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Human Monocyte Chemotaxis: Migrating Cells Are a Subpopulation with Multiple Chemotaxin Specificities on Each Cell

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Only 20 to 40% of human blood monocytes were capable of responding to chemotaxins in vitro. This limit is not due to restrictions of the in vitro system, but is due to the existence of a migrating subpopulation. Over a wide range, the number of cells migrating toward a given concentration of chemotaxin was directly proportional to the number added to the chemotaxis chamber. These monocytes responded to all of the three stimuli used: human serum-derived C5a, human lymphocyte-derived chemotactic factor, and a synthetic peptide. It was possible to deactivate cells to one attractant, leaving the response to other attractants intact. This suggested that these attractants were recognized by different receptors. Several lines of evidence showed that most migrating cells had receptors for all three chemotaxins tested. Thus, if cells were assayed for migration to one attractant, no additional migration occurred when the remaining cells were assayed for migration to a different attractant. Furthermore, the same cells that had migrated toward one attractant were able to respond to other chemotaxins. We also found that a single attractant attracted as many cells as a combination of two or three attractants. Calculations from these data showed that at least 75% of the migrating monocytes have different receptors for all three attractants.

Macrophages play an important role in the immunological response to neoplasms and are found at the site of primary and transplanted tumors. Since peripheral blood monocytes are precursors of these effector macrophages, the ability of monocytes to migrate may be essential for accumulation at tumor sites. Monocytes from cancer patients have abnormal chemotaxis in vitro. Decreased monocyte chemotaxis was associated with poor prognosis (14); the abnormal response was reversed by surgical removal of the tumor (13). Boetcher and Leonard showed differences in monocyte response to two different chemotactic agents, lymphocyte derived-chemotactic factor (LDCF) and activated serum (6). Only 50% of cancer patients with impaired responses to LDCF also had reduced responses to activated serum. These findings suggest that either there are specific subpopulations of migrating monocytes with different chemotaxin receptors or that specific receptors in a homogeneous population with multiple receptors on each cell were blocked. To resolve these alternatives, we examined subpopulations of migrating monocytes in normal subjects. We show by various chemotaxis experiments using 10-μm-thick polycarbonate filters that there is only one subpopulation among all monocytes that is able to migrate in vitro, and that most of these cells respond to all three chemotactic stimuli tested.

MATERIALS AND METHODS

Cell preparation. Blood was drawn from healthy donors and heparinized. Mononuclear cells were isolated by the method of Boyum (7). Blood was diluted with an equal volume of Dulbecco phosphate-buffered saline without calcium or magnesium (PBS) and 35 ml of diluted blood was underlaid with 15 ml of lymphocyte separation medium (Bionetics, Rockville, Md.). After centrifugation at 400 × *g* for 40 min at 20°C, 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 leukocyte recovery was 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 Instruments Co., Washington, D.C.) and observation with a fluorescence microscope. These numbers were used to standardize the cell number added per chemotactic chamber.

Chemotactic factors. *N*-Formyl-methionyl-leu-

cyl-phenylalanine-methylester (referred to as peptide) was a generous gift of K. Ho (Eli Lilly & Co., Indianapolis, Ind.). The optimal concentration range for chemotaxis was 10^{-7} to 10^{-8} M, as described (9). Human serum-derived complement component C5a was prepared as described by Fernandez and Hugli (8) with minor modifications. Briefly, 100 ml of serum was incubated at 37°C with 13 g of epsilon-aminocaproic acid (Sigma Chemical Co., St. Louis, Mo.) and 100 mg of zymosan (Mann Research Laboratories) which was finely suspended in 2 ml of PBS; after 45 min of incubation, it was processed as described (8). The total preparation, about 260 ml after dialysis against starting buffer, was added to a column of CM-Sepharose C1 6B (Pharmacia Fine Chemicals, Inc., Piscataway, N.J., 2.6-cm in diameter, 100 ml of gel) and eluted with 0.1 M ammonium formate buffer (pH 5), containing 0.5 mM ethylenediaminetetraacetic acid. Little or no activity was found in the eluate. The column was then washed with 250 ml of 0.3 M ammonium formate (pH 5), and the activity was eluted with 0.5 M buffer. The pooled fractions were dialyzed against water, membrane filtered (Millipore Corp., Bedford, Mass.), and lyophilized. This material was reconstituted with 10 ml of PBS, and 5 ml was applied to a column of Sephadex G-100 (2.5 by 80 cm) and eluted with 0.1 M ammonium formate buffer, pH 5. Chemotactic activity was detected in the 12,000-dalton region. The active fractions were pooled, dialyzed against water, and lyophilized. The material was solubilized in 10 ml of PBS and stored in samples at −20°C. This preparation is referred to as C5a. A dose-response is shown in Fig. 1. In some experiments, yeast-activated serum was used. For this, baker's yeast was boiled in hot water and washed with water four times. It was added at 1 mg/ml of serum and incubated at 37°C for 50 min in the presence of epsilon-aminocaproic acid. The Fernandez and Hugli procedure for activation was followed, but the acidified sample was dialyzed against PBS and used for chemotaxis without fractionation.

