HUMAN MIGRATION INHIBITORY FACTOR: PURIFICATION AND IMMUNOCHEMICAL CHARACTERIZATION

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Products of activated lymphocytes are known to modulate the function of macrophages (1–3). Migration inhibitory factor (MIF)[1] is one of the best studied mediators of cellular immunity (4). Although many investigators have tried to analyze the biological and physicochemical properties of MIF from different species, the active substance has not been purified to homogeneity. MIF produced by guinea pig lymphocytes appears to be a glycoprotein with a mol wt of ≅ 35,000–55,000 daltons (5). Rocklin et al. (6) were able to differentiate highly purified guinea pig MIF from partially purified human MIF by using gel filtration, disc electrophoresis, isopycnic centrifugation, and enzymatic treatment. They determined MIF activity after Sephadex gel filtration in fractions containing molecules with a mol wt of ≅ 23,000 daltons.

The production of antibodies to guinea pig and human lymphokines was demonstrated by Yoshida et al. (7) and Bendtzen (8), and Geczy et al. (9) produced antibodies to partially purified guinea pig MIF, which inhibited the delayed skin reaction of sensitized guinea pigs challenged with purified protein derivative. These results prompted us to initiate immunochemical studies of the mediators of cellular immunity in man. In view of the role of MIF in cellular immunity, the isolation and characterization of human MIF is of paramount importance for the full evaluation of its role in different diseases, and for the development of rapid qualitative and quantitative methods for its detection.

This paper describes the purification and immunochemical characterization of human MIF produced by concanavalin A (Con-A)-activated lymphocytes. The specificity of anti-MIF antibodies is demonstrated by using sensitive immunoelectrophoretic techniques. MIF activity on guinea pig peritoneal macrophages, as measured by the capillary test, is correlated with its effect on the electrophoretic mobility of macrophages.

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; EF, encephalogenic factor; MEM, Eagle's minimal essential medium; MIF, migration inhibitory factor; MSF, macrophage slowing factor; PBS, phosphate-buffered saline, pI, isoelectric point; SDS, sodium dodecyl sulfate gel.

Materials and Methods

Production of MIF-Rich Material by Human Lymphocytes. Lymphocytes were isolated from human blood by centrifugation in Ficoll-Hypaque gradients (10). After two washes in Hanks' buffer (Microbiological Associates, Bethesda, Md.) at room temperature, the cells were suspended in Eagle's minimal essential medium (MEM) containing 24 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (Grand Island Biological Co., Grand Island, N. Y.), 100 U/ml of penicillin, and 100 μl/ml of streptomycin (Microbiological Associates). The viability of the cells was determined by trypan blue exclusion and was usually found to be ≅ 90%. The cells at a concentration of 10^7 cells/ml (30-ml volume) were then stimulated by the addition of 10 μg of Con A (Pharmacia Fine Chemicals Inc., Uppsala, Sweden) and incubated for 24 h at 37°C in a 5% CO_2 atmosphere in 250-ml plastic tissue flasks (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). After centrifugation at 18,000 *g* for 20 min at 4°C, the supernates were dialyzed against water at the same temperature for 24 h and then lyophilized. The lyophilized products of 40 × 500-ml lymphocyte supernates were each dissolved in 5 ml of 0.01-M phosphate buffer (pH 7.4), cleared by centrifugation for 10 min at 10,000 *g* at 4°C, dialyzed against the same buffer, and applied to a column packed with Sephadex G-100 (2.5 × 100 cm). The columns were precalibrated with albumin and chymotrypsinogen. Appropriate fractions were pooled, extensively dialyzed against water, lyophilized, and tested for MIF activity. For the capillary test, the dry, fractionated material was redissolved in 2 ml of MEM containing penicillin, streptomycin, and 10% heat-inactivated guinea pig serum; 50 or 100 μl of this solution was used. Control supernates were prepared in the same way from cultures to which Con A was added after a 24-h incubation. An equal amount of the same material, prepared as described above, was tested in the cell electrophoresis apparatus according to the methods of Field et al. (11). For this measurement, 50 or 100 μl of MIF-rich material, and equal amounts of control material were used.

Preparative Isotachophoresis. Preparative isotachophoresis was performed according to Svendsen and Rose (12). The lyophilized material obtained from 40 gel filtration experiments was dialyzed against the cathode buffer (terminating electrolyte, 0.23 M ε-aminocaproic acid and 0.23 M Tris, pH 8.9) to which 1 ml of ampholine carrier ampholytes (pH 5–7 and 6–8, diluted 1:1, 40% wt/vol; LKB-Produkter AB, Bromma, Sweden) was added. The preparation was layered on top of the polyacrylamide gel (3.3%) in an LKB 7900 Uniphor, equipped with an LKB 7960 plastic column. Trisphosphate (0.03 M, pH 6.25) was used as the leading electrolyte. Fractions were collected from the isotachophoresis column and they were extensively dialyzed (48 h) against water. Samples of 50 or 100 μl of each fraction (6-ml volumes) were tested for migration inhibitory activity and assayed in the cell electrophoresis apparatus.

