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Chemotaxis of Purified Human Monocytes In Vitro: Lack of Accessory Cell Requirement

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This study was undertaken to determine whether cell cooperation, either among monocytes or between monocytes and lymphocytes, is a prerequisite for monocyte chemotactic responsiveness. We compared Ficoll-Hypaque-separated mononuclear cells and a preparation of 99% pure monocytes obtained by chemotaxis in a newly designed separation chamber. Monocytes of both preparations migrated to chemoattractants without a lag phase, and no further increase in migrated cells was observed after 70 min. The cell dose-response was linear for both preparations over a wide range of cell concentrations in the cell input well of the chemotaxis chamber, suggesting that no monocyte-monocyte interaction was required. Since only 20 to 60% of the monocytes purified by chemotaxis migrated a second time, the possibility of a requirement for an accessory cell was tested. The addition to purified monocytes of several different mononuclear cell preparations comprising lymphocytes or nonmigrating monocytes had no effect on monocyte migration. These experiments show that normal human blood monocytes in vitro do not require stimuli from other cells to respond to chemoattractants. Their behavior is profoundly different from that of mouse peritoneal macrophages, which exhibit a time lag in vitro before migration toward an attractant and become more responsive with either increasing cell concentration or addition of purified lymphocytes.

Macrophages play an important role in the immunological response as both amplifying cells and effector cells. Peripheral blood monocytes are precursors of these macrophages, and their ability to migrate may be essential for accumulation at inflammation sites. Monocytes from patients with a variety of diseases, including cancer, exhibit abnormal chemotaxis in vitro. Although the nature of monocyte chemotaxis defects in disease is still unknown, the literature suggests several interesting possibilities: (i) circulating serum inhibitors of chemotaxis, (ii) specific deactivation, and (iii) requirement for an accessory cell. Investigators have found inhibitors of chemotaxis in serum (for review, see reference 20) or have isolated inhibitors of chemotaxis released by neoplastic cells (15, 17, 18). The possibility of specific deactivation in disease was suggested by the finding that only 50% of the patients with a reduced response to lymphocyte-derived chemotactic factor also had impaired responses to endotoxin-activated serum (3). In studies on normal human monocytes, we showed that the cells capable of migrating into a chemotactic gradient are a subpopulation comprising 20 to 40% of the total blood monocytes (8). They carry multiple chemotaxin specificities on their surfaces, as shown by the fact that a suppressed response can be induced by high concentrations of one chemoattractant without alteration of responses to a different attractant (9). Thus, receptor-specific inhibition of monocyte chemotaxis can be demonstrated in vitro. The possibility of an accessory cell affecting monocyte chemotactic responsiveness is usually not considered, and it has been shown that, over a wide range of lymphocyte-monocyte ratios, chemotaxis depended only on the number of monocytes (2, 8, 19). However, Norris et al. showed that the depressed monocyte response of patients with mycosis fungoides was partially restored by lymphocytes. Furthermore, we reported that the chemotactic responsiveness of mouse macrophages is increased by the addition of autologous lymphocytes (13), and evidence for cell-cell interaction in the chemotactic response has been obtained for both mouse peritoneal macrophages (13) and cell lines (1). These findings stimulated us to determine whether there is any evidence for cell-cell interaction in the chemotactic responses of normal human monocytes. The methods included analysis of the time course of the chemotactic response and development of a new technique for obtaining suspensions of pure monocytes to which other cell fractions could be added.