Lymphocyte-derived chemotactic factors were prepared by stimulation of leukocyte cultures made from Ficoll-Hypaque separated cells as described previously (2). Concanavalin A (Miles Laboratories, Inc., Elkhart, Ind.) was used at a concentration in the culture medium of 1 or 5 μg/ml. Homologous serum was added to 0.5% concentration. Incubation time was 24 h. The supernatants were put through 0.45-μm filters, dialyzed against water, and lyophilized. The residue was solubilized in PBS, applied to a Sephadex G 100 column and eluted with PBS. Peak chemotactic activity was found in the 15,000-dalton region. Fractions were pooled, stored at 4°C, and used as the standard preparation of LDCF. The preparation showed some activity at a dilution of 1:80 and the dose-response curve reached a plateau at 1:10. A 1:5 dilution was used to give optimal chemotaxis.

Chemotaxis protocol. Monocyte chemotaxis was assayed in blind well chambers (Neuroprobe Corp., Bethesda, Md.) with polycarbonate filters (5-μm pores; Nuclepore Corp., Pleasanton, Calif.). Chemotactic agent in the indicated concentrations in Gey-BSA (200 μl, total volume) was added to the lower chamber. The upper chamber was filled with 0.3 ml of cell suspension in Gey-BSA containing 8×10^4 monocytes. The cell dose-response was linear over a 100-fold range (10^4 to 10^6 cells per chamber, Fig. 2). We chose a low cell number to reduce the effort of counting and the number of cells needed. The chambers were incubated for 2 h in humidified air with 5% CO_2 at 37°C. The cells in the top chamber were removed, and the filters were washed for 10 s in absolute methanol before staining as described (10). Counting was done as previously described (6). Each point was assayed in triplicate and results were expressed as the mean ± the standard error of the mean of the number of migrated cells per 20 oil fields. The percentage of migrating cells was calculated as follows: the size of a high power oil field

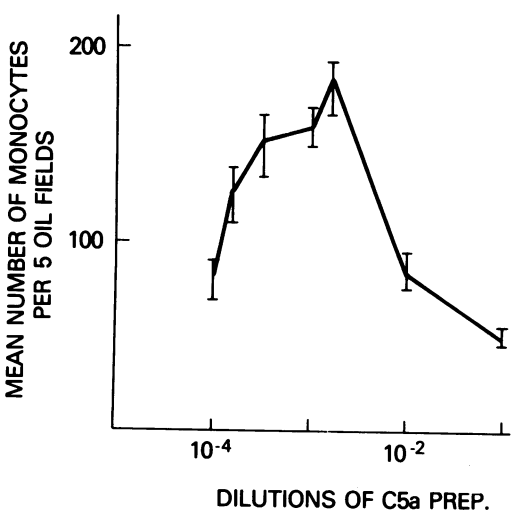


FIG. 1. *Dose-response curve of human peripheral monocytes to purified C5a. C5a in zymosan-activated human serum was purified by CM-Sepharose C1 6B chromatography and Sephadex G-100 gel filtration.*