Chemical Analyses. Protein was determined by the method of Lowry et al. (13). Bovine serum albumin was used as the standard.

Analytical Polyacrylamide Gel Electrophoresis. Analytical polyacrylamide gel electrophoresis was performed using the method of Ornstein (14) on 10% acrylamide gels in Tris-glycine buffer (pH 8.0). The gels were stained with Coomassie Brilliant Blue R 250.

Polyacrylamide gel electrophoresis in 0.1% sodium dodecyl sulfate (SDS) was performed at pH 7.2 in 10% acrylamide using the methods of Shapiro et al. (15), and Weber and Osborn (16). The following proteins were used as standards: bovine serum albumin (mol wt 6.8 × 10^4 daltons), rabbit-muscle phosphorylase (mol wt 9.4 × 10^4 daltons) and chymotrypsinogen (mol wt 2.5 × 10^4). To prevent proteolytic degradation, the samples were boiled for 3 min in a solution of 1% SDS and 1% 2-mercaptoethanol. The marker proteins were prepared in the same way.

Isoelectric Focusing on Thin-Layer Polyacrylamide Gels. The isolated protein was subjected to isoelectric focusing on 5% polyacrylamide gel plate using the method of Awdeh et al. (17). The samples and carrier ampholytes (pH 3–10) were set in the gel. The run was carried out for 16 h at 4°C with a potential difference of 400 V between the two electrodes. After electrophoresis the gel was removed and fixed in 5% trichloracetic acid and stained with Coomassie blue.

Preparation of Antisera. The antigens were dissolved at a concentration of 250 μg/ml. On days 0, 14, 28, and 42, two rabbits weighing 2–2.5 kg were injected intracutaneously with a standard mixture consisting of 50 μl antigen solution and 50 μl incomplete Fruend's adjuvant. On day 50, 45 ml of blood was drawn from an ear vein. Every 6th wk, 45 ml of blood was taken, the animals having received a standard mixture of antigen 8–10 days before each bleeding.

Isolation of Immunoglobulins and Purification by Affinity Chromatography. 25 g of $(NH_4)_2SO_4$ was added to 100 ml of antiserum and the mixture was left at room temperature for about 20 h.

Centrifugation at 4,000 *g* for 30 min precipitated 98% of the antibody activity. The precipitate was washed once with ≅ 25 ml of 1.75 M $(NH_4)_2SO_4$ and the antibody-containing precipitate was transferred in a small amount of water to a 25-ml dialysis bag. The suspension was dialyzed at 4°C for 24 h against two changes of distilled water, for 24 h against 0.021 M acetate buffer (pH 5.0), for 24 h against two changes of distilled water, and finally for 24 h against acetate buffer (0.021 M, pH 5.0). The supernate was transferred to a column containing 24 ml of DEAE-Sephadex A50 (Pharmacia Fine Chemicals, Uppsala, Sweden) and equilibrated with 0.021 M acetate buffer (pH 5.0). The IgG fraction was eluted with ≅ 25 ml of acetate buffer.

Affinity chromatography was used for further purification of the IgG fraction containing anti-MIF antibodies. Control material obtained from unstimulated cultures as described above was coupled to cyanogen bromide-activated Sepharose 2B (Pharmacia Fine Chemicals) according to the method of Porath (18). Lyophilized supernates from 100 ml of unstimulated lymphocytes were dissolved in 5 ml of MEM, added to 5 ml of packed activated beads, and washed in 50 ml of 0.25 M sodium bicarbonate at pH 9. The mixture was stirred for 24 h in the cold. It was then transferred to a glass column and washed with 50 ml of phosphate-buffered saline (PBS), 50 ml of 1 M acetic acid, and again with 50 ml of PBS. 20 ml of the IgG fraction was applied to the column. The flow rate was 0.1 ml/min. The nonbound fraction was used for further investigations.

Immunochemical Techniques for Quantitation of MIF Activity. Antisera were analyzed using the method of Laurell (19). The concentration of the antiserum in the agarose gel was 4.5% (vol/vol). The holes were filled with 5 μl of antigen. After electrophoresis (10 V/cm for 2–3 h or 2.5 V/cm for 18 h), the gel was pressed. The filter paper was then gently removed and the plates were placed for 30 min in 0.9% NaCl, and for 30 min in distilled water. The gel was pressed again, dried, and finally stained with Coomassie Brilliant Blue. Rocket immunoelectrophoresis with intermediate gel was carried out according to Axelsen and Bock (20).

