USE OF ^{51}Cr-LABELED MACROPHAGES FOR THE OBJECTIVE EVALUATION OF MACROPHAGE MIGRATION INHIBITION

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It was shown by correlation analysis of the experimental data that ^{51}Cr-labeled macrophages migrating from capillary tubes and lysed with sodium dodecyl sulfate can be used for the objective quantitative evaluation of the results of the macrophage migration inhibition microtest (MMI) microtest. The degree of migration and sensitivity of the ^{51}Cr-labeled macrophages to the action of migration inhibition factor (MIF) were shown not to differ from those of unlabeled macrophages. Determination of the absolute migration of macrophages and its reduction by the action of MIF formed in the H-2 system, and also of the specificity of this decrease, revealed a high level of correlation between the area of the migration zone and the labeling of the migrant cells.

KEY WORDS: macrophage migration inhibition; H-2 system; ^{51}Cr-labeled macrophages.

The macrophage migration inhibition (MMI) test is widely used as a test of hypersensitivity of delayed type (HDT) in vitro in animals and man and enables interaction between lymphocytes and macrophages to be studied in the course of the immune response. One of the principal disadvantages of the method is the subjectivity of evaluation of the results. To overcome this disadvantage, photometric [13] and telemetric [10] methods of determining the area of macrophage migration and counting the number of cells migrating from the capillary tube by means of a celloscope [2] have been suggested. Recently the method of labeling the cells with radioactive isotopes has been used for the quantitative and objective assessment of some cell immunity reactions.

In the investigation described below it is shown that ^{51}Cr-labeled macrophages can be used to assess the results of MMI as a test for T cells – the effectors of HDT in the H-2 system in mice.

EXPERIMENTAL METHOD

Mice of inbred lines were immunized subcutaneously at five points and intraperitoneally in one session with cells of allogeneic ascites sarcomas S1 of line A or MCh11 of line C57BL ($5 \cdot 10^7$ cells per mouse). From 6 to 10 days later suspensions of regional lymph node cells were used in the direct or indirect MMI test. For the direct MMI test [4], as modified by the writers [3], cells of immune (experimental samples) and normal lymph nodes (control samples) were mixed in the ratio of 1:3 with ^{51}Cr-labeled peritoneal macrophages of the donor's (allogeneic) or recipient's (syngeneic) strain. Glass capillary tubes (from Behringwerke, West Germany) were filled with the mixed cells, centrifuged, cut below the upper boundary of the cell residue, and placed on the base of wells in Microplate II No. 3040 plates (from Falcon Plastics, USA). RPMI-1640 medium or Eagle's medium with L-glutamine (2 mM), HEPES (5 mM), 10% embryonic calf serum (ECS), and antibiotics were used as the incubation medium. After incubation for 18 h at 37°C the capillary tubes were removed from the wells and the migration zones were traced on paper by means of a projector, cut out, and weighed. The culture fluid containing ^{51}Cr spontaneously liberated from the cells during culture was then removed from the wells. Cells migrating from the capillary tube were destroyed with 0.2 ml of 3% sodium dodecyl sulfate (SDS) solution for 20 min at 37°C and the SDS was transferred to counting tubes. The radioactivity of the samples was determined with a β-spectrometer (from Nuclear, Chicago, USA). Indices of MMI for evaluation based on area (MMI_S) and by release of ^{51}Cr (MMI_M) were calculated by the equation:

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TABLE 1. Correlation Analysis of Levels of Migration Determined from Area of Migration and Lysis of ^{51}Cr-Labeled Macrophages

Medium	Level of migration															n	r	r_S
RPMI-1640	M	592	528	526	525	445		437		437	427 ...					30	0,9289 $P<0,05$	0,8818 $P<0,01$
	S	14	12	13	13	8		11		8	10 ...							
Eagle's	M	376	239	238	234	226		221		196	178 ...					39	0,4069 $P<0,01$	0,4274 $P<0,01$
	S	7	12	15	11	9		8		13	7 ...							
RPMI-1640	$\bar{S}$	29	13	11	10	10	9	8	8	8	7	6	6	4		14	0,9919 $P<0,001$	0,9962 $P<0,001$
	$\bar{S}_{ul}$	32	12	11	10	8	9	10	8	8	7	8	6	3				