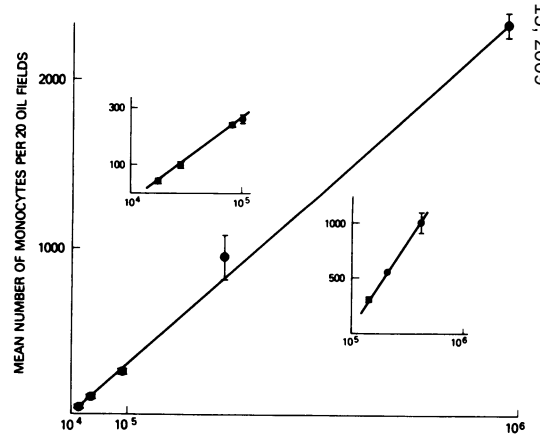


FIG. 2. *Cell dose-response to peptide. The number of monocytes added to the chemotaxis chamber in 300 μl of medium is shown on the abscissa. The two inserts represent different experiments. The concentration of peptide was 10^{-8} M.*

was calibrated; 20 counted fields represented 1/142 of the whole filter area so that the counted number × 142 × 100 divided by the input monocyte number equals the percent of migrating cells. The migrated cells were more than 99% monocytes. Only very few monocytes found on cover slips placed in the lower chamber during incubation showed that the migrated cells remained attached to the filter.

Deactivation procedure. Deactivation was carried out by preincubation of the cells at a concentration of 2.6×10^5 monocytes per ml. The deactivating concentration for the peptide was 10^{-7} M and for activated serum was a dilution of 1:5. Incubation time was 40 to 50 min at room temperature in Gey-BSA. Control populations were preincubated under identical conditions in Gey-BSA alone. After incubation, the cells were centrifuged at $200 \times g$ for 10 min and washed 3 times with Gey-BSA. After the last centrifugation, the cells were suspended in 1 ml of medium for counting. Differential counts and viable counts (by trypan blue dye exclusion) were done, and the suspension was then diluted to provide 8×10^4 monocytes per chamber. The cell recovery was usually 70% with a viability of over 95%.

Filter transfer. The filter transfer procedure was as follows. When the cell layer on the top of the filter was needed, the supernatant was carefully removed with a pipette, the cell layer was washed once with medium, and the filter was transferred to a new chamber. When the top cell layer was not needed, it was removed with a cotton swab. The new chamber was filled with 195 μl of medium or attractant dilution, and the filter was carefully placed in it upside down or up so as to avoid air bubbles. A 300-μl portion of medium was added to the top chamber. All manipulations were carried out very quickly to prevent the filters from drying out. Not more than three chambers were disassembled for filter transfer at a time.

Sephadex G-10 separation of mononuclear cells. Sephadex G-10 was swollen in Gey-BSA for 3 h. Five ml of gel was added to a 35-ml syringe. All manipulations were carried out at 4°C. Five to seven ml of a cell suspension obtained by Ficoll-Hypaque separation and containing 10^7 to 2×10^7 mononuclear cells per ml was placed on top of the syringe columns. The lymphocyte-rich eluant was collected in a 50-ml polypropylene centrifuge tube. The column was washed three times with 5 ml of Gey-BSA, and the washing fluids were combined with the first eluant. The gel was then transferred into a 50-ml centrifuge tube and suspended in 40 ml of medium. After the tube was gently shaken, it was centrifuged for 15 s at $100 \times g$. The supernatant was filtered through loosely packed siliconized glass wool and centrifuged. The cells were suspended in 1 or 2 ml of medium and counted.

RESULTS

Influence of lymphocyte-monocyte ratio. The cell dose-response curve (Fig. 2) shows that the number of migrated cells is directly proportional to the number of monocytes in the top chamber. Since donor variation with respect to the monocyte/lymphocyte ratio is in the range of 15 to 35% monocytes to 65 to 85% lymphocytes in the Ficoll-Hypaque separated fraction, we determined whether this ratio had any influence on the migration. A cell suspension containing 24% monocytes and 76% lymphocytes was fractionated on Sephadex G-10. The cell suspension which was separated from the beads by shaking was composed of 64% monocytes and 36% lymphocytes with a viability of over 99%. Monocyte recovery was about 40%. The dose-responses of this and the original cell suspension to chemotactic peptide were compared (Fig. 3); there was no significant difference in the response.

In the remainder of this paper, we discuss the results which show that human monocytes can be selectively deactivated to different attractants; this implies different chemotaxin receptors. Then, after examining a series of different experimental approaches, we conclude that most of the responding monocytes have receptors for all three of the chemotaxins tested.