Assay for MIF Activity. The lyophilized MIF-rich material was dissolved in MEM medium containing penicillin, streptomycin, and 10% inactivated guinea pig serum, and it was assayed for MIF activity on guinea pig macrophages in capillary tubes. Migration inhibition tests were performed according to the method of David and David (21). The chambers were incubated at 37°C and the area of migration was drawn after 16 h and measured by planimetry. The area of migration of four capillary tubes was averaged and expressed as the percentage of migration according to the formula:

$$\text{MIF activity} = \frac{\text{average area of MIF active preparation}}{\text{average area of control preparation}} \times 100$$

Cell Electrophoresis. The electrophoretic mobility of macrophages was determined according to the method of Field et al. (11) by suspending 10^7 cells in Medium TC 199 (Grand Island Biological Co., Grand Island, N. Y.) in a 2-ml volume. Macrophages were prepared as described by David and David (21) and they were incubated with the test material for 90 min at room temperature. The mobility was measured in the rectangular chamber of a Zeiss cytophotometer (Carl Zeiss, Inc., New York) (22).

Results

Fractionation and Purification of MIF-Rich Material. Fig. 1 shows the elution pattern of 500-ml dialyzed and lyophilized supernates of lymphocytes activated with Con A. Most of the MIF activity was found in fractions 19–24. When pooled, dialyzed, lyophilized, and tested in the migration inhibition assay, MIF activity was 40 ± 9.6% (15 determinations).

Further enrichment of MIF activity was achieved by preparative isotachophoresis. Pooled fractions from 40 gel filtration experiments (≅ 100 mg protein) were used (Fig. 2). Most of the activity eluted in fractions 39–41 (B3) inhibited the macrophage migration by 80 ± 8.5% (6 determinations). The biological activity of fractions 42–44 (B2) was in the range of 18 ± 10.5% (4 determinations), of fraction 45–47 (B1) in the range of 8 ± 5.8% (4 determinations), and fraction 48–50 (A) in the range of 5.8 ± 5% (4 determinations). The pooled

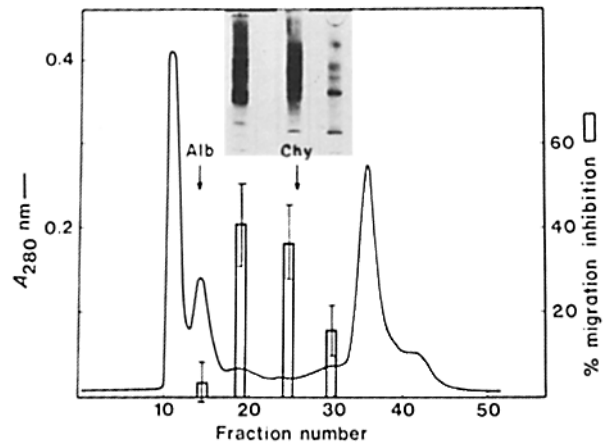

FIG. 1. Activity and elution profile of MIF-rich supernates on Sephadex G-100. The material was eluted at a flow rate of ≅ 32 ml/h, and 12 ml/tube were collected. Protein was measured by its absorbance at 280 nm. For measurements for MIF activity, see Materials and Methods and text. Analytical polyacrylamide gel electrophoresis: 50 μl of 250-fold concentrated fractions (15, 19, 24) were layered on each gel. Vertical bars represent standard deviation. Alb, albumin; Chy, chymotrypsinogen.

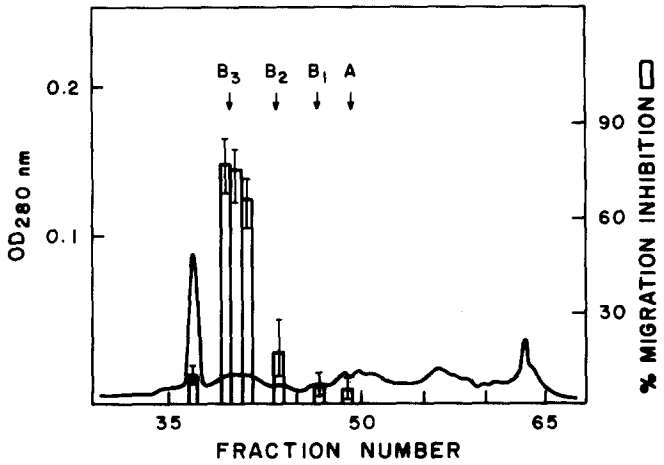

FIG. 2. Biological activity and ultraviolet absorption at 280 nm of the effluent from a Uniphor column in which the MIF protein was separated by preparative isotachophoresis. Vertical bars represent standard deviation.

material was extensively dialyzed, concentrated by vacuum dialysis to 1 ml, and stored at −70°C. To determine the purity of isolated material after gel filtration, analytical polyacrylamide gel electrophoresis was performed. As shown in Fig. 1, gel electrophoresis on 10% polyacrylamide, pH 8.9, of appropriate fractions (15, 19, 24) after gel filtration revealed multiple bands.

