# CORRELATION BETWEEN CYTOTOXIC LYMPHOCYTES AND CELLS SYNTHESIZING MACROPHAGE MIGRATION INHIBITION FACTOR IN THE H-2 SYSTEM

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The high immunologic specificity of direct and indirect macrophage migration inhibition tests in the H-2 system was demonstrated. The ability of immune lymphocytes to produce migration inhibition factor (MIF) was revealed by their incubation with mouse cells of congeneic and recombinant strains containing the separate components of the immunizing complex – both partial and total H-2 specificities. Removal of the cytotoxic lymphocytes by adsorption on the corresponding target cells did not reduce the ability of the remaining cell population to produce MIF. Some of the lymphocytes producing MIF were attached to target cells and could be eluted simultaneously with the cytotoxic lymphocytes. It is postulated that the populations of T cells which synthesize MIF and which destroy target cells differ in the structure of their receptors.

KEY WORDS: cytotoxic effect; inhibition of macrophage migration; H-2 system; partial and total specificities.

Humoral mediators of cellular immunity synthesized by immune lymphocytes during interaction with the corresponding antigens have been closely studied in recent years [8]. The regulatory effect of these mediators on the proliferation, differentiation, migration, and viability of the cells suggests the existence of cell subpopulations, cooperation between which through factors synthesized by them is responsible for the great variety of reactions of cellular immunity. The T cells responsible for these reactions are heterogeneous and they can be divided into at least two subpopulations: $T_1$ and $T_2$, which interact in the course of recognition of antigens in vivo [10] and in vitro [19]. It has also been shown that the subpopulation synthesizing migration inhibition factor (MIF) is not identical with cells that proliferate on contact with antigen [9, 17], and that normal lymphocytes which react in mixed cultures are not identical with cytotoxic immune lymphocytes [18]. Dissociation of "helper" and cytotoxic activities of the T cells has been established [16]. It remains to be discovered whether there are any differences between the immunological specificity of the T cell subpopulations.

An attempt was made in the investigation described below to distinguish two cell subpopulations synthesizing MIF and destroying target cells in the H-2 system. For this purpose a comparative study was made of their immunologic specificity, and the ability of the effector lymphocytes to be adsorbed on the surface of target cells also was used [1].

## EXPERIMENTAL METHOD

The haplotype of mice of the inbred lines, including congeneic and recombinant for H-2, is given on the basis of existing data [6, 12]. Ascites forms of Sal and MKhII sarcomas, induced by carcinogens in mice of strains A ($H\text{-}2^a$) and C57BL/10 ($H\text{-}2^b$) (abbreviated to B10) respectively, were used for a single

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TABLE 1. Immunologic Specificity of MMI Test in H-2 System

| Immune lymphocytes | Expt. No. | Source of macrophages or spleen (antigen)* | MMI index (in %) | P† |
|---|---|---|---|---|
| B10 anti -A | 1 | A | 48 | <0,001 |
| | | B10 | 1 | >0,1 |
| | 2 | A | 30 | <0,001 |
| | | B10 | 14 | >0,05 |
| | 3 | A | 45 | <0,001 |
| | | B10 | —43 | <0,001 |
| B10. D2 anti -A | 4 | A | 34 | <0,001 |
| | | B10.D2 | 13 | >0,05 |
| | 5 | A | 30 | <0,01 |
| | | B10.D2 | —7 | >0,1 |
| B10. D2 anti-B10 | 6 | B10 | 50 | <0,001 |
| | | B10.D2 | 3 | >0,1 |
| | 7 | B10 | 64 | <0,001 |
| | | B10.D2 | —17 | <0,01 |
| | 8 | B10 | 55 | <0,001 |
| | | B10.D2 | 10 | >0,1 |
| | 9 | B10 | 32 | >0,02 |
| | | B10.D2 | —43 | <0,001 |
| Mean ±SE | | Donor | 42,0±4,1 | <0,001‡ |
| | | Recipient | —7,7±7,4 | |

* Direct MMI test (mixture of lymphocytes with macrophages 1 : 3) in experiments Nos. 1 and 4; indirect MMI test (incubation of lymphocytes with spleen cells 4 : 1) in remaining experiments.
† Significance of difference between areas of migration in experiments and control.
‡ Significance of difference between mean MMI indices with antigen of donor and recipient.