MATERIALS AND METHODS

Cell preparation. Blood was drawn from healthy donors and heparinized. After isolation of mononuclear cells by the method of Boyum (6), the interface cell layer was removed and washed twice with Gey balanced salt solution containing 2% bovine serum albumin, Cohn fraction V (Gey-BSA; National Institutes of Health Media Unit, Bethesda, Md.). The cell suspension contained 15 to 35% monocytes, 65 to 85% lymphocytes, and less than 1% granulocytes. Viability was better than 99% as measured by trypan blue dye exclusion. Total and differential counts were made for the final washed preparations. Total leucocyte recovery was 1×10^6 to 3×10^6 cells per ml of whole blood. Differential counts were made after staining cell suspensions with euchrysin 3RX (Roboz Surgical Instrument Co., Inc., Washington, D.C.). To 50 μl of the cell suspension with a monocyte concentration of about 2×10^6 /ml, 10 μl of a euchrysin solution in phosphate-buffered saline (PBS; 0.3 mg/ml) was added, and the mixture was incubated at room temperature for about 5 min. Ten microliters was placed on a microscope slide, a cover slip was added, and the cells were observed with a fluorescence microscope. Monocytes were distinguished by bright red cytoplasmic fluorescence, and the numbers thus determined were used to standardize the monocyte number added per well. The results of this method correlated closely with the counting of monocytes by other criteria: (i) morphology as determined on cytocentrifuged preparations stained with Diff-Quick (Harleco, Philadelphia, Pa.); (ii) peroxidase staining; and (iii) phagocytosis of sheep erythrocytes sensitized with immunoglobulin G anti-Forsmann antibody, 90 to 95% of the monocytes, by morphological criteria, ingesting erythrocytes. Percoll (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) was also used to isolate monocytes as described by Pertoff et al. (16). Briefly, an isotonic stock solution of Percoll in PBS was prepared with a density of 1.024 at 22°C, measured with density marker beads (Pharmacia Fine Chemicals, Inc.). Ficoll-Hypaque-separated mononuclear cells at a concentration of 2×10^6 to 10×10^6 cells per ml in 7 ml of PBS were underlayered with 3 ml of Percoll stock solution diluted 1:1 with PBS. After centrifugation for 60 min at $800 \times g$, the cell band on the Percoll cushion was removed with a pipette. The cells were washed once with Gey-BSA. The purity of this cell suspension was between 84 and 94% as determined by morphological and phagocytic criteria. The contaminating cells were lymphocytes. The monocyte yield in the band was between 30 and 70%. Suspensions enriched for lymphocytes were prepared in two ways. Cells were incubated in a petri dish for 1 h at 37°C in moist air. The dish was then gently shaken, and the nonattached cells were poured off and washed once in medium. The remaining monocyte contamination was about 2%. In other assays, the mononuclear cell suspension was diluted to 2×10^6 cells per ml in Gey-BSA, and a carrageenan (Sea Kem 9; Marine Colloids, Rockland, Maine) stock solution was added to a final concentration of 50 μg/ml to cause destruction of monocytes (12). This suspension was incubated for 2.5 h with frequent mixing; the cells were then centrifuged, washed twice in medium, and then suspended in the appropiate volume of Gey-BSA. The purity of the lymphocytes was 98%.

Chemotaxis assay. Chemotaxis was assayed in a multiwell chamber as described previously (7). Bottom wells were filled with 25 μl of attractant solutions in Gey-BSA. A Nuclepore filter sheet (Polyvinylpyrrolidone coated, 10 μm thick, 5 μm hole size; Neuro Probe Inc., Bethesda, Md.) was placed over the wells, providing a surface area of 8 mm^2 per well. Gasket and top plate were assembled, and 50 μl of cell suspension containing 40,000 to 100,000 monocytes, depending on the experiment, were added to each top well. Chambers were incubated for 90 min in moist air containing 5% carbon dioxide. After incubation, chambers were disassembled and filters were removed. Cells remaining on the top side were wiped off, and filters were immersed in methanol for 15 s and then stained in Diff-Quick (Harleco). The number of cells per square millimeter (between 1,000 and 3,000) was counted with an image analyzer. With optimal concentrations of *N*-formylmethionyl-leucyl-phenylalanine or C5a, 20 to 40% of the monocytes migrated toward attractant, depending on the donor (8). Assay points were in triplicate, and the standard error of the mean was not greater than 15%.