After isotachophoresis, however, pool A (fractions 48–50) showed one band on 10% acrylamide gel, B1 (pooled fractions 45–47) showed two closely spaced bands, B2 (pooled from fractions 42–44) showed one diffuse band, and B3 (pooled from fractions 39–41) showed one single diffuse band (Fig. 3). On SDS gels (Fig. 4) pool A showed one dense band with an apparent mol wt of ≅ 68,000

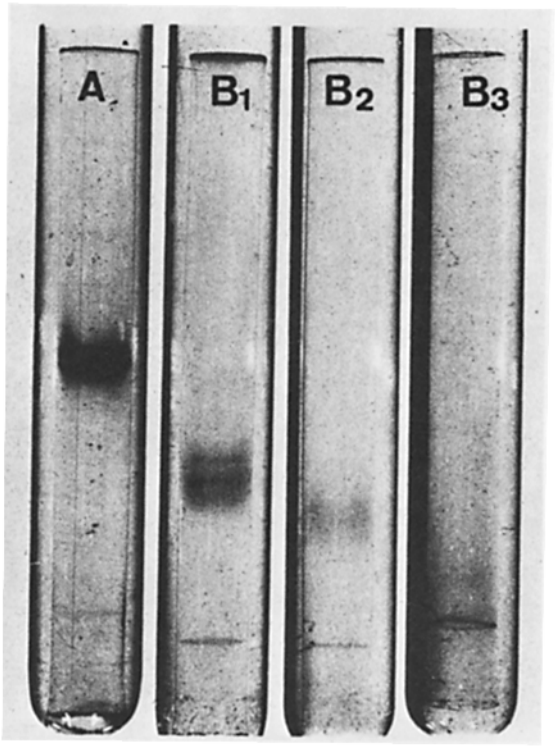

FIG. 3. Polyacrylamide gel electrophoresis of purified material performed after preparative isotachophoresis. 10 μl of the concentrated material (1-ml vol) were placed on each gel. A, pool of fraction 48–50; B1, pool of fraction 45–47; B2, pool of fraction 42–44; B3, pool of fraction 41–39.

daltons (protein A). The pooled fractions B1, B2, and B3 seemed to be contaminated with small amounts of protein A. Pool B1 showed one band with an apparent mol wt of $\cong$ 55,000 daltons, B2 contained the same band in addition to one other component of $\cong$ 28,000 daltons. B3 contained a major band with an apparent mol wt of $\cong$ 25,000 daltons but was contaminated with protein A and an additional minor component with a mol wt of $\cong$ 34,000 daltons. Since almost all of the migration inhibition activity was localized in B3, it was assumed that this fraction contains MIF.

The isoelectric point (pI) of MIF protein (B3) was found to be between 3.5 and 4 (data not shown). Although a second minor band of faint intensity was observed in the same region, the MIF has a purity of 90–95%.

Yields of Purified MIF. The protein content of 40 lymphocyte supernates (500-ml volume of each), as estimated by Lowry et al. (13), was $\cong$1 g (25 mg/supernate; 5 determinations). The MIF-rich material obtained and pooled after 40 gel filtration experiments contained $\cong$100 mg protein (2.5 mg protein/filtration; 5 determinations) or $\cong$10% of the starting material. After preparative isotachophoresis, fraction A yielded $\cong$1,950 μg (or 0.195% of the starting material), fraction B1 contained 750 μg (or 0.075% of the starting material), fraction B2, 100 μg (or 0.01% of the starting material), and fraction B3 yielded 250 μg (or 0.025% of the starting material).

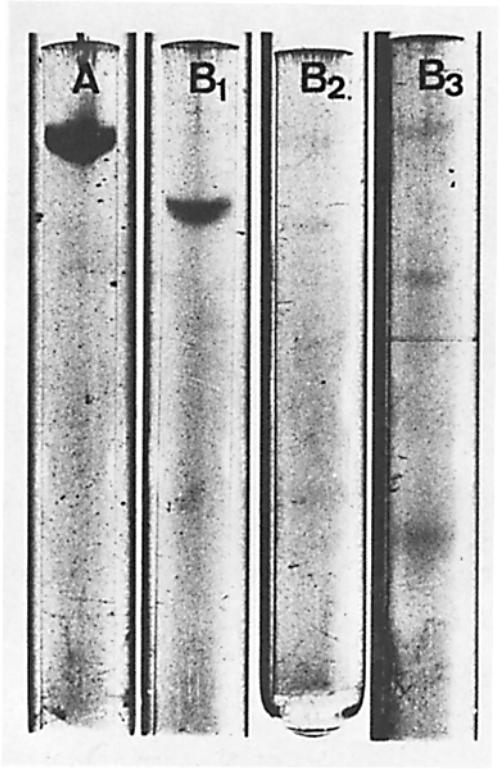

FIG. 4. Polyacrylamide gel electrophoresis in SDS. 10 μl of concentrated fractions (1-ml vol) A, B1, B2, and B3 were loaded onto the gels to facilitate the comparison of the relative proportions of various contaminant proteins.

