MECHANISM OF ACTION OF MIGRATION INHIBITORY FACTOR (MIF)

I. Evidence for a Receptor for MIF Present on the Peritoneal Macrophage but not on the Alveolar Macrophage*

By RICHARD W. LEU,‡ A. L. W. F. EDDLESTON,§ JOHN W. HADDEN,‖ and ROBERT A. GOOD¶

(From the Departments of Pediatrics and Pathology, University of Minnesota, Minneapolis, Minnesota 55455)

(Received for publication 4 May 1972)

The capillary tube technique for assaying macrophage migration (1) has been widely used in studies on cellular immunity and has allowed a better understanding of lymphocyte-macrophage interaction associated with the expression of delayed hypersensitivity. The sensitive lymphocyte on exposure to specific antigen (2, 3) has been shown to produce a soluble effector molecule called migration inhibitory factor (MIF),[1] which has been characterized as an acidic glycoprotein with a molecular weight of 35,000–55,000 (4, 5). MIF inhibits the migration of the peritoneal macrophage, and recent evidence suggests that its primary biological function may be to activate macrophages (6). The mechanism of these actions is not understood.

Understanding of the mode of action of a variety of effector molecules on their respective target cells has been advanced in recent years by recognition of the existence of specific receptors on cell surfaces. The application of basic receptor principles to the effector molecules involved in the expression of cellular immunity appeared to us appropriate. Experiments utilizing pulse

* This investigation and its investigators were supported by The National Foundation-March of Dimes, Minnesota Respiratory Health Association, The Wellcome Trust, the U.S. Public Health Service, National Cystic Fibrosis Research Foundation, and The British Medical Research Council.

‡ Reprint requests should be sent to: Dr. Richard W. Leu, Box 494 Mayo, University of Minnesota Hospitals.

§ Present address: Liver Unit, King's College Hospital, Denmark Hill, London, Southeast 5, England.

‖ Established Investigator, American Heart Association.

¶ Regents' Professor, University of Minnesota.

[1] *Abbreviations used in this paper:* A, active MIF-containing supernatant; A.M., alveolar macrophage; BGG, bovine gamma globulin, Cohn fraction II; C, control supernatant; HBSS, Hanks' balanced salt solution; MEM, Eagle's minimal essential medium; MEM-S, MEM containing 15% decomplemented normal guinea pig serum; MIF, migration inhibitory factor; P.M., oil-induced peritoneal exudate cells; SEM, standardized error of the mean.

exposure of guinea pig macrophages to MIF-containing supernatants provided evidence for a dose, time, and temperature-dependent adsorption of MIF to the peritoneal macrophage. By contrast, adsorption of MIF to the alveolar macrophage was not observed. The interaction of MIF with the peritoneal but not the alveolar macrophage argues for selectivity of lymphocyte modulation of macrophage populations mediated by specific receptors.

Materials and Methods

Sensitization.—Hartley strain guinea pigs weighing 300–400 g were used throughout this study. Each animal was sensitized by intradermal injection into the four footpads with a total of 0.2 ml of an emulsion containing 0.5 mg of bovine gamma globulin (BGG), crystalline, Cohn fraction II, (Armour Pharmaceutical Co., Kankakee, Ill.), and 0.5 mg of heat-killed tubercle bacilli (strain H37Rv) incorporated in Freund's complete adjuvant (Difco Laboratories, Inc., Detroit, Mich.).

Cell Collection.—Peritoneal exudate cells (P.M.) used for assay of MIF activity were collected from normal nonsensitized guinea pigs 72 hr after the intraperitoneal injection of 30 ml of sterile light paraffin oil (Fisher Scientific Company, Fairlawn, N. J.). Animals were sacrificed by cardiac bleeding and cervical dislocation. Exudate cells were collected in 120 ml of prechilled Hanks' balanced salt solution containing 5 units heparin/ml (HBSS) and were washed twice in HBSS by centrifugation at 220 *g* for 5 min at 4°C.

Normal alveolar macrophages (A.M.) were collected by modification of the method of Myrvik (7). To avoid bronchospasm, each donor was injected intraperitoneally with 20 mg of Benadryl (Parke, Davis and Company, Detroit, Mich.) 30 min before sacrifice. The lungs were lavaged with a total of 120 ml of HBSS, and the cells obtained were washed twice in HBSS by centrifugation at 220 *g* for 5 min at 4°C.

The total yields of cells from peritoneal exudate and lungs averaged 120×10^6 and 50×10^6, respectively. The macrophage content from exudates and lungs averaged 70 and 95%, respectively. Cell preparations contaminated with erythrocytes were discarded.

