Migration Enhancement Factor: A New Lymphokine
(lymphocytes/migration inhibitory factor)

RICHARD H. WEISBART, RODNEY BLUESTONE, LEONARD S. GOLDBERG*, AND CARL M. PEARSON

Department of Medicine, University of California at Los Angeles School of Medicine, Los Angeles, Calif. 90024; and the Medical and Research Services, Veterans Administration, Wadsworth Hospital Center, Los Angeles, California 90073

Communicated by Donald B. Lindsley, November 8, 1973

ABSTRACT Production of human migration inhibitory factor by lymphocytes exposed to antigen was studied at intervals over a 7-day period. Migration inhibitory factor was measured by an agarose gel method, with buffy-coat leukocytes as indicator cells. Lymphocyte supernatants from 7-day cultures consistently showed migration inhibitory factor activity; by contrast, enhancement of migration was frequently noted when effector cells were exposed to supernatants from 2- to 5-day cultures. Enhancement activity was manifested either by enhanced migration or by a sequential reduction in inhibitory activity consistent with a factor opposing the action of migration inhibitory factor. When supernatants were subjected to polyacrylamide gel electrophoresis, enhancement activity was regularly found in the beta-globulin region and migration inhibitory factor in the albumin fraction of the gel. The enhancement activity was heat-stable and nondialyzable. These findings characterize a hitherto unreported lymphokine, migration enhancement factor.

Sensitized lymphocytes exposed to specific antigen produce a number of humoral mediators or lymphokines, including migration inhibitory factor (MIF). MIF, a substance that inhibits the migration of phagocytic cells, has been studied extensively and appears to be an in vitro correlate of the delayed hypersensitivity response (1-3). On occasion, however, enhancement of migration rather than the anticipated inhibition has been observed in MIF test systems (4). Although attempts to consistently reproduce this phenomenon have been unsuccessful, certain studies have suggested that the appearance of enhanced migration may be related to the presence of minute concentrations of antigen (4). This enhancement phenomenon has not been further defined, but its presence is believed to represent antigen stimulation of sensitized lymphocytes. A factor that might be responsible for enhanced migration has, however, not been isolated from lymphocyte supernatants.

In this study, production of MIF by lymphocytes exposed to antigen was measured at intervals over a 7-day period. The lymphocyte supernatants consistently demonstrated the presence of an activity that competed with MIF and that enhanced migration. When lymphocyte supernatants were subjected to polyacrylamide gel electrophoresis, enhancement activity was detected in the beta-globulin region, whereas MIF was consistently found in the albumin region of the gel. These findings characterize a hitherto unreported lymphokine, migration enhancement factor (MEF).

METHODS AND MATERIALS

Antigens. Purified protein derivative (PPD) containing 50,000 tuberculin units per mg was provided by H. B. Delvin, Ph.D., Parke-Davis Co., Detroit, Mich. Coccidioidin, Lot no. XV 867F, was supplied by Milton Huppert, Ph.D., Veterans Hospital, Long Beach, Calif.; the antigen was prepared from 24 strains of Coccidioides immitis.

Lymphocyte Separation and Culture Techniques. Lymphocytes were isolated from human peripheral blood as described (5). After the cells were washed with Hanks' buffered salt solution (BSS), lymphocytes were cultured in the presence and absence of PPD (250 tuberculin units per ml) or coccidioidin (0.1 ml of a 1:1000 dilution per ml) as follows: one million lymphocytes were cultured for 7 days at 37° in 12 × 75-mm polypropylene tubes containing 1 ml of Medium 199 with 25 mM Hepes (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer and 2.2 g of NaHCO₃/liter (Grand Island Biological Co., Berkel-y, Calif.), 10% horse serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). After 7 days the tubes were centrifuged for 10 min at 2000 × g and the supernatants were decanted. Cells were viable at 7 days, as determined by trypan blue exclusion. After termination of the cultures, nonstimulated, i.e. antigen-free, supernatants were reconstituted with the same concentration of antigen that was used in the stimulated cultures.

MEF detection by the agarose system inhibits the migration of polymorphonuclear leukocytes. This factor may or may not be identical to MIF that inhibits migration of macrophages.