The protein content of the unfractionated supernate which gave a migration inhibition of ≅35–40% was 0.4 mg/ml. Using 1 μg/ml of the purified MIF protein, ≅45% migration inhibition was measured. Therefore, approximately 400-fold purification in terms of specific MIF activity per protein content was achieved. 50 μl of the concentrated supernates (100-fold) showed 40% inhibition of migration. However, after Sephadex G-100 filtration, 100 μl of the MIF-rich fraction (concentrated 250-fold in comparison with the starting material) also showed 35–45% inhibition. This shows that although the specific activity of MIF-rich material was considerably increased by Sephadex gel filtration, recovery of MIF activity was only about 20%.

Immunoelectrophoresis. The rocket immunoelectrophoresis technique was used according to the method of Laurell (19). Rabbit antisera were produced against protein A and against the pool of B1, B2, and purified protein (B3) containing MIF activity. Fig. 5 shows the immunoprecipitation patterns obtained when purified proteins were run against anti-A antiserum. Visible precipitation lines are formed with pool A, B1, B2 (mixed), and control supernates, but not against protein B3. The immunoelectrophoretic pattern of antibodies prepared against the pool of B1, B2, and B3 is demonstrated in Fig. 6A. Precipitation lines appeared against the antigens B1, B2 (mixed), B3, and the control material. Since anti-B reacted with all the protein fractions tested