Production of MIF by Lymph Node Lymphocyte Culture.—MIF-containing supernatants were produced by modification of the method of Bloom and Bennett (8). The axillary, subclavian, femoral, and popliteal lymph nodes were removed from guinea pigs 14–16 days after BGG sensitization, and the nodes were teased apart in Eagle's minimal essential medium (Grand Island Biological Co., Grand Island, N.Y.) containing penicillin 100 units/ml and streptomycin 100 μg/ml (MEM). The cell suspension was filtered through a No. 100 stainless steel mesh screen, the remaining lymph node fragments were washed once with additional MEM, and the washing fluid was added to the initial cell suspension after filtration. The cells were washed twice in MEM and counted. Approximately 500×10^6 cells were obtained from each animal of which more than 85% appeared to be lymphocytes. The cells were suspended in serum-free MEM containing L-glutamine (2 mM/liter) and cultured at a concentration of 15×10^6/ml and density of $4\text{–}5 \times 10^6$ cm² in Falcon T flasks (Falcon Plastics, Los Angeles, Calif.). BGG was added to half the flasks at a final concentration of 1 mg/ml, and the flasks were incubated for 24 hr at 37°C in a CO_2 incubator. Control and active supernatants were then pooled separately and centrifuged first at 220 *g* for 10 min and then at 3000 *g* for 10 min at 4°C to remove cells and debris. The control supernatant was reconstituted with BGG at a final concentration of 1 mg/ml. Both the reconstituted control (C) and the preincubated active (A) supernatants were sterilized by Millipore filtration (0.45 mμ) (Millipore Corp., Bedford, Mass.), divided into 1.5-ml aliquots, and stored at −70°C. Before in vitro assay the supernatants were supplemented with 15% decomplemented normal guinea pig serum (Grand Island Biological Co.). Dilutions of supernatants were made with MEM containing 15% guinea pig serum (MEM-S).

Standard Assay for MIF Activity.—Macrophage suspensions (12×10^6/ml) were distributed

in 1-ml aliquots to 12 × 75 mm plastic disposable test tubes (Falcon Plastics). After centrifugation, the supernatants were discarded, and the cells resuspended in 0.2 ml of an appropriate dilution of control or active supernatant. Two capillary tubes (1.3–1.5 × 75 mm OD) were filled with the suspension from each tube and plugged with clay (Seal-ease, Clay-Adams Inc., New York). After centrifugation at 90 *g* for 5 min, each capillary tube was cut just below the cell fluid interface. Duplicate capillary tubes were mounted by means of silicone grease in a small tissue culture chamber which was filled with the corresponding dilution of control or active supernatant and sealed with a cover slip. The chambers were constructed by attaching small glass rings (18 × 5 mm) to a glass plate with epoxy glue. After incubation in moist chambers for 18 hr at 37°C, the areas of migration were magnified by a microprojector (Bausch and Lomb, Inc., Rochester, N.Y.), traced, and measured by planimetry. The results were expressed as

$$\text{Per cent inhibition} = 100 - \frac{\text{mean area of migration in A supernatant}}{\text{mean area of migration in C supernatant}} \times 100.$$

The results of each series of experiments were expressed as the mean with its standard error (SEM).

Pulse Exposure Technique.—Small plastic tubes, each containing 12 × 10^6 peritoneal cells, were prepared as described above except that the cells were suspended in 1.5 ml of appropriate dilutions of control or active supernatant containing 15% guinea pig serum. The tubes were rocked on a tilting mixer (Labindustries, Berkeley, Calif.) for 15 min at room temperature (25°C). After pulse exposure, the cells were sedimented by centrifugation, and the supernatants removed. In some experiments these were assayed for residual MIF activity by the standard migration technique. Pulsed cells were prepared for assay by suspension in 0.2 ml of MEM-S and transferred to duplicate capillary tubes. Each chamber was filled with MEM-S, and the assay carried out as previously described.

Macrophage Adsorption of MIF Activity.—Macrophages (15–60 × 10^6/tube) were suspended in 1.5 ml of a 1:4 dilution of control or active supernatant and rocked for 30 min at room temperature. Controls consisted of C and A supernatants incubated without macrophages. After centrifugation, the supernatants were removed and tested for residual MIF activity by the standard assay.

For adsorption experiments with nonviable macrophages both alveolar and peritoneal macrophages were frozen as a dry pellet at −70°C for 72 hr. Cell viability was confirmed to be <5% by trypan blue exclusion.

Enzymatic Treatment of Macrophages.—Peritoneal cells (12 × 10^6/tube) were suspended in 1 ml of a trypsin solution (Grand Island Biological Co.) at a final concentration of 1 or 5 mg trypsin/ml HBSS. Cells suspended in HBSS alone provided the incubation control. After incubation for 30 min in a 37°C water bath with constant shaking, the cells were sedimented by centrifugation, suspended in 1 ml of HBSS containing 2 mg of lima bean trypsin inhibitor (Nutritional Biochemicals Corp., Cleveland, Ohio), and washed three times in HBSS. Peritoneal cells were treated in a similar way with chymotrypsin, 1 mg/ml (Sigma Chemical Co., St. Louis, Mo.), or neuraminidase, 150 μg/ml (Sigma). Because the appropriate enzyme inhibitors were not available, the cells were washed three times in HBSS to remove residual enzyme.

The ability of enzymatically treated cells to respond to MIF was determined by the pulse exposure technique. Their ability to adsorb MIF was then tested by the macrophage adsorption method described above.