Chemotactic factors. *N*-Formylmethionyl-leucyl-phenylalanine was purchased from Sigma Chemical Co., St. Louis, Mo. Stock solutions (10^{-3} M) were prepared in either ethanol or dimethylsulfoxide and stored at −20°C. Human serum-derived complement component C5a was prepared by a modified procedure of Fernandez and Hugli (10) as described previously (8). Briefly, fresh serum was incubated with zymosan (Mann Research Laboratories, New York, N.Y.) in the presence of epsilon-aminocaproic acid (Sigma Chemical Co.). C5a was then purified by consecutive chromatography on CM Sepharose Cl 6B (Pharmacia Fine Chemicals, Inc.) and Sephadex G-100. The final preparation was stored as a solution in PBS at −20°C. Lymphocyte-derived chemotactic factor was prepared as described previously (8). N-Formyl-norleucyl-leucyl-phenylalanine (FNLP) was a generous gift from Elliot Schiffmann.

Cell separation by chemotaxis. To separate migrating from nonmigrating monocytes, a newly constructed separation chamber was used (Neuro Probe Inc.). As in our 48-well chamber (7), upper and lower compartments were separated by a Nuclepore membrane filter (25 by 80 mm), but the area was divided into only four big compartments. The total filter area available for chemotaxis was 1,350 mm^2 (equal to the filter area of 170 wells of the multiwell chamber). The volumes of each of the four bottom and top compartments were 1.3 and 2.0 ml, respectively. The bottom wells were filled with attractant solution; the top wells were filled with a cell suspension containing not more than 2×10^6 monocytes per ml. The chamber was then incubated for 90 min at 37°C in moist air containing 5% CO_2. After incubation, the cells on the top of the filter were collected by rinsing with a pipette. The filter top was then wiped with a cotton swab to eliminate residual cells. The filter was removed and clamped to the inside of a 250-ml polypropylene tube with its neck cut off (no. 25350; Corning Glass Works, Corning, N.Y.), cell side exposed. The migrated cells were rinsed off with medium, washed once to remove traces of chemotactic factor, and counted. The purity of the migrated monocytes was better than 99%. The yield was 20 to 40% of the input monocytes, reflecting the fact that 20

to 40% of the input monocytes can migrate. The total cell yield (top and bottom cells) was between 75 and 90%, depending on the experiment. No trypan blue-positive cells were found.

RESULTS

Cell separation by chemotaxis. To determine whether other leukocytes affected monocyte chemotaxis, it was necessary to obtain pure monocytes. Previously, no methods were available to prepare pure monocyte suspensions suitable for chemotaxis experiments. In these studies, we designed a separation chamber (see Materials and Methods) to obtain purified monocytes by chemotaxis. This method was based on the fact that, with Ficoll-Hypaque-separated cells (comprising monocytes, lymphocytes, and approximately 0.5% neutrophils), the cells migrating toward attractant through the pores of a Nuclepore filter were exclusively monocytes. The few neutrophils that migrated fell off the polyvinylpyrrolidone-coated filters (11). Thus, after 90 min of incubation, the attractant side of the filter was covered with monocytes which were rinsed off relatively easily. No residual cytoplasmic material was seen on the filter after staining. No trypan blue-positive cells were seen, and no gross morphological changes were detected. Typically, 20 to 40% of the input monocytes were recovered from the filter bottom. This percentage corresponds to that of migration in normal individuals in vitro (8). The attractants used for these separations were FNLP at a concentration of 2×10^{-7} M and C5a at a dilution of 1:500. Both concentrations were optimal for chemotaxis, and the effects of specific deactivation under these conditions were small (10 to 15%).

Time course of chemotaxis. To determine whether the removal of lymphocytes had any effect on the time course of monocyte chemotaxis, Ficoll-Hypaque-separated mononuclear cells (approximately 75% lymphocytes and 25% monocytes) and monocytes purified by chemotaxis were compared. One multiwell chamber was set up for each time point. All chambers were put in the incubator at time zero. At the times indicated in Fig. 1 and 2, chambers were removed and migrated cells were counted. As shown in the figures, chemotaxis was complete in both cases after 70 min, and the shapes of the curves were essentially the same. Extrapolation of the curves shows that they intersect the abscissa at about 10 min. Thus, the cells exhibited no time lag before migrating, considering that they needed approximately 5 to 10 min to sediment to the filter surface. The absence of a lag phase suggests that little or no preparatory time was necessary, in contrast to mouse macrophages (4, 13). The time course of the response