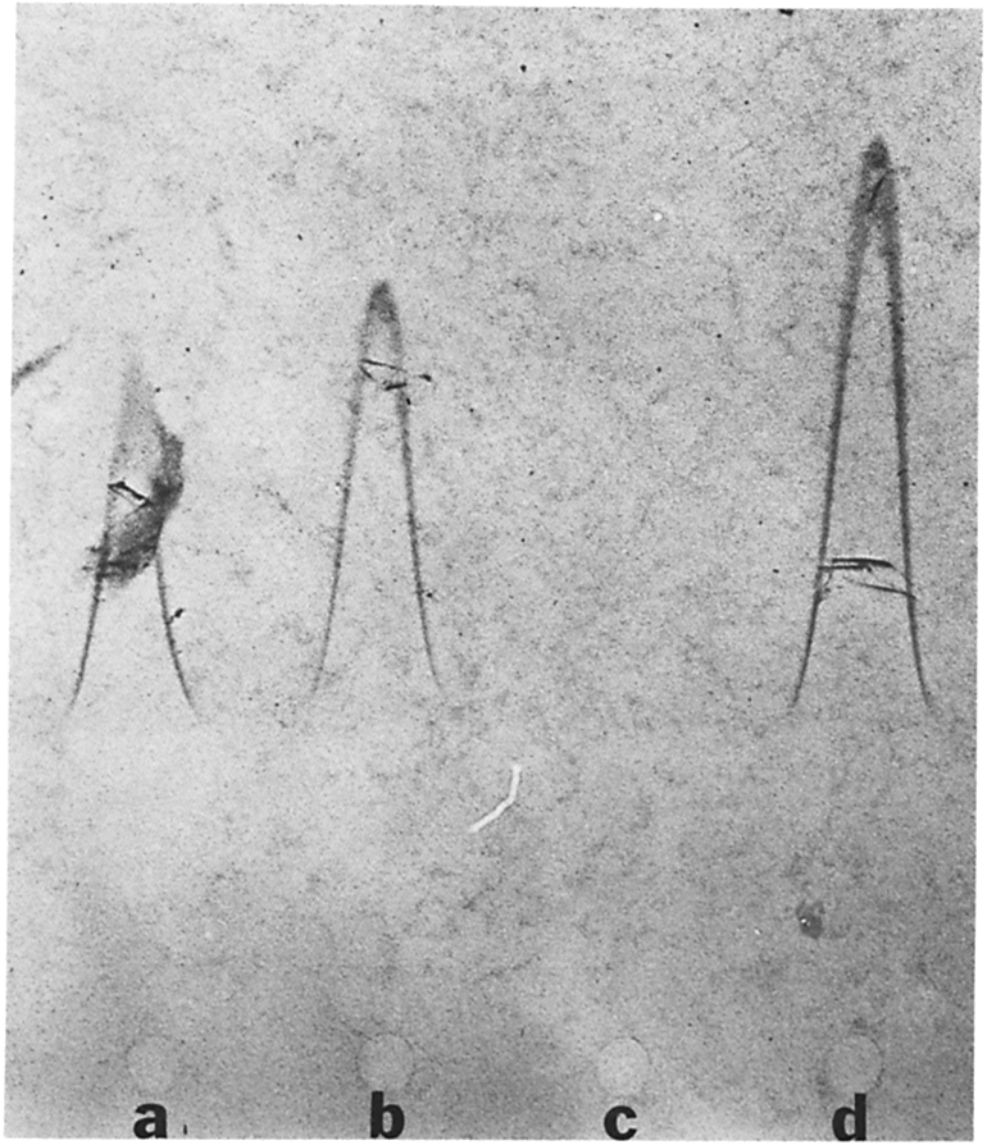

FIG. 5. Rocket immunoelectrophoresis of purified proteins and of nonstimulated control supernates. 5 μl of pools A, B1, B2, and B3 and control supernates were applied. Wells a, b, c, and d contained 0.5 μg/5 μl of substance A, 0.8 μg/5 μl of B1, B2 (mixed), 0.8 μg/5 μl of B3, and 0.8 μg/5 μl of control supernate. The antibody solution was 4.5% immunoglobulins isolated from rabbit antiserum to substance A. Electrophoresis was performed at pH 8.8 in a buffer of 0.0245 M barbital, 0.073 Tris, 0.0003 M calcium lactate, and 0.003 M sodium azide with 2.5 V/cm in the gel at 15°C for 18 h. The gel was stained with Coomassie Brilliant Blue R. The anode was at the top.

and was not specific for fraction B3, contaminating antibodies were present in this antiserum. To remove these contaminating antibodies, affinity chromatography was used.

For this purpose, control supernates obtained from unstimulated lymphocytes

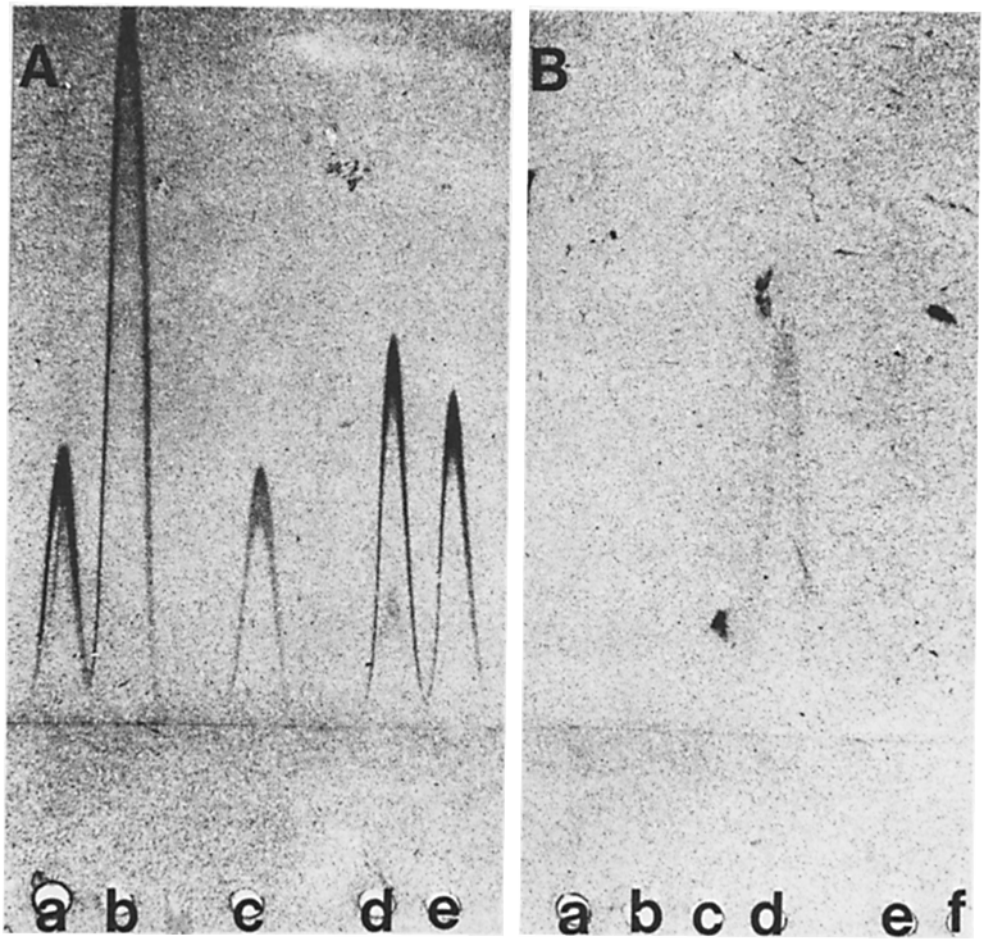

FIG. 6. (A), Rocket immunoelectrophoresis of anti-MIF antibodies before purification. The gel contained 4.5% immunoglobulins obtained from rabbit antiserum to pool of B1, B2, and B3. Antigens, 5 μl/well were: (a) 0.6 μg/5 μl of control I, (b) 1.5 μg/5 μl of control II, (c) 0.5 μg/5 μl of B3, (d) 0.8 μg/5 μl of B1, B2 (mixed), (e) 0.5 μg/5 μl of A. (B) Rocket immunoelectrophoresis after purification of anti-MIF antibodies. The gel contained 4.5% immunoglobulins of modified anti-MIF antibodies. Purification of anti-MIF antibodies was achieved by absorption of anti-B by using affinity chromatography. Well (a) 0.6 μg/5 μl of control I, (b) 1.5 μg/5 μl of control II, (c) 0.5 μg/5 μl of A, (d) 0.8 μg/5 μl of B3, (e) 0.5 μg/5 μl of B1, (f) 0.5 μg/5 μl of B2.

were bound to cyanogen bromide-activated agarose and the immunoglobulin fraction of anti-B was absorbed with this material. Fig. 6B shows the specificity of the absorbed anti-B. A precipitation line is found only with fraction B3 and not with the other protein fractions tested.

