CHANGES IN CROSS-REACTIVITY OF MIF-PRODUCERS IN THE COURSE OF AN IMMUNE RESPONSE IN THE H-2 SYSTEM

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Immune T-lymphocytes, during contact with specific antigen, are activated and produce soluble cellular mediators, including macrophage migration inhibition factor (MIF) [8]. It is suggested that T-cells producing MIF (MIF-producers) are a special subpopulation of T-lymphocytes, not identical with T-killers and T-helpers [5, 11, 13]. One of the properties which distinguishes MIF-producers from T-killers is their marked immunologic cross-reactivity in the H-2 system [4, 11].

In this investigation cross-reactivity of MIF-producers was studied in the course of the immune response.

EXPERIMENTAL METHOD

Cells of an allogeneic MCh-11 ascites tumor, induced in C57BL/10 mice* (abbreviated to B10, genotype H-2: $K^bI^bD^b$) [3], were used to immunize B10 · D2 ($K^dI^dD^d$) mice (abbreviated to D2) and B10 · D2 (R101) $K^dI^dD^d$) mice (abbreviated to R101), by a single injection at five subcutaneous points and intraperitoneally (40-50 million cells per mouse). On the 5th-7th day or on the 13th-15th day after immunization the ability of the lymph node cells to produce MIF during contact with spleen cells of the donor's line (positive control), of the syngeneic line (negative control), and of lines carrying individual components of the $H\text{-}2^b$ immunizing complex was investigated. B10 · D2 anti-B10 lymphocytes were incubated with target cells (TC) of congeneically resistant lines R101 and B10 · D2 (R107) (abbreviated to R107), with H-2 haplotypes of recombinant origin, carrying individual special specificities of the immunizing H-2, and also with TC on lines B10 · A and B10 · M, carrying certain general specificities of this complex (Table 1). R101 anti-B10 (anti-K^bI^b) lymphocytes were incubated with TC of lines B6 · H (Z1) ($H\text{-}2^{ba}$) and B6 · M505 ($H\text{-}2^{bd}$), which differ from the wild-type K^b gene by the point mutation of this gene [2]. MIF activity was determined by the indirect macrophage migration inhibition test (MMIT) [9] in the micromodification described previously [4]. In experiments with congeneic and recombinant lines medium No. 199 was used, whereas in the experiments with mutant lines medium RPMI-1640 with additions [6] was used. The index of the MMIT (MMII) was calculated by the formula:

$$\text{MMII} = \left(1 - \frac{\text{mean zone of migration in experimental samples}}{\text{mean zone of migration in control samples}}\right) \cdot 100$$

EXPERIMENTAL RESULTS

One week after immunization the B10 · D2 anti-B10 lymphocytes, as was shown previously [5], had the ability to produce MIF on contact with TC carrying both special and general specificities of the H-2 immunizing complex (Fig. 1a). The MMIT with cells of the third lines was immunologically specific, for it did not develop in the presence of TC of a syngeneic line. A special feature of the response of these immune lympho-

*The lines of mice used in the experiments were bred in the Department of Genetics, Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR; symbol Y.

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TABLE 1. TC of Different H-2 Haplotypes Used to Stimulate MIF Production

Immune lymphocytes	Source of TC		H-2 specificities potentially capable of participating in the reaction	
	line	haplotype	K-region	D-region
D2 anti-B10 ($anti\text{-}K^{b}I^{b}D^{b}$)	B10	b	33*, 5, 39, 53, 54, 56	2*
	D2	d	None	None
	B10.A	a	5	"
	B10.M	f	39, 53	"
	R107	i7	33*, 5, 39, 53, 54, 56	"
	R101	g1	None	2*
R101 anti-B10 ($anti\text{-}K^{b}I^{b}$)	B6	b	33*, 5, 39, 53, 54, 56	None
	R101	g1	None	"
	B6.H (Z1)	ba	33*, 5, 39,	"
	B6.M505	bd	53, 54, 56, 33*, 5, 39, 53, 54, 56	"

*Special H-2 specificities.