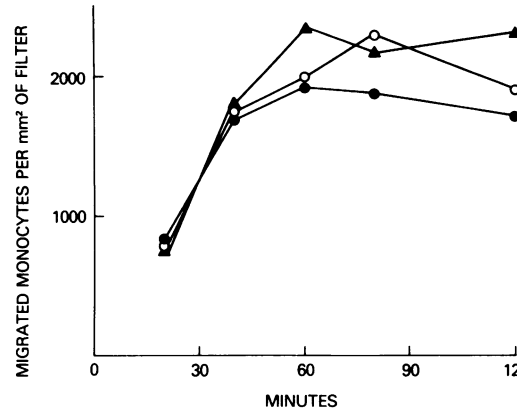

FIG. 1. Time course of chemotaxis of Ficoll-Hypaque-separated human monocytes. Cells were prepared as described in the text. One multiwell chamber was set up for each time point. Monocyte input number was 50,000 per well. All chambers were started at time 0 and removed from the incubator at the indicated times. The filters were immediately prepared for counting. The numbers represent the mean of triplicate determinations. Symbols: ○, C5a (1:800 dilution of stock); ●, lymphocyte-derived chemotactic factor (1:10 dilution); ▲, FNLP (2×10^{-7} M).

was the same for each of the three attractants. We have shown previously that, although the different attractants interact with functionally distinct receptors, the same cell population re-

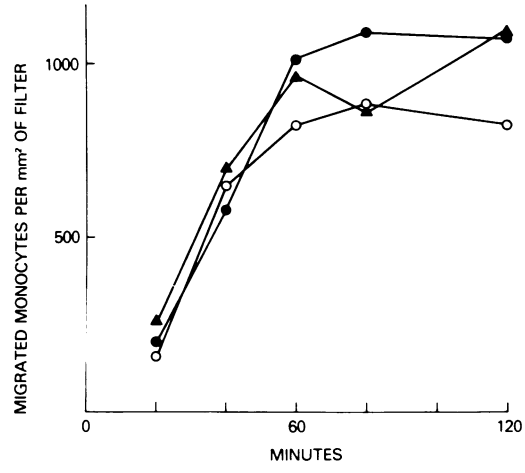

FIG. 2. Time course of chemotaxis of migrated monocytes. Migrated monocytes were separated from Ficoll-Hypaque cells in a separation chamber with 2×10^{-7} M FNLP used as attractant in the bottom compartment. After incubation, the cells were recovered from the attractant side of the filter and used as migrated monocytes. The assay was done as described in the legend to Fig. 1. Monocyte input number was 50,000 per well. ●, Lymphocyte-derived chemotactic factor (1:10 dilution); ○, C5a (1:800 dilution); ▲, FNLP (2×10^{-7} M).

sponds to each attractant. The concentrations used in the figures were optimal. In other experiments with suboptimal concentrations, the curve shape was similar, except for a lower plateau after 70 min.

Dependence of chemotaxis on cell density. If cell cooperation either between different cells or among the monocytes themselves was a prerequisite for chemotaxis, the percentage of input cells that migrate should increase over a certain range of increasing cell input number, as we have shown for mouse peritoneal macrophages (13) and cell lines (1). On the other hand, our published cell dose-response curves for Ficoll-Hypaque-separated unpurified human mononuclear cells were linear over a wide range, showing that the percentage of input monocytes migrating was constant (7, 8). Therefore, in the case of human monocytes, either no cell cooperation occurs or the accessory cell is present in excess over the entire range of the cell dose-response. The cell dose-response curve was also linear for monocytes purified by chemotaxis (Fig. 3). This was true for all attractants used and for monocytes purified on Percoll. The log-log plot lines have a slope of 1.0, indicating that the fraction of cells migrating was independent of the input cell number. This shows that no monocyte-monocyte interaction occurred over the observed range. If an accessory cell had been removed during purification, the cell dose-response should still be linear, but the percentage migrating would be less. In fact, only 20 to 60% of the chemotaxis-purified monocytes migrated to attractant, despite the fact that 100% of them had migrated in the purification procedure. Less than 100% migration was not due to specific deactivation, since similar responses were seen for three unrelated attractants. Furthermore, in separate deactivation experiments, little or no specific deactivation was observed with attractant concentrations used for monocyte separation, i.e., 2×10^{-7} M FNLP or 1:500 C5a. Other causes for suboptimal migration of separated monocytes could be an intrinsic decline in the functional capacity of the monocytes or the removal of an accessory cell. The latter possibility was evaluated as described below by determining whether the addition of various cell populations to the purified monocytes increased their chemotactic responsiveness.