Abbreviations: MIF, migration inhibitory factor; MEF, migration enhancement factor; PPD, purified protein derivative; BSS, buffered salt solution; MI, migration index.

* To whom reprints should be addressed.
Individual cultures not

Lymphocytes of

Hanks' were

Supernatants were collected triplicate cultures from a PPD-sensitive subject and from a PPD-nonsensitive subject at day 7 and assayed simultaneously for MIF activity.

Polyacrylamide Gel Electrophoresis Studies. Lymphocytes were isolated from three subjects who gave positive PPD skin tests and from one subject (W.P.) who gave positive skin tests to both PPD and coccidioidin. Cells were cultured in the presence and absence of PPD for 7 days; in W.P., lymphocytes were also cultured with and without coccidioidin for 7 days. Lymphocytes from one of the PPD-sensitive subjects (R.O.) were also cultured in the presence and absence of PPD for 1 hr. The resultant supernatants were collected and subjected to polyacrylamide gel electrophoresis as described by Remold (8) except that riboflavin (0.4 mg) and bis-acrylamide (0.5 g/100 ml) (upper gel) were used. Glass tubes of identical size, 0.5 × 9.0 cm, served as gel-containing columns. The gel consisted of three parts: the running gel consisted of 1.3 ml of gel solution, the spacer gel of 0.3 ml, and the sample gel of 0.1 ml. After gel polymerization, 50 μl of nonstimulated and 50 μl of the PPD- or coccidioidin-stimulated supernatants were layered onto separate gel columns and subjected to

diated and the supernatants to be recovered at different intervals. In the initial experiments, supernatants were obtained at 24 hr and on days 2, 3, 4, 5, and 7. In subsequent experiments, single cultures were terminated at 1 hr, 6 hr, 18 hr, and on days 2, 3, 4, 5, and 7. Supernatants were tested for MIF activity in the agarose gel system. To ascertain the reproducibility of MIF production and detection in multiple supernatants, we collected triplicate cultures from a PPD-sensitive subject and from a PPD-nonsensitive subject at day 7 and assayed simultaneously for MIF activity.

Fig. 1. Production of migration inhibitory factor by lymphocytes in response to purified protein derivative. Multiple aliquots of lymphocytes were cultured with and without antigen, and individual cultures (stimulated and nonstimulated) were terminated at 1 hr to 7 days. Lymphocyte supernatants from two subjects not sensitive to purified protein derivative, R.W. (●—●) and C.C. (○—○), were assayed for migration inhibitory factor by the agarose gel method. Vertical lines, SEM.

Agarose Technique. Supernatants from lymphocyte cultures were replenished with an equal volume of Medium 199 with 10% horse serum before testing. Seven million buffy-coat cells were suspended in 0.2 ml of each replenished supernatant. The cell suspension was incubated at 37° for 30 min, and adjusted to 3 × 10⁶ leukocytes per ml. Five-microliter aliquots of the suspension were dispensed into four alternate wells. Tests were performed in quadruplicate on a single plate. Plates were incubated at 37° for 18 hr in a CO₂ incubator. The amount of migration, excluding the area of the well, was measured with an ocular micrometer. The migration index (MI) was calculated by dividing the mean area of migration in the presence of antigen-stimulated supernatants by the mean area of migration in the presence of nonstimulated supernatants. Standard error of the mean (SEM) of the MI was computed from the equation for SEM for x/y (7). P values for the difference between the migrations produced by stimulated and nonstimulated supernatants were determined by the Student’s t-test.