RESULTS

Comparison of Peritoneal and Alveolar Macrophage Sensitivity to MIF.—The sensitivity of normal peritoneal or alveolar macrophage populations to MIF generated from sensitive lymph node lymphocytes was determined by the

standard migration technique. The effect of twofold serial dilution of control and active supernatants on macrophage migration is shown in Fig. 1, which summarizes the results of eight experiments with peritoneal exudate cells and six experiments with alveolar macrophages. The migration of peritoneal macrophages was markedly inhibited in the presence of active supernatants; however, the migration of alveolar macrophages was not significantly inhibited at any of the dilutions of supernatants tested. The inhibition of peritoneal cell migration by MIF-containing supernatant exhibited a dose-response relationship within the range of dilutions tested. Each twofold dilution of supernatant resulted in a mean decrease of per cent inhibition of migration of approximately 10.4. A linear relationship is demonstrable between the dilution of supernatant

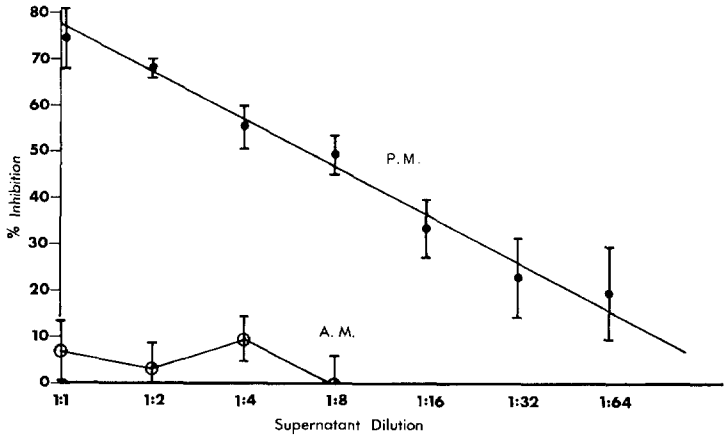

FIG. 1. Effect of dilution of MIF-containing supernatants generated from BGG-sensitive guinea pig lymph node lymphocytes on the migration of normal peritoneal (P.M., ●—●) and alveolar (A.M., ○—○) macrophages. The response was measured by the standard MIF assay with continuous exposure to MIF and is expressed as percentage inhibition of migration ± SEM.

and the inhibition of migration of these cells (Fig. 1). The data on the abscissa were replotted as MIF concentration in arbitrary units so that 1 unit of MIF equals the least amount of detectable inhibitory activity (i.e. that amount, present in 1.5 ml of a 1:64 dilution of supernatant). Fig. 2 shows the relationship of MIF in units to the per cent inhibition of migration. MIF, at low concentration, produced a progressive inhibition of migration; however, as inhibition approached 50%, further increases in MIF concentration produced little additive effect. This relationship suggests that a saturation process is involved. The formula which describes this rectangular hyperbola is $y = ax/b + x$ where y = per cent inhibition of migration, x = units of MIF, a = maximal percentage inhibition of migration, and b = number of units of MIF to give ½ maximal inhibition. From the formula, MIF in units = $5y/78 - y$,

we were able to estimate the quantity of MIF present in a given supernatant based on a measured inhibition of migration.

Pulse Exposure of Peritoneal Macrophages to MIF.—In order to investigate the early interaction between MIF and its target cells, normal peritoneal cells were exposed for 15 min at 25°C to active supernatants and their migration subsequently determined in medium containing no MIF. The results of eight pulse exposure experiments are shown in Fig. 3 and are compared with those obtained by the standard assay technique. The pulse exposure technique produced a linear dose-response relationship parallel to that obtained with the standard assay for MIF activity. These parallel results indicate that under the

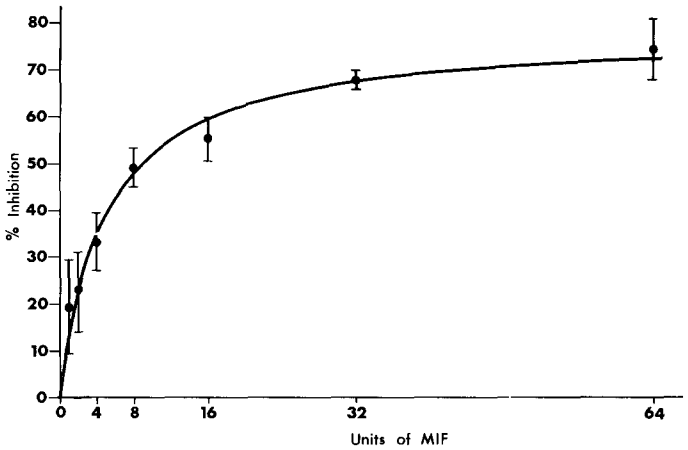

FIG. 2. Relationship of the migration inhibition response of normal peritoneal macrophages and the quantity of MIF expressed in arbitrary units (1 unit = amount of MIF in 1.5 ml of a 1:64 dilution of active supernatant derived from the effect of supernatant dilution on migration in the standard assay).

conditions used, pulse exposure results in an average reduction of the expression of MIF units of 50%, which is independent of MIF concentration.

Adsorption of MIF Activity by Peritoneal Macrophages.—The results of the previous experiments suggested that MIF is adsorbed by peritoneal macrophages during exposure to active supernatants. To explore this possibility, selected dilutions of control and active supernatants were incubated with 12×10^6 peritoneal macrophages for 15 min at 25°C and then tested for residual activity by the standard assay. The results of four experiments were analyzed to determine the amount of MIF adsorption in units and are shown in Fig. 4. MIF units removed by adsorption are plotted against the total number of units available in unadsorbed control supernatants. The results confirm that pulse exposure resulted in removal of MIF from active supernatants. This adsorption was dose dependent at low concentrations of MIF but showed saturation at

higher concentrations. These results correspond to those obtained for migration inhibition (Fig. 2) and indicate that the observed plateau in migration inhibition may result from saturation in binding of MIF.