Biological Activity of MIF. It was speculated by Preece and Light (23) that macrophage slowing factor (MSF) produced by sensitized lymphocytes after incubation with the encephalogenic factor (EF) (24) and MIF may be the same substance. Therefore, the effect of MIF in the capillary test was correlated with its effect on the electrophoretic mobility of macrophages. 100 μl of 100-fold concentrated supernates showed 45% migration inhibition and the same concen-

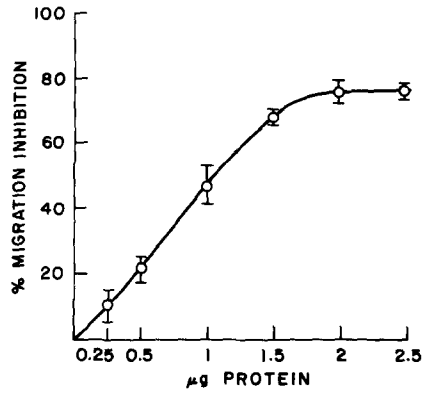

FIG. 7. Dose-response relationship of MIF protein in the capillary test. Concentrations used are calculated per ml MEM medium and used per chamber. Each point represents the average of six assays. The vertical bars represent standard deviation.

tration, and the same material resulted in 15±8.5% (8 measurements) reduction of the electrophoretic mobility of macrophages. As indicated above, MIF-rich material (appropriate fractions after gel filtration) inhibited the migration of macrophages ≅40±9.6% (7 determinations). The electrophoretic mobility of macrophages in the presence of this MIF-rich fraction was 17.5±6.2% (6 measurements) lower than that of cells treated with control material. The migration inhibition activity of B3 was 80±8.5% (6 determinations). Fraction B3 at the same concentration decreased the electrophoretic mobility of macrophages as well, to ≅24.5±6.3% (5 experiments), whereas other fractions had no effect. Since biological activity of the MIF and the MSF may be identical, the dose-response relationship of B3 was studied for both tests. For the migration inhibition test the amount of protein was varied from 0.25–2.5 μg in 1 ml MEM. For the cell electrophoresis measurements, the same amounts of protein were diluted with 2 ml Medium TC 199. Figs. 7 and 8 show the effect of protein B3 in both tests. While in the capillary test, a protein concentration of 2–2.5 μg/ml caused 77.4±5.4% inhibition (6 determinations), the same amount of protein in 2 ml of Medium TC 199 lowered the electrophoretic mobility of macrophages to a maximum of 27.8±6.3% (5 measurements).

Effect of Neutralization by Anti-MIF Antibodies. 50 μl of lymphocyte supernates causing migration inhibition of ≅35–40% and 50 μl of MIF protein which inhibited the migration by ≅75% were incubated with different dilutions of anti-MIF antibodies for 1 h at 37°C. Then the solutions were centrifuged at 10,000 *g* for 10 min and the supernates were used for the capillary test. In using antibody dilutions of 1:1 and 1:2, the inhibition effect of the lymphocyte supernate and the purified MIF was neutralized (95–100% migration). After treatment with 1:3 diluted antibodies, the supernate affected migration inhibition by ≅20%, and the purified MIF by ≅35% (5 experiments). The electrophoretic mobility of macrophages was lowered by using the same concentrations of supernates to ≅10%, and of purified MIF protein to ≅25% (6 measurements). This effect of the supernates on electrophoretic mobility was fully neutralized by using antibody dilutions 1:1, 1:2, and 1:3 (4 measurements). The effect on

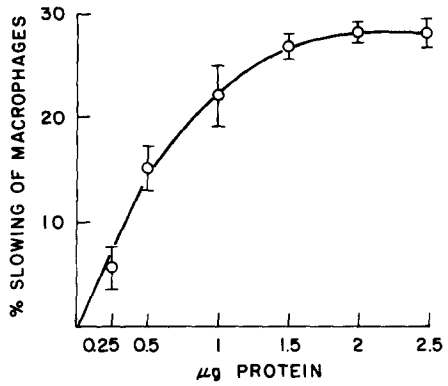

FIG. 8. Effect of different concentrations of purified MIF on the electrophoretic mobility (EM) of macrophages. The cells at a concentration of 10^7 were incubated in 2 ml of medium TC 199 at 23°C before measurements were taken. Each point represents the average of five measurements. The vertical bars represent standard deviation.

electrophoretic mobility of the purified MIF protein was neutralized when antibody dilutions 1:1 and 1:2 were used (4 measurements). In using an antibody dilution of 1:3, the electrophoretic mobility of macrophages was lowered to ≅10% (4 experiments).