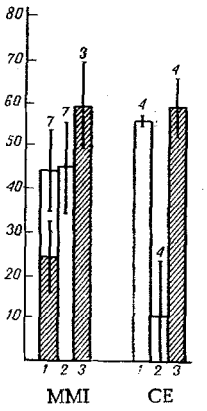


Fig. 1. MIF production and CE of B10 anti-A lymphocytes after adsorption on monolayer of A macrophages and subsequent elution. Abscissa: 1) intact, 2) unattached, 3) eluted immune lymphocytes; ordinate, MMI index or CE (in %). Ratio of lymphocytes to A cells in indirect MMI test 4:1 or 1:4 (unshaded columns); 1:9 (shaded columns). Dose of lymphocytes in cytotoxic test $4 \cdot 10^6$ (unshaded columns) and $1 \cdot 10^6$ (shaded columns). Vertical lines show confidence limits with 95% level of significance. Number of experiments shown above columns.

immunization of allogeneic mice by subcutaneous injection at 5 points and intraperitoneally ($5 \cdot 10^7$ cells per mouse).

The methods of experiments to study the cytotoxic action of immune lymphocytes on allogeneic target cells (peritoneal macrophages), grown in Leighton's tubes, and of quantitatve determination of the cytotoxic effect (CE) were described previously [1]. Adsorption of immune lymphocytes on a monolayer of macrophages grown in plastic flasks (No. 3024, Falcon Plastics, USA) was carried out for 3 h at 30°C, once or twice [1, 2]. After incubation, the flasks were agitated on a shaker (New Brunswick Scientific Co., USA) for 5 min at 60 rpm, the unattached lymphocytes were collected, and the attached lymphocytes were eluted with pronase (25-100 μg/ml) [5]. After repeated washing and counting of the living cells, the ability of 3 types of lymphocytes (intact, unattached, and eluted) to exert a cytotoxic action on target cells and to form MIF was determined.

The mouse macrophage migration micromethod [15] was used with several modifications. Washed peritoneal macrophages were suspended in 30% (by volume) concentration in medium No. 199 or MEM (BDH, Bethesda, USA), containing 2 mM glutamine, 10% calf embryonic serum, and antibiotics, and drawn by a syringe into polyethylene tubes measuring 300 × 0.6 mm. The tubes were cut into 2-cm lengths, sealed at one end, and centrifuged for 5 min at 1000 rpm. Capillary tubes 2.5 mm long containing the cell residue were fixed with petrolatum to the floor of plastic chambers (Lexy Culture Chamber, Mini-Lab. Co., Canada), which were then filled with medium (see above) and incubated for 18 h at 37°C in an atmosphere of 5% $CO_2$. The migration zone was projected on paper by means of a photographic enlarger, cut out, and weighed.

Macrophage Migration Inhibition (MMI) Test. For the writers' modification of the direct MMI test [7] cells of immune lymph glands (in the experimental samples) and of normal lymph glands (control) were mixed with peritoneal macrophages of mice of the test strain in the ratio of 1 : 3, introduced into capillary tubes, and incubated, after which the size of the migration zone was determined as stated above. For the indirect MMI test [13], mixtures of immune or normal lymph gland cells with spleen cells from mice of

TABLE 2. MIF Production on Incubation of Immune Lymphocytes with Spleen Cells of Mice Containing Separate Partial or General Specificities of the H-2 Immunizing Complex*

| Source of spleen cells | Immune lymphocytes: B10.D2 anti-B10 | | | | | | Immune lymphocytes: B10 anti-A | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B10 | B10.D2 | B10.A | B10.M | R107 | R101 | A | B10 | BALB/c | CBA | DBA/1 | CBA+ BALB/c |
| H-2 alleles taking part in the reaction | $K^bD^b$ P(2, 33) + G | Absent | $K^\kappa$ G (5) | $K^f$ G (39,53) | $K^b$ P (33) + G | $D^b$ P (2) | $K^dD^b$ P(23, 4) + G | Absent | $D^d$ P(4) + G | $K^\kappa$ P(23) + G | $K^dD^d$ G (1,3, 11, 13, 49, 52) | $K^\kappa+D^d$ P (23+ +4) +G |
| H-2 specificities† | | Absent | | | | | | Absent | | | | |
| MMI index (in %) | 50‡ | 17‡ | 39‡ | 32‡ | 49‡ | 45‡ | 56 | 1†† | 51 | 41** | 31 | 49,5 ** |