Effect of Time, Temperature, Cell Number, and Cell Viability on Adsorption of MIF to Peritoneal Macrophage.—To determine the time-course of MIF adsorption to peritoneal macrophages, pulse exposures were performed for 2, 5, 15, and 30 min using a 1:4 dilution of active supernatant. The cells were assayed as described for pulse exposure, and the adsorbed supernatants were tested for residual MIF activity by the standard assay. The results of three such experiments are shown in Fig. 5. Extending the time of exposure of cells to MIF resulted in increasing cellular adsorption as indicated by increasing

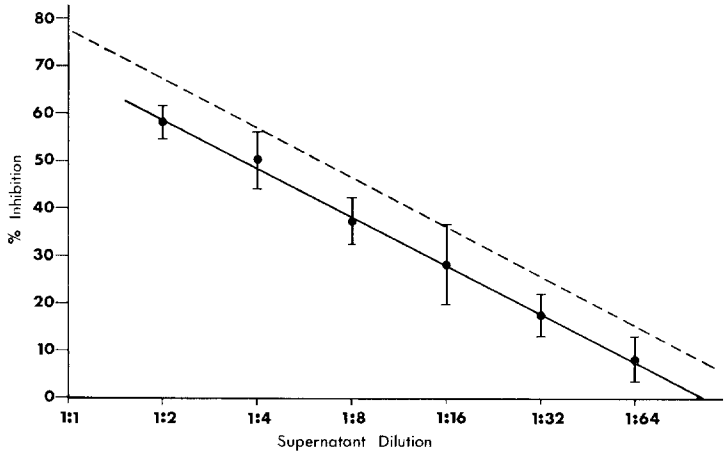

FIG. 3. The effect of pulse exposure (●—●) compared with continuous exposure (○—○) of dilutions of MIF-containing supernatants to inhibit the migration of peritoneal macrophages.

inhibition of migration of adsorbing cells and declining residual supernatant MIF remaining to inhibit a second population of macrophages. A replot of the data on the ordinate in units of MIF would accentuate the rate at which MIF is removed from the supernatant. These data indicate that the MIF adsorption during the 30 min period approaches equilibrium and a plateau of migration inhibition results.

Results of five experiments on the effect of temperature on MIF binding are depicted in Fig. 6. In these experiments temperatures of 4°, 25°, and 37°C were maintained during the 15 min exposure of peritoneal cells to both active and control supernatants. Temperature during the pulse exposure did not significantly affect the subsequent migration of macrophages exposed to control supernatants; however, increasing temperature is associated with increasing adsorption of MIF as expressed in migration inhibition. The effect of tem-

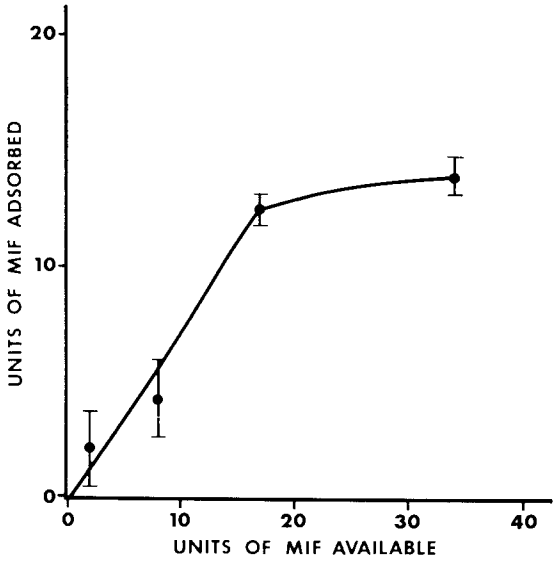

FIG. 4. The relationship of the units of MIF available and the amount bound by 12×10^6 peritoneal macrophages during pulse exposure for 15 min at 25°C. Total MIF available and residual MIF after pulse exposure were determined by the standard assay, and MIF bound was calculated as the difference between total and residual MIF.

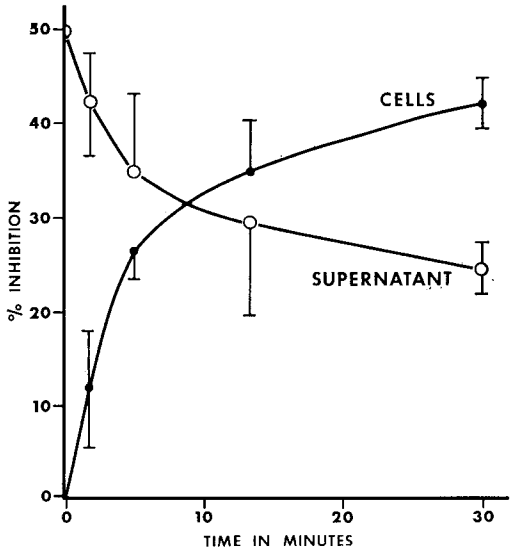

FIG. 5. Effect of time of adsorption on the removal of migration inhibitory activity from active supernatants (○—○) and the corresponding increase of migration inhibition of the cells used for adsorption (●—●).

peratures of 4°, 25°, and 37°C on binding corresponds to 1.25, 4.0, and 6.0 units of MIF bound, respectively.