Discussion

As mentioned by Taylor (25) the preparation of MIF from Con A-stimulated lymphocytes appears to be rather inefficient. Conventional methods such as Sephadex chromatography and disc electrophoresis on polyacrylamide gels were not successful in purifying preparative amounts of MIF. Therefore, we attempted the purification by using preparative isotachophoresis which allows fractionation of proteins on the basis of charge and molecular weight. The application of preparative isotachophoresis resulted in the successful purification of a protein with MIF-like activity obtained from Con A-activated lymphocyte supernates. The degree of purification of human MIF was 400-fold, if the capillary test can be considered as a criterion for the estimation of specific MIF activity. The recovery of specific MIF activity after purification of Sephadex G-100 was ≅20%, in agreement with the observations of Remold et al. (5). Calculation of the recovery at various stages during purification was not possible in the absence of a reliable quantitative radioimmunoassay. Fraction B3 contained MIF, and this was concluded from the observation that migration inhibitory activity of ≅75–85% was detected only in this fraction with a specific dose-response relationship. The purified protein appeared to be relatively homogeneous on 10% acrylamide gel with a mol wt of $\cong 2.5 \times 10^4$ daltons, as estimated by SDS gel electrophoresis and Sephadex G-100 chromatography. This finding is in agreement with Taylor et al. (25), Rocklin et al. (6), and Preece and Light (23), who detected MIF activity in partially purified supernates of activated lymphocytes in a similar region of the chromatogram. On SDS gels, B3 seems to be composed of two proteins; one with a mol wt of ≅25,000 daltons, and a small band with a mol wt of 34,000 daltons. In addition, fraction B3 is contaminated with low amounts of protein A, which is also found

as a major component in control supernates. Isoelectric focusing of fraction B3 resulted in two bands in the region of (pI) 3.5–4, which leads to the further possibility of microheterogeneity of MIF apart from the possibility of contamination and/or multiple subunits. As indicated earlier, the lymphocytes used for MIF production were purified on a Ficoll-Hypaque gradient. Despite careful washing of the cells, it seems likely that the supernates were still contaminated by serum proteins or hemoproteins, as mentioned by Bendtzen (8), and Geczy et al. (9). The existence of a protein in lymphocyte supernates in the mol wt range of ≅68,000 daltons was observed in both studies.

Isolation of specific antibodies was performed by initial preparation of a mixed population of antibodies against fractions B and removal of nonspecific antibodies by its selective absorption with proteins of control material. Although the titer of the antiserum was low, it was efficient enough to neutralize MIF activity in the capillary test and in the cell electrophoresis apparatus.

Our studies show that MIF is a relatively heat-stable protein since its incubation for 30 min at 56°C resulted in loss of no biological activity, although boiling for 20 min destroyed its activity completely (unpublished data). This is in agreement with earlier findings of Rocklin et al. (6). Our studies with purified MIF show its biological effect is caused by very small amounts of protein, as predicted by Sorg and Bloom (26) and Remold et al. (5). However, the relatively small yield of MIF (250 μg from Con A-stimulated lymphocytes, prepared from 80 liters of fresh human blood) could be the result of a loss of material during preparative procedures such as dialysis, and/or the result of heterogeneous origin of the human blood samples.

It was speculated by Preece and Light (23) and Field et al. (11) that the MSF produced from sensitized lymphocytes after incubation with the EF (24), and MIF may be the same substance. The dose-response relationship of purified MIF protein studied in the cell electrophoresis apparatus correlated very well with the previously reported dose-response relationship for MSF, studied by Carnegie et al. (27). A highly purified MSF preparation was detected in the same range of molecular weight where MIF activity was found (23). The cause for the slowing effect appears to be a change in the surface charge (24). Since MIF protein can cause the same effect in a comparable dose, it is likely that MIF is identical to MSF. Remold (28) found that during the early steps of MIF action, MIF appears to bind to the surface membrane of macrophages. This may indicate that MIF interacts with membrane receptors, which could change the surface charge of macrophages. Therefore, we suggest that the first step of MIF action could be a change in the surface charge of macrophages.

The availability of specific antibodies against human MIF will provide a possible tool for further studies on MIF and its mode of action. By using a senitive radioimmunoassay (studies in progress), precise quantitation can be achieved which should allow studies of its role in disease.

Summary

Using gel filtration and preparative isotachophoresis, the migration inhibitory factor (MIF) was highly purified from human lymphocytes activated with concanavalin A. MIF is an acidic protein with a mol wt of ≅25,000 daltons as

determined by gel filtration and analytical polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The protein inhibits migration of macrophages in the capillary test and in addition, has a slowing effect on the electrophoretic mobility of guinea pig peritoneal macrophages. Rabbit antibodies specific for this protein, as determined by immunochemical techniques, neutralized the biological effect of MIF on migration and on the electrophoretic mobility of macrophages.

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