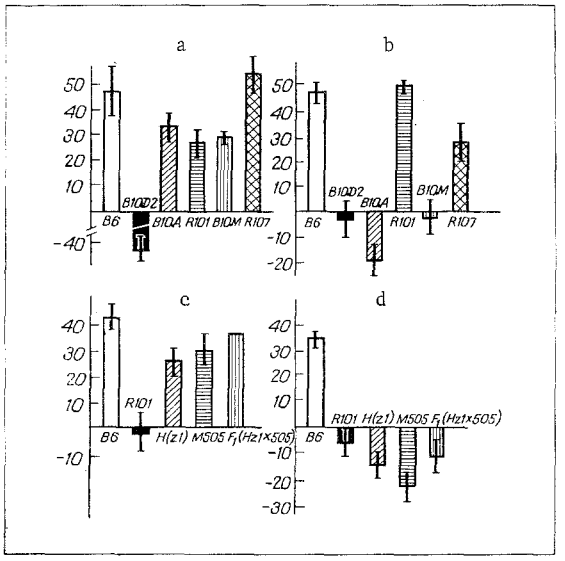

Fig. 1. MIF production by B10 · D2 anti-B10 (a, b) and R101 anti-B10 (c, d) lymphocytes on contact with TC carrying individual components of the H-2 immunizing complex (a, b) or with TC of mice of mutant H-2 haplotypes and their F_1 hybrids (c, d). Ordinate MMII. Columns show mean with standard error for 3 (a, b) and 5-6 (c, d) experiments.

cytes 1 week after immunization was the significant ($P < 0.05$) excess of intensity of MMIT in the presence of TC of the R107 line, possessing K- and I-regions of the H-2 immunizing complex, over the reaction to TC of the R101 line, possessing the D-region of the $H\text{-}2^{b}$ complex. In two of the three experiments the MMIT to TC of the R107 line was stronger, and that to TC of the R101 line was weaker than the MMIT to TC of the donor's line. In the 2nd week the intensity of MMIT in the presence of TC of the donor's line remained the same as before (Fig. 1b). Meanwhile, the character of MMIT discovered during incubation of B10 · D2 anti-B10 lymphocytes with TC of other lines showed a considerable change: It disappeared in the presence of TC of the B10 · M line, was converted to stimulation of migration in the presence of TC of the B10 · A line, was considerably potentiated in the presence of TC of the R101 line, and was sharply reduced in the presence of TC of the R107

line. The difference between the MMIT to TC R101 and R107, which was opposite to that found after 1 week, also was significant ($P < 0.05$).

Changes in cross-reactivity of the MIF-producers also was found in experiments with lines of mice carrying the mutant H-2 haplotype. It was shown previously that antibodies against normal H-2 · 33 (K^b) antigen distinguish it only weakly or not at all from mutants K^{ba} and K^{bd} antigens [9, 12]. Ability to discriminate from mutant antigens is much more marked in T-killers [1]. Like T-killers, MIF-producers 1 week after immunization reacted to mutant H-2^{ba} and H-2^{bd} antigens, although less strongly than to normal H-2^b antigen (Fig. 1c). However, 2 weeks after immunization the MMIT of immune anti-K^b lymphocytes to mutant antigens not only disappeared completely, but changed to a reaction of stimulation of migration (Fig. 1d), whereas the intensity of MMIT in the presence of TC of the normal haplotype decreased only very little. Similar changes took place in MMIT to H-2 antigens of the [H(Z1) × M505]F_1 hybrids in the course of the immune response. The absence of MMIT to TC of hybrids found 2 weeks after immunization (Fig. 1d) is evidence that mutant genes H-2 K^{ba} and H-2 K^{bd} are not complementary, as was shown previously in skin grafting experiments [2], and it confirms the suggestion that the two mutations, although not identical, have taken place in the same gene.

In the course of the immune response of MIF-producers in vivo in the H-2 system changes thus take place in immunologic specificity of their reaction: The intensity of the response changes with respect to products of the K- and D-regions of H-2 and cross-reactivity to "foreign" H-2 haplotypes disappears. As a result of disappearance of cross-reactivity the ability of the MIF-producers to differentiate the mutant H-2 antigen from the initial antigen increases, even when only one aminc acid residue in the antigen molecule is changed as a result of mutation (the H-2^{ba} haplotype) [8]. The increase in immunologic specificity of the reaction of the MIF-producers in the course of the immune response was described previously during an investigation of MMIT on conjugates of haptens with similar chemical structure and a protein carrier [1]. A change in specificity could arise only through the selection of clones with receptors with the highest affinity, or by a change in the affinity of the lymphocyte receptors. The possibility of participation of antibodies, of specific T- or B-suppressors, and also of a subpopulation of lymphocytes producing a factor with activity opposite to MIF – stimulation of macrophage migration – in this process cannot be ruled out. This last hypothesis is supported by the discovery of stimulation of macrophage migration in both series of experiments after contact with TC of "foreign" (B10 · A) or mutant H-2 haplotypes. As a result of the increase in discriminitive power the MIF-producers can evidently acquire immunologic specificity, distinguishing them from other lymphocyte subpopulations, including from killers: 2 weeks after immunization the MIF-producers acquired the ability to discriminate determinants of H-2 antigens more clearly than T-killers [1]. The change discovered in cross-reactivity of MIF-producers in the H-2 system, tested by a set of congeneically resistant lines and lines with recombinant and mutant H-2 haplotypes can serve as a model with which to study mechanisms of formation of the immunologic specificity of the response of this T-cell subpopulation.

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