To what extent do the antibodies and their Fab fragments preserve their activity as a result of modification by polymers, and does the presence of chelated metal affect this process? Typical curves for titration of modified antibodies in indirect solid-phase radioimmunoassay using myosin [5] are given in Fig. 3. They demonstrate that activity of the whole antibodies was virtually unchanged as the result of their one- or two-point modification by chelating polymers. The presence or absence of metal had no effect on preservation of activity (data for the remaining preparations are not given because they were exactly the same as those described above). In the case of Fab fragments (Fig. 3), their activity was reduced a little by modification, but as before, it still remained sufficiently high (about 60% of the original value).

It can thus be concluded from the results that, first, modification of antibodies or their fragments by chelating polymers enables as many as several tens of atoms of a heavy metal to be bound to one protein molecule, which is a much higher figure than has hitherto been obtained, and second, that modification has virtually no effect on the specific properties of the antibodies. The possibility of using these conjugates *in vivo* is currently being studied by the present writers.

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IN VIVO PRODUCTION OF MACROPHAGE MIGRATION INHIBITION AND STIMULATION FACTORS DURING THE INDUCTIVE PHASE OF THE ALLOIMMUNE RESPONSE

A. P. Suslov, A. K. Yazova, and N. P. Berkova

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The macrophage migration inhibition factor (MIF), one of the most thoroughly studied immunomediators, is usually obtained in culture *in vitro*, where it is produced by immune T lymphocytes, stimulated by a specific antigen, and also by normal lymphocytes, activated by an alloantigen or mitogen [7]. Investigation of the production of MIF and other lymphokines *in vivo* demonstrated activity of these factors in the serum of animals immunized with MCG 4 h after a second intravenous injection of the antigen [11]. The appearance of MIF in the serum or lymph leads to adhesion of the macrophages to the walls of vessels or serous cavities, and to accumulation of cells in regional lymph nodes [10, 12, 16], thereby evidently facilitating cellular cooperation in lymphoid organs.

N. F. Gamaleya Research Institute of Epidemiology and Microbiology, All-Union Oncologic Research Center, Academy of Medical Sciences of the USSR, Moscow. M. M. Shemyakin Institute of Biological Chemistry, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.)

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It was shown previously that radiosensitive Lyt 1^+2^+ T cells, producing MIF in culture *in vitro*, may appear after intravenous alloimmunization of mice in the inductive phase of the immune response, namely during the first few days after injection of the antigen [1, 6]. However, it remained unclear whether early production of the immunomediator is a phenomenon that exists *in vivo*, or whether it can be detected only *in vitro*.

The aim of this investigation was to study production of MIF, and also of the alternative macrophage migration stimulation factor (MSF), *in vivo*.

EXPERIMENTAL METHOD

CBA ($H\text{-}2^k$) mice were given an intravenous injection of $9 \cdot 10^6$ BALB/c ($H\text{-}2^d$) mouse spleen cells, irradiated with a dose of 1,500 rads [6]. The immunized animals were divided into three groups: 1) these animals were exsanguinated 1-2 days after immunization, and 4 or 16 h after a second injection of irradiated BALB/c spleen cells in a dose of $20 \cdot 10^6$ cells per mouse (groups 1.4 and 1.16, respectively); 2) these animals were exsanguinated 6-7 days after immunization, and 4 or 16 h after second injection of irradiated BALB/c spleen cells into them in a dose of $20 \cdot 10^6$ cells per mouse (groups 6.4 and 6.16, respectively); 3) control immunized animals were exsanguinated on the 1st or 6th days after immunization, without restimulation by the antigen (groups 1.0 and 6.0, respectively).

Experimental and control sera of individual mice were fractionated by electrophoresis in polyacrylamide gel in both preparative [2] and analytical [3] versions. The apparatus for preparative electrophoresis [2] was divided by means of rubber partitions into 5 compartments for parallel fractionation of 5 samples. The original volume of the sera for fractionation was 0.1-0.2 ml. Electrophoresis was carried out in an interrupted system [8] in Tris-glycine electrode buffer (pH 8.3) at a constant voltage of 160 V for 2.5-3 h. After electrophoresis and staining of the control sections with a 1% solution of Amido black the gels were divided into six fractions: 1) the cathode end of gel up to the reference substance; 2 and 3) two fractions in the prealbumin zone; 4) a zone including albumin and postalbumins as far as transferrin; 5) the zone from transferrin to immunoglobulin; 6) concentrating 4% gel. The fractionated preparations were eluted from the gels by buffered physiological saline (pH 7.2) for 24 h, then dialyzed successively against distilled water and medium 199. The final volume of fractionated preparations was 1.5-2 ml. The dialyzed samples were treated with 10% embryonic calf serum, 2 mM L-glutamine, HEPES, and antibodies and tested by the micro-version of the macrophage migration inhibition test [4]. The macrophage migration inhibition index (MMI) was calculated by the equation:

$$MMI = \left(1 - \frac{\text{mean migration in experiment}}{\text{mean migration in control}}\right) \times 100.$$

EXPERIMENTAL RESULTS

Samples of all fractions obtained by electrophoresis of sera of unimmunized mice had no significant effect of macrophage migration (Fig. 1). Unfractionated sera of immunized mice, obtained after a second injection of alloantigen and tested in a dilution of 1:5, as a rule stimulated macrophage migration (Figs. 2 and 3).

Fractionation of the sera of immunized CBA mice led to discovery of both MIF and MSF activity compared with migration of these same cells in the presence of the corresponding fractions of control normal CBA mouse sera (Figs. 2 and 3). For instance, fractionation of the sera of the mice in group 1.4 showed that during the first day of formation of the alloimmune response reinjection of the alloangiten stimulated MIF production considerably (Fig. 2a). In this case, as was shown previously for this factor in mice [13], MIF was discovered in the prealbumin zone. MSF activity was found in the transferrin–immunoglobulins zone. MIF was found in mouse sera of the 1.16 group in the prealbumin zone, immediately next to albumin, whereas MSF was found in the zone of albumin and postalbumins (Fig. 2b). Thus mouse MSF, not previously studied biochemically, was found in the same fraction as human MSF [15]. Fractionation of control group 1.0 sera, obtained 22 h after a single injection of alloantigen, revealed no MIF activity, but MSF activity was found in the transferrin–immunoglobulins zone (Fig. 2c).

Unfractionated sera of the group 6.4 mice stimulated macrophage migration considerably. After their fractionation, high MIF activity was found in the prealbumin zone, immediately next to the albumin zone (Fig. 3a). MSF activity was found in the zone of albumin and postalbumins. These results are similar to those obtained by electrophoresis of the group 1:16

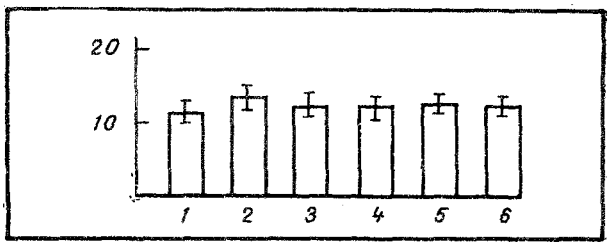

Fig. 1

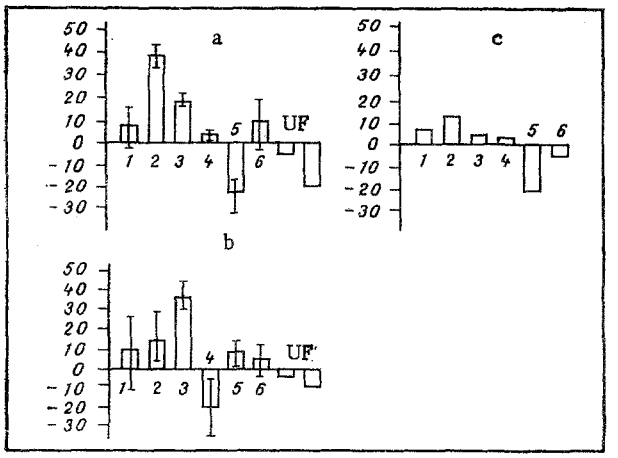

Fig. 2

Fig. 1. Migration of peritoneal macrophages in presence of fractions of normal mouse serum (mean of 6 experiments). Abscissa, nos. of fractions; ordinate, extent of projection of migration zone (in mg, M ± m).