Selective deactivation by preincubation. Mononuclear cells were incubated in diluted activated serum, in a chemotactic peptide solution, or in medium as a control. After extensive washing, the cells were counted, adjusted to equal monocyte numbers, and assayed for their responses to activated serum and peptide (Table 1). The response of the cells to the deactivating chemotaxin was suppressed, whereas the response to the new chemotaxin was not altered. This was true for both deactivating agents. The deactivation was therefore chemotaxin specific and migration to other chemotaxins was not inhibited.

Selective migration in the presence of deactivating concentrations of chemotax-

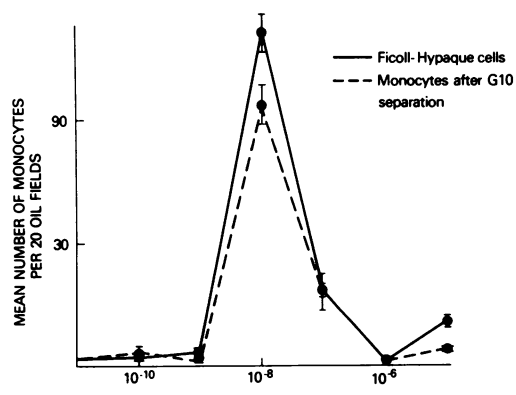


FIG. 3. *Comparison of the dose-response curves of Ficoll-Hypaque cells and a Sephadex G-10 enriched monocyte suspension. The two cell suspensions were adjusted to the same monocyte number per ml. The chemoattractant was peptide.*

ins. An experiment similar to the above was done with a three-attractant system. Cells were suspended in medium containing different chemotaxins in deactivating concentrations. These suspensions were placed in the top chambers. The bottom chambers were filled with chemotaxin solutions as shown in Table 2; again, the chemotaxin response was specific. The attractants in the mixture of all three had the same concentrations as when used alone. Little or no response was observed to attractant in the lower chamber if the same attractant was present in the upper one (Table 2, italicized data). However, if the attractants in the top and bottom chambers were different, the monocytes responded to the stimulus by migration. With a mixture of all three attractants in the top chamber, there was little or no migration to any stimulus in the bottom (Table 2, bottom line). This shows that these three attractants are specifically recognized by the cells and that it is possible to desensitize specifically to one attractant, leaving responsiveness to other attractants intact. At the desensitizing concentrations shown, capacity of the cells for movement was not affected.

TABLE 1. *Specific deactivation of monocytes*

Cells preincubated for 40 min at 22°C in[a]	Attractant	Mean no. of monocytes per 20 oil fields ± SEM[b]
Medium	Activated serum[c]	90 ± 10
	Peptide	115 ± 15
Peptide (10^{-7} M)	Activated serum	110 ± 15
	Peptide	35 ± 5
Activated serum (1:5 dilution)	Activated serum	30 ± 5
	Peptide	150 ± 10

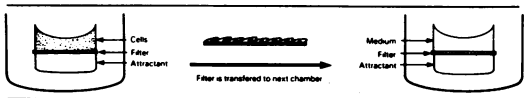
[a] After preincubation, the cells were washed three times with medium and then assayed.
[b] SEM, Standard error of the mean.
[c] Yeast-activated human serum; dilution 1:40.

Evidence for different chemotaxin receptors on the same monocyte. The previous experiments led to the questions of were different populations responding to different attractants, or was one population capable of responding to all of the three stimuli used? To evaluate these questions, we set up an experiment to test for migration of additional cells after a standard chemotaxis incubation. Cells in the first run migrated to medium, peptide, or C5a (Fig. 4). After the 2-h incubation period, the supernatants in the top chambers were carefully removed and the filter top surfaces were gently rinsed so as not to remove adherent cells. The filters were then placed in new chambers containing medium or different attractants in the bottom wells. Medium was added to the top well. The chambers were incubated for 2 h to determine if additional cells migrated during this period. Results for the sequences medium-peptide or medium-C5a were the same as for peptide-medium or C5a-medium, showing that the cells remained viable during the transfer and were capable of responding to a chemotactic stimulus after a first incubation period (Fig. 4). No increase in numbers of migrated cells was observed when the cells migrated towards peptide or C5a in the first run and were then tested for response to either stimulus in the second run. This lack of additional migration suggests that there is predominantly a single population of cells capable of being attracted by either peptide