Time-Course Studies on MIF Production. Isolated lymphocytes obtained from seven PPD-sensitive and four PPD-insensitive subjects were cultured in the presence and absence of PPD (250 tuberculin units per ml). In one individual (S.N.), separate cultures were incubated with 250 and 500 tuberculin units per ml. Multiple cultures were prepared on each subject, thereby permitting single cultures to be termi-
Objects, showed remaining between studies no produced 7 Lymphocyte time-course Hanks' BSS and with Hanks' BSS. Tubing (24-A (8), electrophoresis and section C the albumin, contained 90 for amido black. 199 and for migration inhibitory factor by the agarose gel method.

electrophoresis simultaneously. A third gel containing Medium 199 and 10% horse serum was used as a control and stained with amido black. A current of 2.0 mA per column was applied for 90 min. Gels were sectioned into equal portions; section A contained albumin, section B contained the beta-globulins, and section C the gamma globulins. Gels were eluted at 4° by electrophoresis (8), and the eluates were dialyzed in dialysis tubing (24-Å pore size) for 24 hr at 4° against three changes of Hanks' BSS. The volume of eluate was adjusted to 0.3 ml with Hanks' BSS and stored at -20° until assayed in the agarose system.

Properties of Activities Within Eluates. Selected eluates were subjected to 56° for 30 min and/or to extensive dialysis for 24 hr and retested in the agarose system. The activities of certain eluates were again assayed after storage at -20° for periods up to 10 months.

RESULTS

Time-course studies (1 hr to 7 days) on MIF production

Lymphocyte supernatants studied at intervals between 1 hr and 7 days in four subjects with negative PPD skin tests produced no significant alteration of migration. The time-course studies on two of these subjects are depicted in Fig. 1; the remaining two individuals showed similar results. Lymphocyte supernatants from seven PPD-sensitive individuals showed several different patterns of response. In three subjects, enhancement of migration was initially observed between 1 hr and 2 days with peak enhancement migration indexes of 1.18 ± 0.07 (P < 0.05) (P.B.), 1.20 ± 0.04 (P < 0.01) (R.O.), and 1.09 ± 0.03 (P < 0.05) (G.D.). Inhibition of migration followed between 2 and 4 days with MI of 0.80 ± 0.05 (P < 0.02) (P.B.), 0.87 ± 0.05 (P < 0.01) (R.O.), and 0.55 ± 0.02 (P < 0.001) (G.D.). In two of these subjects (R.O. and G.D.) there ensued a period of decreasing inhibition at days 4 or 5 with MI of 1.00 ± 0.00 (R.O.) and 1.00 ± 0.00 (G.D.), but all subjects showed significant inhibition at day 7 with MI of 0.71 ± 0.00 (P < 0.001) (P.B.), 0.63 ± 0.04 (P < 0.001) (R.O.), and 0.55 ± 0.02 (P < 0.001) (G.D.). Fig. 2 shows the results of the sequence studies on P.B. and R.O. G.D. gave results similar to those of R.O. except that the 1-hr supernatant did not alter migration (MI of 1.00 ± 0.04). In three other individuals with positive skin tests, inhibition of migration was the initial response between 18 and 24 hr with MI of 0.57 ± 0.03 (P < 0.001) (E.S.), 0.68 ± 0.00 (P < 0.001) (S.N.), and 0.69 ± 0.04 (P < 0.001) (L.P.). This was followed by decreasing inhibition between 2 and 5 days with MI of 0.86 ± 0.00 (P < 0.001) (E.S.), 1.00 ± 0.00 (S.N.), and 1.00 ± 0.04 (L.P.). Inhibition of migration increased again by 7 days with MI of 0.57 ± 0.00 (P < 0.001) (E.S.), 0.78 ± 0.00 (P < 0.001) (S.N.), and 0.72 ± 0.03 (P < 0.001) (L.P.). Fig. 3 shows the results for E.S. and S.N.; L.P. gave results intermediate between those of E.S. and S.N. The 1-hr and 6-hr results for L.P. showed no significant alteration of migration with MI of 0.97 ± 0.05 and 0.95 ± 0.04, respectively. The 18-hr sample gave an MI of 0.77 ± 0.04 (P < 0.01). In one PPD-sensitive subject (D.C.) no significant alteration of migration was evident from 1 hr to 2 days. Inhibition of migration appeared at day 3 with MI of 0.86 ± 0.02 (P < 0.05) and was greatest at day 7 with MI of 0.81 ± 0.04 (P < 0.01). Thus, the whole supernatants of six of the seven PPD-sensitive subjects appeared to show enhancement of migration at certain times over a 7-day course. Enhancement was manifested either as a MI of significantly greater than 1.00 or as a sequential diminution in inhibitory activity consistent with the presence of a factor opposing the action of MIF.