Fig. 2. Effect of fractionated sera obtained on 1st-2nd day after alloimmunization of mice on migration of peritoneal macrophages. Abscissa, nos of fractions; ordinate, macrophage migration inhibition index (in percent). UF) unfractionated sera in dilution of 1:5. *a*) 18 h after first and 4 h after second injection of alloantigen; b) 18 h after first and 16 h after second injection of alloantigen; c) 22 h after single injection of alloantigen (M ± m for 5 individually tested mice).

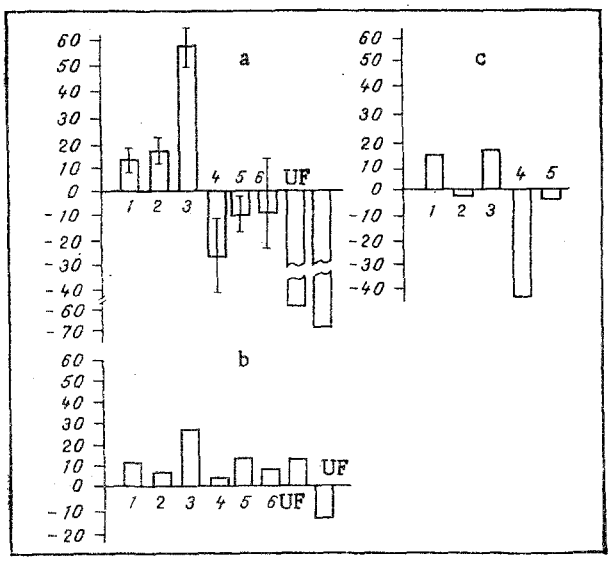

Fig. 3. Effect of fractionated sera obtained on 6th day after alloimmunization of the mice on migration of peritoneal macrophages. *a*, b, c) the same as Fig. 2, but 6 days after primary injection of alloantigen. Remainder of legend as to Fig. 2.

sera (Fig. 2b). Unlike group 1.16, electrophoresis of the group 6.16 sera revealed weak MIF activity, and MSF activity was absent (Fig. 3b). Consequently, the kinetics of MIF and MSF secretion into serum in the inductive phase differs from the kinetics of secretion of these factors in later stages of the alloimmune response. The latter more closely resembles the kinetics of exhibition of lymphokine activity in the serum, discovered previously [11]. In control sera of group 6.0, just as in group 1.0, considerable MSF activity was revealed after fractionation, whereas MIF activity was absent (Fig. 3c). This means that single alloimmunization without reinjection of the alloantigen does not induce MIF production in the serum, although it stimulates MSF secretion.

As a result of intravenous immunization followed by reinjection of the alloantigen, MIF thus appears in the animals' serum. Just as in investigations by other workers, who used bacterial antigen [11], MIF activity was found in the serum 4 h after the second injection of the alloantigen. Meanwhile, in those investigations the antigen was reinjected 3 weeks after primary immunization only. In our experiments activity of MIF-producing cells could be detected both in the induction phase (1st day) and in the early phase of the alloimmune response (6th day). However, Neta et al. [11] found MIF in immune serum without fractionation in dilutions up to 1:256. In our experiments MIF activity could not be found in unfractionated sera, and it was evidently masked by the action of a factor with MSF activity alternative to it, and which was differentiated from MIF by electrophoresis. Since only MSF activity was found in the sera of animals not receiving a second injection of alloantigen, it can be tentatively suggested that secretion of MIF into the circulation preventing the outflow of macrophages to the periphery and inhibiting the development of a local delayed-type hypersensitivity reaction [16], is under strict control in the body, to ensure that MSF activity predominates over MIF activity in the serum. This can also explain the nonlinearity of the dilution curves of MIF-containing supernatants [5, 14]. As a result of this predominance local gradients of high MIF activity, connected with the production of this factor by antigen-stimulated "early" Lyt 1^+2^+ T lymphocytes, which are MIF producers [1] in lymphoid organs, could bring about a local increase in the number of macrophages per unit volume of the organ, essential for cellular cooperation and immunoregulation.

The results as a whole are evidence that T cells, which are early producers of lymphokines, may function *in vivo* during the period before development of the antigen-specific proliferative response of the T cells. The technique used to approach the problem, described above, can be used for preparative isolation of purified MIF and MSF without contamination by embryonic calf serum proteins, which are usually present in culture *in vitro*.

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