TABLE 2. *Selective migration of human monocytes*

Attractant in upper chamber[a]	Mean no. of migrated monocytes ± SEM[b] with the following attractant in lower chamber:				
	Medium	LDCF[c]	C5a[d]	Peptide[e]	LDCF + C5a + Peptide[f]
Medium	*65 ± 7*	266 ± 57	184 ± 20	247 ± 47	252 ± 52
LDCF 1:5[g]	85 ± 10	*75 ± 5*	160 ± 15	170 ± 35	270 ± 30
C5a	20 ± 10	190 ± 20	*40 ± 15*	300 ± 20	340 ± 20
Peptide	10 ± 5	75 ± 25[h]	240 ± 10	*35 ± 5*	340 ± 15
LDCF + C5a + Peptide	10 ± 5	45 ± 20	10 ± 5	35 ± 15	*40 ± 5*

[a] The cells were mixed with the indicated attractants and placed into the upper chamber.
[b] SEM, Standard error of the mean. For each row in the table, the response with different attractants in upper and lower chamber was significantly greater than the response with the same attractants in both chambers.
[c] A 1:5 dilution of stock solution in both chambers.
[d] A 1:50 dilution in upper chamber; 1:500 dilution in lower chamber.
[e] A 10^{-7} M concentration in upper chamber; 10^{-8} M concentration in lower chamber.
[f] The concentration of single attractants in the mixture was the same as when used alone.
[g] See text for preparation of stock solution dilutions.
[h] The low response to LDCF when peptide is on top was observed in two other experiments and has not been explained.



Attractants		Mean number of migrated monocytes per 20 oil fields ± SEM
First run	Second run	
Medium	Medium	20 ± 5
	Peptide	70 ± 5
	C5a	85 ± 10
Peptide	Medium	75 ± 10
	Peptide	85 ± 10
	C5a	90 ± 5
C5a	Medium	90 ± 10
	Peptide	85 ± 10
	C5a	85 ± 30

C5a prep. 1:500 Peptide 10^{-8}M

FIG. 4. *Absence of cell populations with specificity for only one chemotaxin. A normal chemotaxis experiment was performed during the first run with the indicated attractants. The filters with cells remaining on both surfaces were then transferred to new chambers with the indicated chemotaxins, incubated for 2 h, stained, and counted to determine if the presence of different chemotaxins in the second run increased the number of migrated cells. SEM, Standard error of the mean.*

or C5a and thus having receptors for both.

To obtain direct evidence for the presence of multiple receptors on the same cell, we set up the following experiment. During the first 2-h incubation period the cells were tested for response to activated serum or peptide in the usual way (Table 3). Supernatant and cells were then removed from the top chamber. Control medium, activated serum, or peptide was then placed in the top chambers in the same concentrations as used for the first period. The cells which migrated to the bottom of the filter in response to the first stimulus now responded to the second stimulus by upward migration to the top of the filter. After the second incubation period, the cells remaining on the filter bottom were counted. These numbers are presented in Table 3; reduction in number compared to the medium response means back migration. Cells selected by response to one attractant were capable of back migration to a different attractant (see italicized data in Table 3). In the first row of the table, cells selected by response to activated serum migrated back to peptide and vice versa for the second row. Similar results were obtained for a three attractant system (LDCF, peptide, and C5a).

Reduction in cell number on the bottom of the filter is, of course, an indirect measure of back migration, and it is possible that cells were simply falling off the filter into the bottom well. However, as noted above, migrated monocytes did not fall off filters onto cover slips placed in the bottom wells. A second migratory response was also shown directly by removing the filter after the first incubation and placing it upside down in a new chamber with a new attractant. The migrated cells, now on the top of the filter, migrated a second time and were counted on the bottom of the filter after the second incubation.

Quantitation. The previous experiments demonstrate in a qualitative way that there is only one population of migrating monocytes that responds to the stimuli used. The results are qualitative because they may be affected by many factors such as diffusion of chemotaxins, aging of cells, or exhaustion of the motility apparatus. To get quantitative information, we arranged an experiment with all possible combinations of 3 attractants in a normal chemotaxis assay. Seven different attractant combinations are possible with respect to 3 attractants and the corresponding receptors on the cells (Table 4). With the attractant combinations indicated and counting the numbers of cells attracted by these combinations, one can set up seven equations for seven unknowns, as outlined in the table (e.g., $x + u + v + t = 130$). It is apparent that the number of cells responding to a mixture of all three attractants is not much greater than the number responding to a single attractant. Thus, most of the cells have receptors for the three attractants used; $t = 75\%$. The results from the solutions of the equations are shown in the rightmost column of the table. The fact that the total percentage exceeds 100 is attributable to the standard errors of the counted numbers.

