[15]. Removal of both these factors, just as culturing PMN in medium with a reduced IgM concentration, revealed the above-mentioned receptors, and this is confirmed by controls with 2-mercaptoethanol and myeloma IgM. It may be that the combined action of hypotonic shock and acetate buffer solution was suitable to detect T_{μ} immediately after isolation of the pure population of T cells.

The results are evidence that early antibodies can play an opsonizing role. This is essential for our understanding of the mechanism of defence against ubiquitous microbes because of their ability to stimulate formations predominatly of IgM under ordinary conditions [2, 6, 10].

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NONIDENTITY OF Lyt PHENOTYPES AND RADIOSENSITIVITY OF "EARLY" AND "LATE" MIF PRODUCERS RESPONDING TO H-2 ANTIGENS

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Macrophage migration inhibition factor (MIF) is a lymphokine produced by T lymphocytes in the course of the delayed-type hyerpsensitivity reaction and responsible for concentration and activation of macrophages in the zone of the immune response, which has not been studied in detail. Nevertheless, T cells which produce MIF, unlike other T subpopulations, have not yet been adequately characterized. These cells, induced by microbial antigens or soluble proteins [10], and also by mitogens [8], carry the T lymphocyte phenotype Lyt-$1^{+}2^{-}$. However, MIF producers are in fact heterogeneous. Their different variants differ in size and affinity for lymphoid organs [9], immunologic specificity [1], and sensitivity to drugs [2] and irradiation [11]. In all cases MIF producers have been investigated 1 week or more later. However, as early as 16-18 h after intravenous immunization with allogeneic cells T lymphocytes producing MIF and γ-interferon on repeated contact *in vitro* with the same alloantigen, appear in the recipient's spleen [3]. Activity of "early" MIF producers has been

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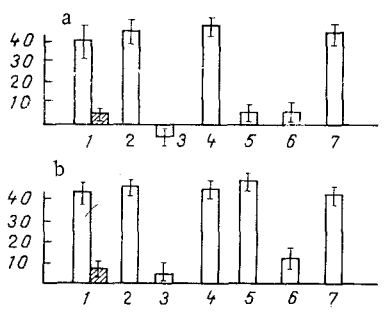

Fig. 1

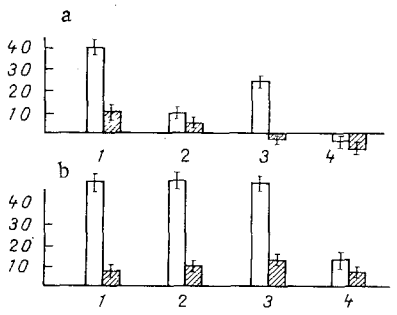

Fig. 2

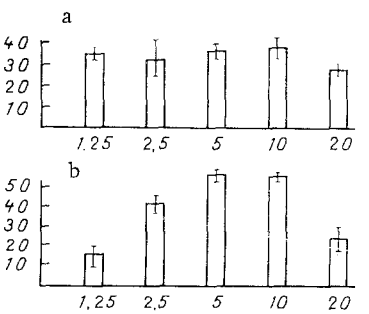

Fig. 3

Fig. 1. Sensitivity of "early" (a) and "late" (b) MIF producers to treatment with monoclonal anti-Lyt-antibodies and anti-Thy-serum with complement. Abscissa: 1) initial activity of MIF producers; 2) activity of MIF producers after treatment with anti-Lyt-1.1; 3) the same after treatment with anti-Lyt-1.2; 4) anti-Lyt-2.1, 5) anti-Lyt-2.2; 6) anti-Thy-serum; 7) medium with complement. Ordinate, MIF (in %). Unshaded column — allogeneic, shaded — syngeneic stimulation.

Fig. 2. Sensitivity of "early" (a) and "late" (b) MIF producers to irradiation. Abscissa: 1) initial activity of MIF producers; 2-4) activity of MIF producers irradiated before secondary mixed culture in doses of 500, 1500, and 3000 rads respectively; ordinate, MIF (in %). Remainder of legend the same as to Fig. 1.

Fig. 3. Dependence of MIF production on concentration of "early" (a) and "late" (b) MIF producers. Abscissa, concentration of responding cells in culture ($\times 10^6$) with constant ratio to stimulator cells (10:1); ordinate, MIF (in %).

found to be much higher than that of "late," if they were induced by minor or mutant antigens of the mouse H-2 complex.

The aim of this investigation was to compare Lyt markers and some properties of "early" and "late" MIF producers specific for H-2 antigens.

EXPERIMENTAL METHOD

BALB/c mice were immunized by injection of spleen cells of C57BL/6 mice, irradiated in a dose of 20 Gy (^{137}Cs, 7.4 Gy/min), into the caudal vein or retro-orbitally [4]. Spleen cells of immune (in the control — normal) mice in a concentration of $5 \cdot 10^6$ cells/ml were incubated 1 or 21 days after immunization *in vitro* with the donor's spleen cells (in the control — with syngeneic cells) in the ratio of 10:1 in a volume of 2 ml, in 24-well plastic plates (from "Linbro," England) in medium RPMI-1640 with the addition of 10% embryonic calf serum, 2 mM L-glutamine, HEPES buffer, and antibiotics. Incubation was carried out at 37°C in an atmosphere of 5% CO_2 for 16-18 h. The culture fluids were separated from the cells by centrifugation at 1000g and tested for their ability to inhibit migration of peritoneal macrophages obtained from intact mice 2-3 days after intraperitoneal injection of sterile mineral oil, using a microtest developed by the writers [5]. The migration inhibition index (MII) was calculated by the equation:

$$\text{MII} = \left(1 - \frac{\text{average zone of migration in experiment}}{\text{average zone of migration in control}}\right) \times 100\%.$$