Legend. M, S) values of migration estimated from liberation of ^{51}Cr and area of migration zone respectively; n) number of pairs compared; r) linear coefficient of correlation; r_S) rank correlation coefficient; $\bar{S}$ and $\bar{S}_{ul}$) mean areas of migration zones for five measurements for ^{51}Cr-labeled and unlabeled macrophages respectively, tested under identical conditions.

$$MMI = \left(1 - \frac{a}{b}\right) \cdot 100,$$

where a and b are the mean areas of the migration zone (in mg; for MMI_S) or mean radioactivity (in cpm; for MMI_M) respectively in the experimental and control series (five parallel tests for each cell sample). For the indirect test [7] the mixed cells from immune and normal lymph nodes with mouse spleen cells of the test line, taken in the ratio of 5:1, were incubated for 40 h at 37°C in an atmosphere of 5% CO_2 in a concentration of $20 \cdot 10^6$ living cells in 1 ml medium. After incubation the suspensions were centrifuged for 30 min at 1000g and the supernatant was used as the culture medium for migration of ^{51}Cr-labeled peritoneal macrophages. The cells were labeled as follows. Cells obtained from peritoneal exudate 1-3 days after intravenous injection of 1.5 ml mineral oil were washed twice and incubated in the proportion of $20 \cdot 10^6$-$30 \cdot 10^6$ cells to 1 ml medium 199 containing 10% ECS, 10% lactalbumin hydrolysate, 5 mM HEPES, 20 units/ml heparin, and 20 μCi/ml ^{51}Cr (specific activity of ^{51}Cr 50 mCi/mg). The cells were washed three times 30 min after the beginning of incubation at 37°C and used for the MMI test. The experimental results were subjected to statistical correlation analysis [12]. The distribution of the migration zones in the control and experimental samples was normal [5, 11].

EXPERIMENTAL RESULTS

Peritoneal exudate macrophages labeled with ^{51}Cr had exactly the same ability to migrate as unlabeled cells (r and r_S for $\bar{S}$ and $\bar{S}_{ul}$ are close to 1; see Table 1) and their sensitivity to the action of MIF was not less than that of the unlabeled macrophages (r and r_S for MMI_S and MMI_{Sul} are close to 1; Table 2). The scatter in the parallel assessments of MMI based on the quantity of label (M) liberated during lysis of cells migrating from the capillary tube during incubation for 18 h did not exceed that for evaluation based on area of the migration zone (S): the mean standard error for 52 sets of five parallel determinations was 9.2 ± 0.5% for M and 8.7 ± 0.6% for S of the absolute values of the mean levels of migration. The level of migration based on determination of M correlated with that based on determination of S (Table 1).

The action of MIF, as revealed in both the direct and the indirect MMI tests, led as a rule to a decrease in M in the experimental samples compared with the control. This decrease, just as the increase or absence of change which were found in some cases, correlated with the corresponding inhibition or stimulation of migration of the macrophages or absence of effect calculated on the basis of the change in S (Table 2). As this table shows, MIF formation, as demonstrated by M, was immunologically specific. The degree of correlation was higher in cases when MIF activity was discovered. A high degree of correlation both for M and S and for MMI_M and MMI_S was established only when RPMI-1640 medium was used, and not with Eagle's medium. The difference between the results obtained by the use of the different media can be explained by the much less effective synthesis of MIF in Eagle's medium than in RPMI medium, and by the substantial difference in the

TABLE 2. Correlation Analysis of Indices of Inhibition of Migration Based on Area of Migration and Lysis of ^{51}Cr-Labeled Macrophages

