6 form MIF in the reaction with stimulators not only of each mutant, but also of the (Hz1 × M505)F_1 hybrid, evidence of absence of the complementarity of mutations $H\text{-}2^{bm1}$ and $H\text{-}2^{bm3}$, established previously by skin grafting [1]. Absence of complementarity also indicates that both mutations took place in the same gene.

The study of the kinetics of MIF activity in medium of MLC of individual mice shows that MIF appears as a pointed peak on the second, third, or fourth day after the beginning of culture (Fig. 1). The kinetics of reaction of B6 lymphocytes to stimulators of allogeneic lines (D2 and Af) and of mutant Hz1 could either coincide or not coincide.

MIF production in MLC with both allogeneic and mutant lines stopped completely after treatment of the responding cells with anti-Thy-1,2 serum and complement (Fig. 2), i.e., it was T-dependent.

MIF producers can thus not only distinguish mutant H-2 antigens from normal during the immune response to normal H-2 alloantigens [5], but they can also recognize mutant H-2 antigens by reacting to them directly. Consequently, MIF producers possess fine immunologic specificity, enabling them to react to mutant antigens, changes in which compared with the original H-2 antigen are very slight. For instance, mutation $H\text{-}2^{bm1}$ is characterized by replacement of only one amino acid residue [7].

The reaction of MIF producers to mutant antigens, just as to alloantigens, develops and reaches a maximum mainly in the early period of MLC, when the cytotoxic activity of the T-killers is minimal or is absent altogether [4]. This indicates nonidentity between MIF producers and T-killers and that the former are less mature than T-killers [3], and also that generation of MIF producers may be a more sensitive indicator of the reaction of the immune system to the "altered self" than the formation of the T-killer response. The difference in the kinetics of generation of MIF producers in MLC with allogeneic and mutant stimulators is evidence in support of independent differentiation of alloreactive and mutant-reactive MIF producers.

Since the original B6 line and mutant lines Hz1 and M505 are identical for all regions of the H-2 complex except the K-region [1], it is evident that the assistance of other T-cells reacting to products of the I region of the H-2 complex is not required for the reaction of MIF producers to H-2K antigens.

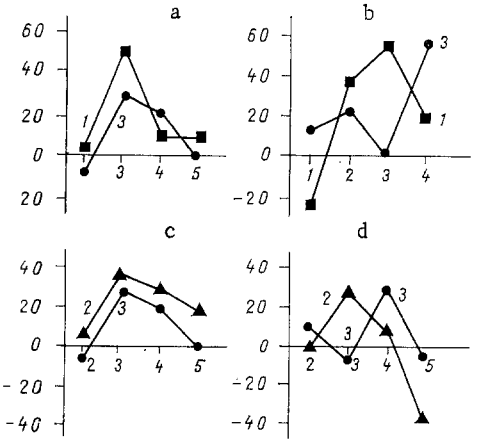

Fig. 1

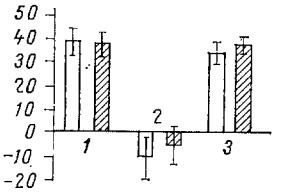

Fig. 2

Fig. 1. Coincidence (a, c) and difference (b, d) of reaction kinetics of MIF producers in MLC of spleen cells of original line B6 to stimulating cells of allogeneic lines D2 (1), Af (2), and mutant line Hz1 (3). Abscissa, age of MLC (in days); ordinate, MMI (in percent).

Fig. 2. Sensitivity of MIF producers reacting to mutants and alloantigens H2 against anti-Thy-1,2 antibodies. 1) Untreated B6 spleen cells; 2) B6 spleen cells treated with anti-Thy-1,2 serum with complement; 3) B6 spleen cells treated with normal mouse serum and complement. Unshaded columns — Hz1 stimulator cells; shaded columns — D2 cells. Vertical axis — MMI (in percent with standard error of four determinations).

MIF production in response to mutant and alloantigens H-2 in MLC evidently is affected by T-cells, because this reaction is sensitive to the action of anti-Thy-1,2 and anti-Ly-antibodies [9] and it develops in response to serologically poorly distinguishable mutant H-2 antigens.

The ability of MIF producers to distinguish normal and mutant H-2 antigens demonstrates the high discriminative power of the receptors of the MIF producers and the high sensitivity of their reaction and it indicates that these cells may participate effectively in the mechanism of immunologic surveillance.

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