Treatment of immune lymphocytes with anti-Thy-1,2-serum and also with monoclonal anti-Lyt-1 and anti-Lyt-2 antibodies with complement (from "Cedarline," Canada) was carried out by the method described previously [4]. Day-old lymphocytes were treated with anti-Lyt-antibodies in a dilution of 1:500, 21 day-old lymphocytes — in a dilution of 1:100. Immune lymphocytes were irradiated on a ^{137}Cs apparatus (7.4 Gy/min), in doses of 500, 1500, and 3000 rads.

EXPERIMENTAL RESULTS

To study subpopulations of MIF producers cells formed in the course of secondary allogeneic stimulation were investigated. Immune splenocytes obtained from animals 1 and 21 days after intravenous immunization with allogeneic spleen cells were incubated in mixed

lymphocyte culture for 16 h, and MIF activity was measured in the culture medium (see "Experimental Method").

Phenotypic markers of MIF producers (Lyt-antigens), sensitivity to irradiation, and dependence of lymphokine production on the density of MIF producers were studied.

MIF production by 1- and 21-day-old alloimmune lymphocytes was inhibited by treatment of the cells with anti-Thy-serum with complement. This is evidence that the MIF producers were T lymphocytes. "Early" producers were more sensitive to treatment with anti-Thy-serum than "late" producers, evidence of a higher density of θ-antigens on these cells.

Treatment of 1- and 21-day-old immune lymphocytes with anti-Lyt-antibodies and complement revealed differences between the "early" and "late" MIF producers as regards their Lyt phenotype (Fig. 1): MIF production by 1-day alloimmune lymphocytes was highly sensitive to the action of anti-Lyt-1.2 and anti-Lyt-2.2 monoclonal antibodies with complement (Fig. 1a), whereas MIF production by 21-day lymphocytes was inhibited only by treatment with anti-Lyt-1.2, and not with anti-Lyt-2.2 monoclonal antibodies plus complement (Fig. 1b). The distinction between "early" and "late" MIF producers with respect to their Lyt phenotype also was expressed in the fact that anti-Lyt-antibodies acted on these cells in different dilutions: "late" MIF producers were sensitive to treatment with anti-Lyt-1.2 antibodies only in a dilution of 1:100, but not of 1:500, whereas the "early" producers were sensitive to the action of anti-Lyt-1.2 (and also of anti-Lyt-2.2) in a dilution of 1:500 (results not shown). This means that Lyt-1.2-antigens are expressed on "early" MIF producers with a higher density than on "late." The results are thus evidence that "early" and "late" MIF producers have different phenotypes for cell surface antigens: Thy-1^+, Lyt-1^+2^+ for "early" and Thy-1^+, Lyt-1^+2^- for "late" MIF producers.

The study of radioresistance of the MIF producers showed that "early" and "late" MIF producers differ in their sensitivity to γ-irradiation (Fig. 2). MIF production by "early" producers was inihibited by irradiation in a dose of 500 rads, and partially by a dose of 1500 rads (Fig. 2a), which can evidently be explained by the inactivation of T-suppressors acting on other subpopulations of MIF producers. Meanwhile "late" producers were insensitive to this dose and were inhibited only by a dose of 3000 rads (Fig. 2b). Irradiation did not induce spontaneous MIF production by immune lymphocytes in the presence of syngeneic stimulators (Fig. 2). "Early" MIF producers thus differ from "late" by being highly radiosensitive.

To study activity of "early" and "late" MIF producers, dependence of MIF production on the concentration of responding cells in a secondary mixed lymphocyte culture was investigated, with a stable ratio to stimulator cells of 10:1 (Fig. 3). MIF formation by "early" producers was found to change only negligibly if the cell concentration exceeded $1.75 \cdot 10^6$ (Fig. 3a), whereas in the case of "late" producers, with a cell concentration of $1.75 \cdot 10^6$ no MIF activity was observed, and with an increase in the cell concentration, to $10 \cdot 10^6$, MIF rose to 54 ± 3% (Fig. 3b). With a cell concentration of $20 \cdot 10^6$, MIF production by both "early" and "late" producers was a little lower than with lower concentrations, possibly because of a change in pH of the medium (Fig. 2). These data may be evidence that "early" and "late" MIF producers differ in activity and frequency of occurrence in a secondary mixed lymphocyte culture. Further investigations are needed to shed light on this problem.

The results confirm the previous hypothesis of the existence of two nonidentical cell types — "early" and "late" producers of lymphokines, which succeed one another in the course of the immune response in the H-2 system.

Since "early" MIF producers with the Lyt-1^+2^+ phenotype appear very quickly after immunization with minor and mutant transplantation antigens [6, 7], it can be postulated that their precursors are activated on contact with antigen without proliferative expansion, and on repeated contact with the same antigen they perform the highly specialized function of producers of lymphokines, activated macrophages, and MK cells.

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