To study the effect of increasing cell numbers on adsorption of MIF activity, supernatants containing 10 units of MIF (1:4 dilution) were exposed to 15, 30, or 60 × 10^6 peritoneal macrophages for 30 min at room temperature. Residual MIF supernatant activity was determined by the standard assay. The results of five experiments are summarized in Fig. 7. The migration data has been translated into MIF units and the MIF units adsorbed are plotted against cell number. Increasing the number of peritoneal cells progressively depleted the supernatant of MIF activity and a plateau of binding was seen at

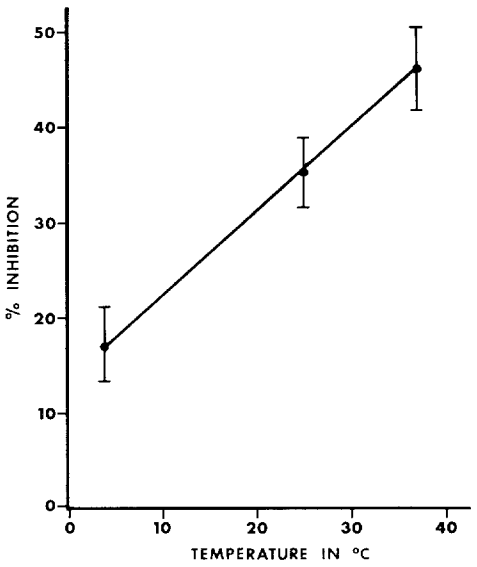

FIG. 6. Effect of temperature during pulse exposure on the ability of normal peritoneal macrophages to express migration inhibition.

higher cell numbers as MIF concentration became limiting. Alveolar macrophages at high concentrations were ineffective in adsorbing MIF.

To determine the effect of cell viability on the adsorption of MIF to macrophages, peritoneal and alveolar macrophages were rendered nonviable by freezing. Nonviable macrophages (60 × 10^6) were exposed to MIF for 45 min at 25°C, the cells sedimented, and the supernatants tested for residual MIF activity. The results of three experiments indicated that nonviable peritoneal macrophages adsorbed 80% of the MIF activity, which was equal to that adsorbed by viable cells under these conditions. Equivalent numbers of nonviable alveolar macrophages failed to adsorb MIF.

Effect of Enzymatic Treatment of Peritoneal Macrophages on their Ability to Respond to and Adsorb MIF.—If adsorption of MIF by peritoneal macrophages

were due to surface binding of the effector molecule, enzymatic pretreatment of these cells might be expected to interfere with their ability both to respond to and to adsorb MIF from active supernatants. The effect of pretreatment of peritoneal macrophages with trypsin, chymotrypsin, and neuraminidase on their ability to respond to pulse exposure of 1:4 dilution of active supernatant are shown in Table I. After exposure to trypsin at a concentration of 1 mg/ml for 30 min at 37°C, the peritoneal macrophages were significantly impaired in their ability to respond to MIF pulse although their ability to migrate was

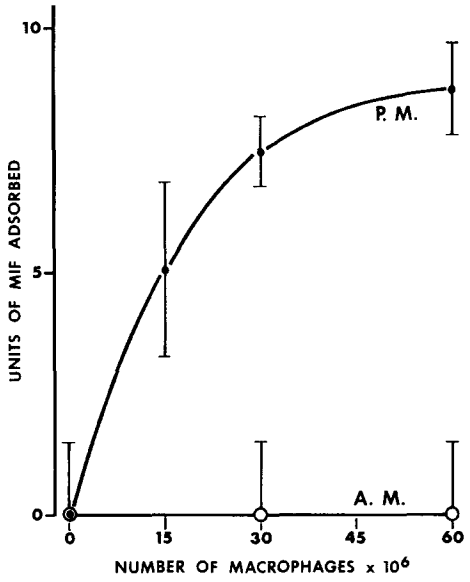

FIG. 7. Effect of the number of adsorbing alveolar macrophages (A.M., ○—○) and peritoneal macrophages (P.M., ●—●) on the removal of MIF from active supernatants containing a total of 10 units of MIF. Pulse adsorption was performed for 30 min at 25°C with varying numbers of cells, and the supernatants were retested for MIF activity in the standard assay.

unaffected. Cells treated with 5 mg/ml trypsin were even less responsive. The active supernatants which had been exposed to trypsinized cells showed no loss of MIF activity, thereby excluding the possibility that the failure of trypsinized macrophages to respond to MIF was due to destruction of MIF by residual cell-associated enzyme. The results of experiments with chymotrypsin (1 mg/ml) were similar. Neuraminidase (150 μg/ml) not only failed to reduce the ability of peritoneal macrophages to respond to MIF, but tended to increase their sensitivity.

Because trypsin at a concentration of 5 mg/ml was most effective in reducing the response of peritoneal macrophages to MIF, this concentration of the enzyme was used to study the ability of enzyme-treated cells to adsorb MIF.

Active supernatants were exposed to 30×10^6 trypsinized and control peritoneal macrophages for 30 min at room temperature and subsequently tested for MIF in the standard assay. Cells subjected to no enzyme treatment adsorbed 75% of the MIF present while trypsinized cells adsorbed none. The difference between the adsorptive capability of trypsinized *versus* control cells was highly significant ($P < 0.001$).