Fig. 3. Production of migration inhibitory factor by lymphocytes in response to purified protein derivative. Multiple aliquots of lymphocytes were cultured with and without antigen, and individual cultures (stimulated and nonstimulated) were terminated at 1 hr to 7 days. Lymphocyte supernatants from two subjects sensitive to purified protein derivative, E.S. (————) and S.N. (———O), were assayed for migration inhibitory factor by the agarose gel method.

Fig. 4. Polyacrylamide gel of lymphocyte supernatant. Lymphocytes from a subject sensitive to purified protein derivative were cultured with and without antigen for 7 days. Lymphocyte supernatants were collected and subjected to polyacrylamide gel electrophoresis. Gels were divided into three equal portions (A, B, and C) and eluates were obtained from each section. Eluates were assayed in the agarose gel method.
The MI of triplicate supernatants from a PPD-sensitive individual (S.N.) at 7 days were: 0.82 ± 0.04 (P < 0.01), 0.78 ± 0.00 (P < 0.001), and 0.85 ± 0.03 (P < 0.01). The MI of triplicate supernatants from a PPD-insensitive individual showed no significant alteration of migration. The results, which do not differ significantly from one another, demonstrate the reproducibility of both the MIF production in multiple cultures and the agarose system. Moreover, in one subject (S.N.), time-course studies performed on separate cultures with 250 and 500 μg/ml of PPD showed comparable results.

**Polyacrylamide Gel Studies.** Supernatants obtained at 7 days from the cultures of four PPD-sensitive individuals and one coccidioidin-sensitive individual were subjected to gel electrophoresis (Fig. 4). In all but one instance, enhancement activity was detected in the eluates obtained from the beta-globulin portion of the gels. The single exception was the supernatant from 1-hr and 7-day cultures of subject R.O.; enhancement activity was found in the gamma globulin fraction when these supernatants were subjected to polyacrylamide gel electrophoresis. The MI in response to PPD were 1.38 ± 0.06 (P < 0.001) (D.F.), 1.30 ± 0.10 (P < 0.02) (W.P.), 1.32 ± 0.09 (P < 0.01) (R.O.), and 1.47 ± 0.06 (P < 0.001) (D.C.). Enhancement activity was detected in the eluate from the 7-day supernatant of subject D.C., the only individual whose time-course study failed to show the presence of this activity. In response to coccidioidin, the eluate from the supernatant of subject W.P. gave an MI of 1.16 ± 0.07 (P < 0.05). MIF, produced in response to PPD, was uniformly found in the eluates from the albumin section of the gels. The MI were 0.58 ± 0.03 (P < 0.001) (D.F.), 0.74 ± 0.04 (P < 0.01) (W.P.), 0.81 ± 0.05 (P < 0.02) (R.O.), and 0.89 ± 0.05 (P < 0.05) (D.C.). MIF produced in response to coccidioidin also migrated to the albumin portion of the gel (MI 0.77 ± 0.06, P < 0.02). In one of these subjects (R.O.), a supernatant obtained at 1 hr was subjected to electrophoresis and enhancement activity was eluted from the gamma globulin region of the gel with a MI of 1.21 ± 0.04 (P < 0.01); however, no MIF activity was uncovered in the albumin fraction (MI of 1.00 ± 0.05). The 7-day supernatant from R.O. showed enhancement activity in the gamma globulin eluate with a MI of 1.32 ± 0.09 (P < 0.01) and MIF in the albumin region with a MI of 0.81 ± 0.05 (P < 0.05).

**Other Properties of Eluates with Enhancement Activity.** In two subjects (D.C. and W.P.), selected eluates with enhancement activity were tested before and after heating at 56° for 30 min. Before heating, the MI were 1.19 ± 0.05 (P < 0.01) and 1.19 ± 0.04 (P < 0.01), respectively. After heating, the MI were 1.23 ± 0.06 (P < 0.01) and 1.25 ± 0.05 (P < 0.01), respectively. No significant alteration of the enhanced response resulted from heating at 56° for 30 min.