Medium		Level of migration														n	r	r_S
RPMI=1640	MMI_M	96	47	46	41*	41*	38	36	28	26	24	14	9*	—7*	—18*	—33		0,8647†	0,8546†
	MMI_S‡	94	52	53	54	43	21	28	35	20	28	54	25	—1	—29	—12	23	$P<0,01$	$P\leqslant 0,01$
	MMI_M‡	—	—10	—	—	6	1	—4	—	3	—	—	—	10	—	—13		0,8304	0,8187
	MMI_S	—	—1	—	—	—6	11	—45	—	0	—	—	—	—14	—	—29		$P<0,01$	$P<0,01$
Eagle's	MMI_M	20	25*	18	15	8	8	2	—5								8	—0,2620	—0,4702
	MMI_S	39	20	29	—18	53	33	52	37									$P>0,05$	$P>0,05$
RPMI=1640	MMI_S		63	54	21	11	—29	53									6	0,9116	0,9428
	MMI_{Sul}		55	48	—23	—29	—35	50										$P<0,05$	$P<0,01$

Legend. MMI_{Sul} denotes MMI_S for unlabeled macrophages.
*Direct variant of MMI test.
†r and r_S calculated for values of $MMI_M > 20$ (n = 10).
‡Specificity control (mixtures of immune lymphocytes with recipient's spleen cells and mixtures of normal lymphocytes with the same cells).

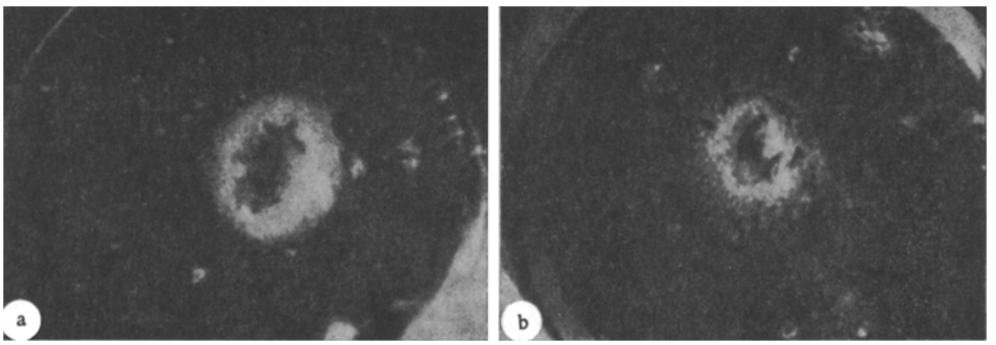

Fig. 1. Difference in character of migration of macrophages in RPMI-1640 medium (a) and in Eagle's medium (b).

character of cell migration from the capillary tubes in these media: cells migrate in several layers in RPMI, but in Eagle's medium the area of migration is divided into two zones: the zone proximal relative to the capillary tube consists of several layers, whereas the distal zone consists of a single layer (Fig. 1). These differences are evidently due to the greater viability of the cells in RPMI medium than in Eagle's medium. MIF in Eagle's medium inhibits migration only in the monolayer without affecting the number of cells in the zone with several layers, whereas MIF formed in RPMI inhibits migration of a large majority of cells even when migrating in several layers. The following ways of increasing sensitivity of the isotope test in Eagle's medium are possible: enrichment of the medium; subtracting the value of M obtained during the first hours of migration, as the background, and by preincubating the macrophages with the test MIF.

The experimental results confirm that ^{51}Cr-labeled macrophages can be used for the objective and quantitative evaluation of the results of MMI test. Correlation between MMI_S and MMI_M shows that evaluation of the inhibition of migration by means of these parameters characterizes the same phenomenon. The apparatus required for the method suggested in this paper is widely used in other cell reactions, for example, during testing for the cytotoxic effect [1] and chemotaxis [8], unlike the apparatus used in variants suggested previously [10, 13], which is used for that purpose alone. The modification described above, unlike that proposed by Novikov et al. [2], does not differ in principle as regards the procedure of the test from the classical variant [9]. Evaluation of the results of the MMI test not only makes this test objective, but also provides the opportunity for quantitative assessment of macrophage migration and its immunological inhibition among cells of different cultures, for it distinguishes between cells migrating from a capillary tube and cells of the culture

itself. Spontaneous liberation of ^{51}Cr into the culture fluid can also be used to judge the toxicity of the medium and so to distinguish between the action of MIF and of nonspecific toxic agents on macrophages.

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