DISCUSSION

The assay for macrophage migration inhibitory factor (MIF) has been used for several years as an in vitro model of delayed hypersensitivity. Initially, peritoneal exudates containing sensitive lymphocytes and macrophages were incubated with antigen and the effect of progressive generation of MIF on macrophage inhibition was measured (1). Later, crude MIF-containing supernatants generated from sensitive lymphocytes incubated with antigen were used to inhibit macrophage migration (2, 3). More recently, efforts to purify MIF from crude supernatants have yielded more specific preparations, unfortunately, of diminished activity resulting from the process of purification (5, 9, 10). This gain in specificity has allowed approaches to physicochemical characterization but the diminution in activity has made difficult quantitative correlation with in vivo sensitivity. Although these developments offer the advantage of direct study of interaction of preformed MIF with its effector cell, the characteristics of this interaction have not been fully analyzed.

TABLE I

Effect of Enzyme Pretreatment of Peritoneal Macrophages on their Ability to Respond to MIF

Enzyme treatment	Response to MIF as per cent inhibition of migration	No. of experiments	*P* value compared with control
Control	37.9 ± 3.7	8	
Trypsin (1 mg/ml)	13.1 ± 6.7	6	$P < 0.01$
Trypsin (5 mg/ml)	5.6 ± 12.5	2	$P < 0.01$
Chymotrypsin (1 ml/ml)	15.3 ± 13.4	2	$P < 0.01$
Neuraminidase (150 μg/ml)	47.6 ± 9.1	2	$P < 0.30$

It has been assumed that migration inhibition has a linear relationship with MIF concentration and, therefore, is a direct correlate of the degree of delayed hypersensitivity. Our observations on the effect of diluting crude supernatants containing MIF on inhibition of migration indicate that the dose-response relationship involved in MIF expression shows saturation characteristics. These observations indicate that this limitation of the migration system makes

impossible direct and quantitative correlation of activity in the migration inhibition assay with the degree of delayed hypersensitivity. The calculation of MIF in arbitrary units based on the equation for a rectangular hyperbola provides a means of quantitation which should be applicable to capillary tube migration methods used by other workers.

Several mechanisms have been described to mediate the inhibition of macrophage migration. Our data indicate that MIF as a mediator of delayed hypersensitivity inhibits the migration of the peritoneal macrophage but not that of the alveolar macrophage. Cytophilic antibody with antigen has been shown to inhibit the migration of both the peritoneal (11) and alveolar (12) macrophage. Immune serum and antigen also inhibit migration of both peritoneal (13) and alveolar (R. W. Leu, unpublished data) macrophage. Evidence for other inhibitory factors which differ in physical properties and antigen dependence from either MIF, cytophilic antibody, or immune serum have been presented (14–16) but they remain unclarified. Thus, two or more different mechanisms involving both humoral and cellular immunity act to inhibit the migration of peritoneal macrophage, while only humoral mechanisms appear to inhibit the migration of aveolar macrophage.

Crude supernatants prepared from sensitive lymph node lymphocytes can be expected to contain varying amounts of both MIF and antibody depending on the methods of immunization and in vitro culture. These differential characteristics of alveolar and peritoneal macrophages should be useful as an alternative to physicochemical separation for separating MIF from the humoral factors.

The use of crude supernatants is prevalent in work with lymphocyte mediators. Although purification techniques are available, their yields of semipurified factors are small. In addition to multiple lymphocyte factors, known and unknown, these supernatants can be envisioned to contain a variety of nonspecific metabolites and to show varying degrees of nutrient depletion as a result of incubation for 24 hr with metabolically active cells. We approached this problem with the presumption that if a macrophage receptor for MIF existed, then brief exposure of the macrophage to an active supernatant should concentrate MIF onto the cell and exclude those factors in medium which would not be specifically adsorbed. One would expect that any other factors which affect the macrophage might be bound during pulse exposure to an active supernatant; these would include macrophage chemotactic factor, aggregating factor, and activating factor in addition to MIF. We acknowledge that these factors which have been defined biologically may not represent different biochemical entities. Current evidence suggests that only chemotactic factor and MIF are different (17). We observed that pulse exposure for 15 min at 25°C removes more than ½ of the MIF activity in the original supernatant. On the basis of approximate cell-to-supernatant volume ratios, this transfer of activity represents at least sixfold concentration. Increasing the time and tem-

perature of pulse exposure to 30 min at 37°C, while inviting the occasional complication of cell clumping, increased adsorption of the MIF activity. The pulse exposure technique offers, therefore, the advantage of a purification step in which the reduction of MIF activity from supernatant is predictable. The extent to which other lymphocyte mediators are adsorbed to the macrophage is under study. The pulse principle, in addition to offering considerable support for the existence of a receptor, provided the experimental means by which such a receptor concept could be explored.

Receptor models have been used for years to explain the actions of a variety of drugs and hormones. Our approach to the macrophage was guided by observations in a number of tissues that the specificity of action of many hormones and mediators lies in specific binding to receptors on their target cell surfaces. The ideal approach to mediator-receptor interaction involves purified, assayable components. The lymphocyte and macrophage provided neither. MIF is secreted in infinitesimally small quantities and only the biological assay of migration is available as an indication of interaction of MIF with the macrophage. With these admitted liabilities we offer the following as evidence for the presence of a MIF receptor on the peritoneal macrophage: (*a*) Both viable and nonviable peritoneal macrophages selectively adsorb MIF from an active superantant. (*b*) The adsorption has a direct dose-response relationship at low concentration of MIF, shows saturation characteristics when the quantity of MIF exceeds available receptor sites, and shows a plateau of binding when the number of receptors exceeds available MIF at equilibrium. (*c*) The adsorption is time and temperature dependent. (*d*) Pretreatment of macrophage with proteolytic enzymes removes their ability to adsorb and to respond to MIF while leaving their ability to migrate unaffected. (*e*) Alveolar macrophages do not remove MIF activity from an active supernatant thus demonstrating specificity of the MIF receptor.