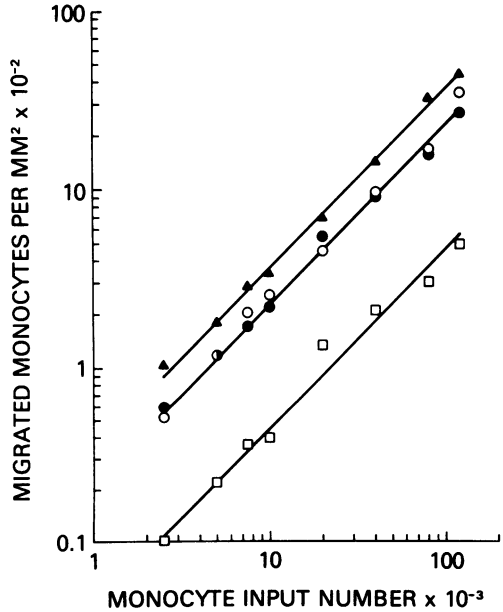

FIG. 3. Cell dose-response of migrated monocytes. Migrated monocytes were prepared as described in the legend to Fig. 2. A log 2 dilution series of the cells in Gey-BSA was prepared, and chemotaxis was measured in multiwell chambers in duplicate. The reagents in bottom wells were: lymphocyte-derived chemotactic factor, 1:10 (▲); FNLP, 2×10^{-7} M (○); C5a, 1:800 (●); Gey-BSA (□).

Recombination experiments. To determine whether lymphocytes or nonmigrating monocytes affected monocyte chemotactic responses, we prepared the following cell suspensions. Cells recovered from the top of a separation chamber filter after all of the chemotactically responsive monocytes had migrated to the bottom of the filter were used in three different ways: (i) untreated, comprising lymphocytes and nonmigrating monocytes; (ii) treated by adherence to plastic, the nonadherent cells comprising lymphocytes and residual (approximately 4%) monocytes; and (iii) treated with carrageenan, which destroyed monocytes but did not affect lymphocytes (12). These cells included plastic adherent lymphocytes that were not present in the second preparation. To a constant number of purified monocytes, increasing numbers of these cell fractions were added, and the suspensions were incubated for 10 min before they were pipetted into the chemotaxis wells. The nonadherent cell fraction neither enhanced nor suppressed chemotaxis (Table 1). To test the possibility that adherent lymphocytes could affect monocyte chemotaxis, cells from the top of the separation chamber were treated with carrageenan for 2.5 h to destroy nonmigrated monocytes. Only 2% of the monocytes remained intact. Lymphocytes were not damaged, as determined by their response to three different mitogens. [^{3}H]thymidine uptake after the addition of concanavalin A, phytohemagglutinin, or lipopolysaccharide was 160, 130, and 80%, respectively, of the response by mononuclear cells not treated with carrageenan. A recombination assay identical to that shown in Table 1 was performed and yielded the same result (Table 2).

TABLE 1. Effect of nonadherent cells on chemotaxis of monocytes purified by chemotaxis[a]

No. of nonadherent cells per well[b]	Chemotactic response to[c]:		
	Medium	FNLP	C5a
0	256 ± 12	1,960 ± 175	2,290 ± 285
5,000	224 ± 17	1,980 ± 190	2,315 ± 150
10,000	317 ± 25	2,050 ± 130	2,260 ± 210
20,000	202 ± 10	1,850 ± 45	2,370 ± 280
40,000	230 ± 25	2,230 ± 280	2,400 ± 125
80,000	270 ± 45	1,770 ± 210	2,530 ± 70

[a] Migrating monocytes were separated from the Ficoll-Hypaque mononuclear cell mixture by chemotaxis to C5a. The effect of nonadherent cells on the chemotactic response of the separated monocytes was determined in a multiwell chamber by adding 40,000 separated monocytes per well plus nonadherent cells as indicated.