The MI for five eluates from polyacrylamide gels are listed in the section on polyacrylamide gel studies; all showed significant enhancement of migration. Each of these eluates was extensively dialyzed before it was assayed. The persistence of significant enhancement activity after the dialysis indicated that this factor was nondialyzable.

Enhancement activity was consistently demonstrated in supernatants stored at −20° for as long as 5 months. However, supernatants stored at −20° for 8 months from one individual appeared to lose all enhancement activity. In contrast, all supernatants stored at −20° for as long as 10 months retained full MIF activity.

**DISCUSSION**

This report describes a new lymphokine, termed migration enhancement factor (MEF). MEF enhanced the migration of phagocytic cells, and was consistently found in supernatants from lymphocytes exposed to specific antigen. Furthermore, MEF activity was partially isolated by polyacrylamide gel electrophoresis and was distinct and separate from MIF. Like MIF, MEF appeared to be heat-stable and nondialyzable.

The recognition of MEF resulted from our previous attempts to standardize the agarose gel technique for the detection of MIF. In an earlier report from this laboratory (6), it was shown that discrimination between PPD-sensitive and -nonsensitive individuals was maximized by assaying the supernatants from 7-day lymphocyte cultures. By contrast, in most methods described for the detection of MIF, supernatants are assayed from 1- to 3-day cultures (1, 9). These contrasting findings prompted the investigation of the time-course of MIF production and release. In the present report, lymphocytes were cultured with antigen for periods of 1 hr to 7 days and the supernatants were tested in the agarose system. The results indicated that MEF appeared to be produced as early as 1 hr and that MEF activity seemed to peak between 3 and 5 days; however, MIF activity invariably predominated at day 7. Curves constructed from the time-sequence studies were consistent with the presence of two substances with opposing activities on cell migration, and the isolation of these separate activities by polyacrylamide gel electrophoresis confirmed this interpretation.

Both MIF and MEF were consistently present and distinguishable by polyacrylamide gel electrophoresis of 7-day culture supernatants. In subject R.O., the 1-hr supernatant, which induced enhanced migration, yielded only MEF but not MIF on polyacrylamide gel electrophoresis; the 7-day supernatant, which produced inhibition of migration, contained both MIF and MEF when studied by gel electrophoresis. MEF recovered from the 1-hr and 7-day supernatants appeared in the same fraction of the gel. These findings support the contention that the alteration in the MI observed from 1 hr to 7 days resulted from the opposing action of two distinct lymphokines.

Considerable controversy exists regarding the reproducibility of various methods currently used to detect MIF. Moreover, enhancement of migration is occasionally observed instead of the anticipated inhibition. The data presented here tend to explain the variability inherent in the various MIF assays and to account for the appearance of enhanced migration on occasion instead of inhibition of migration. It would appear that production of MEF like MIF is the result of lymphocyte stimulation by specific antigen.

This study also demonstrates the need to assay lymphocyte cultures at different time intervals in order to consistently detect MIF and MEF. Two separate factors with opposing activities may obscure the presence of either activity. Although MIF activity was uniformly observed in 7-day cultures, the detection of MEF required sequential analysis of lymphocyte supernatants. However, in one subject (D.C.), MEF was not detected, even though sequential analysis of multiple supernatants had been performed. Therefore, accurate detection of both MIF and MEF may be possible only
by electrophoretic separation of these factors. Distinguishing these separate and opposing activities is an important first step in defining their biological functions.

Jo Ellen Cunningham, Beverly Sullivan, Antoinette Jelusich, and Judy Isaacson provided skilled technical assistance. This work was supported by grants from the United States Public Health Service (AM CA 15220-01 and GM-15759), the Veterans Administration and the Kroc Foundation. R.H.W. is a Research and Education Associate of the Veterans Administration.


6. Weisbart, R. H., Cunningham, J. E., Bluestone, R. & Gold- 


7. Dahlberg, G. (1940) Statistical Methods for Medical and Bio-


tical Students (George Allen and Unwin Ltd., London), 95 pp.