The features of interaction of MIF with the peritoneal macrophage correspond remarkably to the interactions described for insulin and the fat cells (18). It is of interest that the fat cell regains its insulin receptors within hours after trypsinization (19). We suggest this relationship is the explanation for the observation (20) that the trypsinized macrophage will respond to continuous exposure to MIF.

The MIF receptor differs from the macrophage receptor described for antibody in that the latter resists proteolytic digestion (21). Both insulin (18) and cytophilic antibody (21) bind to their receptors to form dissociable complexes. Our preliminary attempts to elute MIF from peritoneal cells by repeated washing, heat treatment (56°C for 30 min), and chelation (5 mM ethylenediaminetetraacetate for 30 min) have been unsuccessful suggesting either that MIF is bound with unusual avidity to its receptor or that it is inactivated after initial binding.

The role of MIF as a participant in immune responses remains unclear. One

questions the biological function of MIF to inhibit migration in the presence of a chemotactic factor to attract actively migrating macrophages. Evidence has been presented that MIF is indistinguishable in its physical properties from a factor which activates macrophages (6). We have shown in preliminary work that macrophages pulsed with active supernatants subsequently undergo those morphological and adherence changes described by Mooney and Waksman (22) thus indicating that macrophage activation is a concomitant of migration inhibition and results from the adsorption of MIF. The inhibition of migration may well be secondary to the more important process of activation. Thus, the primary biological role of MIF may lie in its action to activate macrophages and thereby expand the expression of cellular immunity.

The role of macrophage activation in the expression of cellular immunity in vivo has recently been reviewed by Mackaness (23). Lymphocyte-induced macrophage activation in vivo is associated with enhanced phagocytic and bactericidal capacities. The study of the activation process in vitro has provided evidence that MIF-rich supernatants or semipurified MIF enhance phagocytic capability of peritoneal macrophages (24) and their bactericidal capacity (25, 26). The demonstration that the lymphocyte can be induced by antigen to produce a soluble mediator which activates macrophages supports a concept of immune modulation of macrophage function which would operate to varying degree in all cellular immune responses.

An important aspect of this modulation would appear to be its specificity. The action of MIF on the peritoneal macrophage indicates that the circulating blood monocyte which is the precursor for the peritoneal macrophage is the population subject to regulation by the sensitive lymphocyte through MIF. The lack of a receptor for MIF on the alveolar macrophage suggests that the alveolar macrophage functions with a certain autonomy with respect to cellular immunity. Recent evidence supports the lack of participation of the alveolar macrophage in acquired resistance to pulmonary infection with the facultative intracellular pathogens, bacille Calmette Guérin and *Listeria monocytogenes* (27, 28). Additional data suggest that another fixed macrophage population, the Kupffer cells, may not participate in acquired resistance to the facultative pathogen *Brucella abortus* (29). Further elucidation of mechanisms of immune modulation of various fixed and free macrophage populations represents an important key to understanding local and systemic immunity.

SUMMARY

The initial interaction between migration inhibitory factor (MIF) and the guinea pig alveolar and peritoneal macrophage was studied. MIF-containing supernatants were generated from sensitized lymph node lymphocytes obtained from guinea pigs immunized with bovine gamma globulin in complete Freund's adjuvant. MIF-containing supernatants were markedly inhibitory for the migration of the peritoneal macrophage but had no effect on the alveolar macro-

phage. A linear relationship was observed between per cent inhibition of migration and serial twofold dilution of supernatant. Reexpressed in arbitrary MIF units, this relationship reflects a dose-response relationship with saturation characteristics. Pulse exposure of peritoneal macrophages to MIF resulted in adsorption of MIF onto both viable and nonviable cells with corresponding depletion of supernatant MIF. The alveolar macrophage did not adsorb MIF. Pulse adsorption of MIF onto the peritoneal macrophage is dependent on time, temperature, and cell number. Pretreatment of the cells with proteolytic enzyme prevents the adsorption of MIF while leaving migration unaffected. These observations support the existence of a specific cell surface receptor for MIF. The existence of such a receptor provides selectivity of immune modulation of macrophage populations by lymphocytes in delayed hypersensitivity reactions.