* Indirect MMI test.
† H-2 specificities potentially capable of reacting with immune lymphocytes (P – partial, G – general).
‡ Mean of 3 experiments.
** Mean of 2 experiments.
†† $P > 0.1$, in other cases $P < 0.05$.

the test strain, in different ratios, were incubated for 36 h at 37°C in an atmosphere of 5% $CO_2$ in a concentration of $14 \cdot 10^6$ living cells/ml medium (see above), enriched with unessential amino acids. After incubation the cell suspensions were centrifuged for 30 min at 1000 g and the supernatant was used as culture medium for migration of B10 or CC57BR(H-$2^b$) macrophages. The MMI index was determined by the formula $(1 - a/b) \times 100$, where $a$ and b represent the migration zones (mean of 6 capillary tubes) in the experimental and control series respectively. Statistical significance was determined by Student's t test.

## EXPERIMENTAL RESULTS

Investigation of the immunologic specificity of MMI in the H-2 system (Table 1) showed that in all combinations of strains used incubation of immune lymphocytes with cells of the corresponding donor induced highly significant MMI in the direct and indirect tests compared with incubation of normal lymphocytes with cells of the same donor. It follows from Table 1 that this MMI was highly specific, for it was not observed in any of 8 experiments in which the same immune lymphocytes were incubated with syngeneic cells. On the contrary, in the last case migration of macrophages was stimulated in three experiments.

To study the immunologic specificity of cells synthesizing MIF more exactly, B10.D2 anti-B10 (d anti-b) lymphocytes were incubated with spleen cells of the donor (positive control), recipient (negative control), and third strains containing the separate components of the immunizing complex: total specificities H-2.5 (strain B10.A), H-2.39,53 (strain B10.M), or partial specificities H-2.2 (strain R101) and H-2.33 (strain R107).

It follows from Table 2 that the total and partial H-2 specificities determined by either locus (K or D) induce MIF formation almost equally. Similar results were obtained with B10 anti-A lymphocytes: MIF formation was induced not only by A cells, but also by strains BALB/c, CBA, DBA/1; DBA/1, moreover, gave a cross reaction with A only on account of general H-2 specificities (Table 2). In the last case, however, the possibility of participation of antigens of other loci (not H-2) in the reaction could not be ruled out, because the strains were not congeneic for H-2.

To separate lymphocytes killing target cells and producing MIF, they were adsorbed on target cells and then eluted. As Fig. 1 shows, after removal of the cytotoxic B10 anti-A lymphocytes by adsorption on A macrophages, the ability of the unattached lymphocytes to form MIF remained completely untouched. In two experiments the CE of the unattached lymphocytes was 3 and 9% respectively of the CE of the originals, whereas their ability to produce MIF was only slightly reduced. At the same time, the cytotoxic activity of the attached and subsequently eluted immune lymphocytes was more than 4 times higher than the initial activity (based on calculation of the dose of lymphocytes giving a 50% CE). Meanwhile the ability to produce MIF also increased: With a ratio of lymphocytes to spleen cells of 1 : 9 the MMI index for the eluted lymphocytes was 55-65% but only 16-33% for the intact (Fig. 1).

Unlike cytotoxic lymphocytes, which did not react with the general specificities H-2 (3, 4), MIF was produced during incubation with both partial and general H-2 specificities, and removal of the cytotoxic lymphocytes by adsorption on target cells did not reduce the ability of the remaining population to produce MIF. Nevertheless, some cells forming MIF were attached to target cells and could have been eluted along with the cytotoxic lymphocytes.

The results can be explained by the greater sensitivity of the MMI reaction than of the cytotoxic test, by the participation of B cells in MIF production, and also by the fact that MIF synthesis and CE against target cells are produced by two corresponding populations of effector T lymphocytes which differ in the structure of their receptors. The first two hypotheses seem unlikely because of qualitative differences in the specificity of the two tests and synthesis of MIF by T cells in other systems [11, 14]. The third hypothesis assumes that the heterogeneity of the effector lymphocyte population is connected with differences in affinity of the receptors for H-2 antigens: Whereas a stable and prolonged contact between lymphocytes and target cells is essential for CE and is secured by receptors for the "configuration" partial specificity or the determinant carrier linked with it [4], for the induction of MIF formation all that is required is contact between a low-affinity receptor with "single" general H-2 specificity, i.e., stong adsorption on target cells is not essential in this case. This means that cytotoxic lymphocytes are a component part of the cell population synthesizing MIF on contact with antigen.

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