[b] Cells that were recovered from the top well of a separation chamber after chemotaxis were incubated for 30 min in a culture dish in Gey-BSA. Nonadherent cells were then poured off, counted, and used. Cells were mixed, preincubated for 10 min, and then assayed.

[c] Number of cells migrated per square millimeter of filter ± standard error of the mean. FNLP and C5a were used at concentrations of 2×10^{-7} and 1:500, respectively.

In a third type of recombination assay, cells that were recovered from the top of the separation chamber filter were added back to the migrated cells without any further treatment. There was no effect on monocyte migration (Table 3). Other recombination experiments were carried out at lower monocyte densities (20,000 per well) and higher densities (80,000 per well). An effect of added lymphocytes was not seen. These experiments show that there is no accessory cell in the nonmigrated population that enhances monocyte responsiveness.

All of the above experiments were also done with monocytes separated on a Percoll cushion (16). The highest purity we achieved was 94% at a rather low yield (30%). The results of adding lymphocytes (in the form of carrageenan-treated cells or nonadherent cells) to this preparation were comparable to those shown in Tables 1 and 2.

DISCUSSION

The experiments reported in this paper were designed as a result of our finding that accessory cells play a role in the chemotaxis of mouse macrophages in vitro. For both mouse resident peritoneal macrophages (13) and mouse macrophage-like cell lines (1), the percentage of cells that migrated increased with cell density. Furthermore, addition of lymphocytes to a constant number of mouse peritoneal cells in the chemo-

TABLE 2. Effect of lymphocytes on chemotaxis of monocytes purified by chemotaxis[a]

No. of lymphocytes per well[b]	Chemotactic response to[c]:		
	Medium	FMLP	C5a
0	156 ± 13	2,075 ± 50	2,360 ± 390
5,000	140 ± 20	1,890 ± 100	2,360 ± 340
20,000	135 ± 5	1,890 ± 60	2,140 ± 185
80,000	150 ± 5	2,170 ± 160	2,260 ± 210

[a] Migrating monocytes were separated by chemotaxis to C5a. The effect of lymphocytes on the chemotactic response of the separated monocytes was determined in a multiwell chamber by adding 40,000 separated monocytes per well plus lymphocytes as indicated.

[b] Lymphocytes were prepared from nonmigrating cells recovered from the top well of a separation chamber after chemotaxis. They were incubated for 2 h in Gey-BSA containing 50 μg of carrageenan per ml to destroy monocytes. The cells were then washed twice and counted. The monocyte contamination was less than 2%. Lymphocytes incorporated [^{3}H]thymidine upon stimulation with B- or T-cell mitogens. Cells were mixed, preincubated for 10 min, and then assayed.

[c] Number of cells migrated per square millimeter of filter ± standard error of the mean. *N*-Formylmethionyl-leucyl-phenylalanine (FMLP) and C5a were used at concentrations of 2×10^{-7} M and 1:500, respectively.

TABLE 3. Effect of a lymphocyte-nonmigrated monocyte cell mixture on chemotaxis of purified monocytes[a]

No. of lymphocytes per well[b]	Chemotactic response to[c]:		
	Medium	FNLP	C5a
0	300 ± 30	1,950 ± 65	2,080 ± 105
5,000	150 ± 35	1,815 ± 90	1,900 ± 85
10,000	200 ± 25	1,850 ± 180	1,850 ± 330
20,000	220 ± 25	1,490 ± 110	2,160 ± 320
40,000	295 ± 40	1,870 ± 260	2,120 ± 115
80,000	250 ± 20	2,110 ± 290	2,200 ± 230

[a] Migrating monocytes were separated by chemotaxis to C5a. The effect of the lymphocyte-nonmigrated monocyte cell mixture on the chemotactic response of separated monocytes was determined in a multiwell chamber by adding 40,000 separated monocytes per well plus the lymphocyte-monocyte mixture as indicated.