REFERENCES

1. George, M., and J. H. Vaughan. 1962. *In vitro* cell migration as a model for delayed hypersensitivity. *Proc. Soc. Exp. Biol. Med.* **111**:514.
2. Bloom, B. R., and B. Bennett. 1966. Mechanism of a reaction *in vitro* associated with delayed-type hypersensitivity. *Science* (*Wash. D.C.*). **153**:80.
3. David, J. R. 1966. Delayed hypersensitivity *in vitro*: its mediation by cell-free substances formed by lymphoid cell-antigen interaction. *Proc. Natl. Acad. Sci. U. S. A.* **56**:72.
4. Remold, H. G., and J. R. David. 1970. Cellular hypersensitivity: characterization of migration-inhibitory factor by enzymatic treatment. *Fed. Proc.* **29**:305.
5. Remold, H. G., A. B. Katz, E. Haber, and J. R. David. 1970. Studies of migration inhibitory factor (MIF): recovery of MIF activity after purification by gel filtration and disc electrophoresis. *Cell. Immunol.* **1**:133.
6. Nathan, C. B., M. L. Karnovsky, and J. R. David. 1971. Alterations of macrophage functions by mediators from lymphocytes. *J. Exp. Med.* **133**:1356.
7. Myrvik, Q. M., E. Leake, and B. Farriss. 1961. Studies on pulmonary alveolar macrophages from the normal rabbit: a technique to procure them in a high state of purity. *J. Immunol.* **86**:128.
8. Bloom, B. R., and B. Bennett. 1971. *In* In Vitro Methods in Cellular Immunity. B. R. Bloom and P. R. Glade, editors. Academic Press, Inc., New York. 242.
9. Bennett, B., and B. R. Bloom. 1968. Reactions *in vivo* and *in vitro* produced by a soluble substance associated with delayed-type hypersensitivity. *Proc. Natl. Acad. Sci. U. S. A.* **59**:576.
10. Dumonde, D. C., R. A. Wolstencroft, G. S. Panayi, M. Matthew, J. Morley, and W. T. Howson. 1969. Lymphokines: non-antibody mediators of cellular immunity generated by lymphocyte activation. *Nature* (*Lond.*). **224**:38.
11. Amos, H. E., B. W. Gurner, R. T. Olds, and R. R. A. Coombs. 1967. Passive generalization of tissue cells. Ability of cytophilic antibody to render the migration of guinea pig peritoneal exudate cells inhibitable by antigen. *Int. Arch. Allergy Appl. Immunol.* **32**:496.
12. Heise, E. R., S. Han, and R. S. Weiser. 1968. *In vitro* studies on the mechanism of macrophage migration inhibition in tuberculin sensitivity. *J. Immunol.* **101**: 1004.

13. Spitler, L., H. Huber, and H. H. Fudenberg. 1969. Inhibition of capillary migration by antigen-antibody complexes. *J. Immunol.* **102:**404.
14. Pollock, E. M., C. N. Pegram, and J. J. Vazquez. 1971. A comparison of the *in vitro* migration properties of alveolar and peritoneal macrophages. *J. Reticuloendothel. Soc.* **9:**383.
15. Bartfeld, H., and R. Kelly. 1968. Mediation of delayed hypersensitivity by peripheral blood lymphocytes *in vitro* and by their products *in vivo* and *in vitro*: morphology of *in vitro* lymphocyte-macrophage interaction. *J. Immunol.* **100:** 1000.
16. Amos, H. E., and P. J. Lachman. 1970. The immunological specificity of a macrophage inhibition factor. *Immunology.* **18:**269.
17. Ward, P. A., H. G. Remold, and J. R. David. 1970. The production of antigen-stimulated lymphocytes of a leukotactic factor distinct from migration inhibitor factor. *Cell. Immunol.* **1:**162.
18. Cuatrecasas, P. 1971. Insulin-receptor interactions in adipose tissue cells: direct measurement and properties. *Proc. Natl. Acad. Sci. U. S. A.* **68:**1264.
19. Kono, T. 1969. Destruction and restoration of the insulin effector system of isolated fat cells. *J. Biol. Chem.* **244:**4777.
20. Pochyly, D. F. 1967. The role of trypsin in interfering with *in vitro* delayed hypersensitivity reactions. *Fed. Proc.* **26:**478.
21. Davey, M. J., and G. L. Asherson. 1967. Cytophilic antibody. I. Nature of the macrophage receptor. *Immunology.* **12:**13.
22. Mooney, J. J., and B. H. Waksman. 1970. Activation of normal rabbit macrophage monolayers by supernatants of antigen-stimulated lymphocytes. *J. Immunol.* **105:**1138.
23. Mackaness, G. B. 1971. *In* Immunobiology. R. A. Good and D. W. Fisher, editors. Sinauer Assoc. Inc., Stamford, Conn. 45.
24. David, J. 1971. Lymphocytic factors in cellular hypersensitivity. *Hospital Pract.* **6:**79.
25. Salvin, S. B., and S. Cheng. 1971. Lymphoid cells in delayed hypersensitivity. II. *In vitro* phagocytosis and cellular immunity. *Infect. Immun.* **3:**548.
26. Simon, H. B., and J. N. Sheagren. 1971. Cellular immunity in vitro. I. Immunologically mediated enhancement of macrophage bactericidal capacity. *M. Exp. Med.* **133:**1377.
27. Truitt, G. L., and G. B. Mackaness. 1971. Cell-mediated resistance to aerogenic infection of the lung. *Amer. Rev. Respir. Dis.* **104:**829.
28. Shima, K., A. M. Dannenberg, M. Ando, S. Chandrasekhar, J. A. Seluzicki, and J. I. Fabrikant. 1972. Macrophage accumulation, division, maturation, and digestive and microbicidal capacities in tuberculous lesions. I. Studies involving their incorporation of tritiated thymidine and their content of lysosomal enzymes and bacilli. *Am. J. Pathol.* **66:**365.
29. North, R. J. 1970. The relative importance of blood monocytes and fixed macrophages to the expression of cell-mediated immunity to infection. *J. Exp. Med.* **132:**521.