[b] A cell mixture consisting of nonmigrated monocytes (25%) and lymphocytes (75%) was recovered from the top well of a separation chamber after chemotaxis. Cells were mixed, preincubated for 10 min, and then assayed.

[c] Number of cells migrated per square millimeter of filter ± standard error of the mean. FNLP and C5a were used at concentrations of 2×10^{-7} and 1:500, respectively.

taxis chamber increased the number of macrophages that migrated (13).

In contrast, we reported previously that the number of human monocytes migrating to chemoattractant was proportional to the number of input monocytes. Thus, the percentage of input monocytes that migrated was independent of cell density and remained constant over a wide range of input cell numbers. In addition, over a twofold range of lymphocyte numbers, monocyte chemotaxis did not depend on the lymphocyte/monocyte ratio (8). This was also shown by others using different methods (2, 19). The possibility still remained that chemotaxis responsiveness of normal human monocytes is affected by lymphocytes but that the number required is smaller than the number present at the lowest lymphocyte/monocyte ratio in previous experiments. Also, Norris et al. showed that the defective monocyte chemotaxis of patients with mycosis fungoides was partially restored by addition of lymphocytes from normal donors (14).

We evaluated this point in studies on monocytes purified in a chemotaxis separation chamber, after which the cell suspension comprised 99% monocytes. Since only 20 to 60% of these cells migrated to chemotaxins, we tested the effect of adding various mononuclear cell preparations on chemotactic responsiveness. There was no effect on the migration of the monocytes (Tables 1–3). Thus, the capacity of human monocytes from normal donors to migrate in a chemotactic gradient does not appear to require signals in vitro from enhancing or suppressing cells.

Our studies suggest that one can distinguish two distinct groups of mononuclear phagocytes with respect to chemotactic responses: monocyte type, characterized by no time lag before migration toward attractant and no effect of cell concentration on chemotactic response; and resident peritoneal macrophage type, characterized by a time lag before response to attractant occurs and an increase in the percentage of cells migrating when the input cell concentration is increased. Human blood monocytes are in the first category. Except for the time required for settling of the cells, they were capable of an immediate response to attractant, and the rate at which the cells accumulated on the attractant side of the filter was nearly constant until the plateau was reached (Fig. 1 and 2). Also, changing the cell concentration did not change the percentage of monocytes that migrated (Fig. 3). Mouse blood monocytes are in this category in that they exhibited no time lag in response to attractant (5). The mouse monocyte-like cell line WEHI-3 also behaved like the human blood monocyte, exhibiting neither a time lag nor an increased chemotactic response with increased input cell concentration (1). In the resident peritoneal macrophage category are mouse resident macrophages (13) and mouse macrophage-like cell lines (1). They exhibit a time lag and require a minimum critical cell concentration in the upper well before they respond to attractant.

What accounts for the inability of some of the migrated monocytes to respond again to chemoattractant? In many respects, these cells are normal. They all exclude trypan blue. Their capacity to ingest immunoglobulin G-coated erythrocytes is unimpaired (Falk and Leonard, manuscript in preparation). Like unseparated cells, their response to attractants begins without a time lag (cf. Fig. 1 and 2). Thus, the inability of a fraction of the migrated cells to migrate again remains an enigma. Although a formal explanation could be that there is a requirement for an accessory cell and that the ability to respond to this cell is lost in the migrated population, there is no evidence that would lead us to favor this possibility over any other.

Our results showing no cell-cell interaction with monocytes from normal subjects are in contrast to the report that lymphocytes from normal donors partially restore chemotactic responses of monocytes from patients with mycosis fungoides (14). It is possible that, at one stage of their development, either in the bone marrow or shortly after their release into the circulation, monocytes require a stimulus by another cell to differentiate into a chemotactically responsive monocyte. The monocyte chemotaxis defects that have been observed in disease may be due to an impaired differentiating stimulus by another cell type.

LITERATURE CITED

1. **Aksamit, R. R., W. Falk, and E. J. Leonard.** 1981. Chemotaxis of macrophage cell lines. J. Immunol. **126**:2194–2199.
2. **Altman, L. C., L. T. Furukawa, and S. J. Klebanoff.** 1977. Depressed mononuclear leukocyte chemotaxis in thermally injured patients. J. Immunol. **119**:199–205.
3. **Boetcher, D. A., and E. J. Leonard.** 1974. Abnormal monocyte chemotactic response in cancer patients. J. Natl. Cancer Inst. **52**:1091–1099.
4. **Boetcher, D. A., and M. S. Meltzer.** 1975. Mouse mononuclear cell chemotaxis: description of system. J. Natl. Cancer Inst. **54**:795–799.
5. **Boumsell, L., and M. S. Meltzer.** 1975. Mouse mononuclear cell chemotaxis. I. Differential response of monocytes and macrophages. J. Immunol. **115**:1746–1748.
6. **Boyum, A.** 1962. Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Clin. Lab. Invest. Suppl. **21**:77–89.
7. **Falk, W., R. H. Goodwin, Jr., and E. J. Leonard.** 1980. A 48-well micro chemotaxis assembly for rapid and accurate measurement of leucocyte migration. J. Immunol. Methods **33**:239–247.
8. **Falk, W., and E. J. Leonard.** 1980. Human monocyte chemotaxis: migrating cells are a subpopulation with

multiple chemotaxin specificities on each cell. Infect. Immun. **29**:953–959.
9. **Falk, W., and E. J. Leonard.** 1981. Specificity and reversibility of chemotactic deactivation of human monocytes. Infect. Immun. **32**:464–468.
10. **Fernandez, H. N., and T. E. Hugli.** 1976. Partial characterization of human C5a anaphylatoxin. I. Chemical description of the carbohydrate and polypeptide portion of human C5a. J. Immunol. **117**:1688–1694.
11. **Harvath, L., W. Falk, and E. J. Leonard.** 1980. Rapid quantitation of neutrophil chemotaxis: use of a polyvinylpyrrolidone-free polycarbonate membrane in a multiwell assembly. J. Immunol. Methods **37**:39–45.
12. **Lake, W. W., D. Bice, J. Schwartz, and J. Salvaggio.** 1971. Suppression of *in vitro* antigen-induced lymphocyte transformation by carrageenan, a macrophage-toxic agent. J. Immunol. **107**:1741–1745.
13. **Leonard, E. J., and A. H. Skeel.** 1981. Effects of cell concentration on chemotactic responsiveness of mouse resident peritoneal macrophages. RES J. Reticuloendothel. Soc. **30**:271–282.
14. **Norris, D. A., R. E. Perez, L. E. Goliz, L. E. Seitz, and W. L. Weston.** 1979. Defective monocyte chemotaxis in Mycosis fungoides. Lack of essential helper lymphocytes. Cancer **44**:124–130.
15. **Pasternack, G. R., R. Snyderman, M. C. Pike, R. J. Johnson, and H. S. Shin.** 1978. Resistance of neoplasms to immunological destruction: role of a macrophage chemotaxis inhibitor. J. Exp. Med. **148**:93–102.
16. **Pertoff, H., A. Johnson, B. Wärmegard, and R. Seljelid.** 1980. Separation of human monocytes on density gradients of Percoll. J. Immunol. Methods **33**:221–229.
17. **Pike, M. C., and R. Snyderman.** 1976. Depression of macrophage function by a factor produced by neoplasms: a mechanism for abrogation of immune surveillance. J. Immunol. **117**:1243–1249.
18. **Snyderman, R., and M. C. Pike.** 1976. An inhibitor of macrophage chemotaxis produced by neoplasms. Science **192**:370–372.
19. **Territo, M. C., and M. J. Cline.** 1977. Monocyte function in man. J. Immunol. **118**:178–192.
20. **Van Epps, D. E., and R. C. Williams, Jr.** 1978. Serum inhibitors of leukocyte chemotaxis and their relationship to skin test anergy, p. 237–254. *In* J. I. Gallin and P. G. Quie (ed.), Leukocyte chemotaxis: methods, physiology and clinical implications. Raven Press, New York.