TABLE 3. *Evidence for more than one receptor for chemoattractants on a single cell*

Attractant for first run in lower chamber	Mean no. of migrated monocytes per 20 oil fields ± SEM with the following attractants in upper chamber for second run:[a]		
	Medium	Activated serum (1:40 dilution)	Peptide (10^{-8} M)
Yeast-activated serum (1:40 dilution)	200 ± 10	200 ± 50	*90 ± 5*
Peptide (10^{-8} M)	320 ± 20	*90 ± 15*	140 ± 20[b]

[a] SEM, Standard error of the mean.

[b] The response to the same attractant is possible because the attractant in the bottom chamber may be diluted by diffusion into the top chamber or destroyed by migrated cells (4, 12) during the first period. Addition of attractant to the top chamber thus creates a concentration gradient in the direction of the top chamber.

TABLE 4. *Estimation of the fraction of migrating cells with a peculiar set of receptors*

Possible receptor combination on a single cell	No. of cells with receptor combination	No. of cells migrating to corresponding attractant combination	Observed no. of cells migrating	Calculated values for column 2[a]
C5a[b]	x	$x + u + w +$ t	130[c]	10
LDCF	y	$y + u + v + t$	150	5
Peptide	z	$z + v + w + t$	130	25
LDCF + C5a	u	$x + y + u + v + w + t$	130	10
LDCF + peptide	v	$y + z + u + v + w + t$	170	0
C5a + peptide	w	$x + z + u + v + w + t$	160	0
C5a + LDCF + peptide	t	$x + y + z + u + v + w + t$	180	75

[a] As percentage of total cells migrating to the combinations of all three attractants (180). Due to the standard errors, the percentages can vary by ±15%.

[b] Optimal concentrations were used: C5a, 1:500; LDCF, 1:5; peptide, 10^{-8} M.

[c] Mean number of monocytes per 20 oil fields.

DISCUSSION

Only 20 to 40% of human peripheral blood monocytes responded by migration to chemotactic stimuli. These findings raised two questions that we attempted to answer. First, do the responsive monocytes represent a distinct subpopulation among the total blood monocytes in vivo? Second, are there migrating monocytes with different chemotaxin receptors or combinations of receptors on their surfaces? An approach to the first question is to consider the possibility that the limit in the number of migrating monocytes is not due to a subpopulation, but that the population is uniform and the limitation is due to an aspect of the in vitro system, such as restriction for movement by small pore diameter or low pore density, cell crowding on the filter, inadequate time for migration, disappearance of chemotaxin gradient, or loss of cellular capacity to respond. The in vitro system was therefore analyzed in several ways. It was shown that the number of migrated cells was always proportional to the number of monocytes in the upper chamber over at least a 100-fold range (Fig. 2) and thus, over this range, migration was not limited by cell crowding. Furthermore, the result was unaffected by the proportion of lymphocytes in the cell suspension (Fig. 3). This is in agreement with two studies in the literature (1, 15). The possibility that the number of migrating cells was restricted by the pore density on the filter was ruled out by our finding that there was no significant increase in the number of cells migrating through filters with twice the usual number of pores per unit area (unpublished data). We also showed that the limit in the number of migrated cells was not due to selection of a population responsive to only one chemoattractant (the remaining cells being responsive to another attractant) since in the filter transfer experiment (Fig. 4), a significant number of cells with the ability to migrate to another attractant could not be detected. This also rules out duration of incubation or disappearance of chemotaxin gradient as limiting factors. Furthermore, the medium control shows that the cells could still migrate after the first incubation period and therefore the limit in migration numbers was not due to loss of migratory capacity during the experiment. These studies provide the most compelling evidence to date that the monocytes capable of migrating to chemotaxins represent a subpopulation of the total blood monocytes.

The analysis of monocyte receptor specificity was made with three attractants that may have importance in immune reactions: LDCF, made by stimulation with concanavalin A of human lymphocytes; C5a from human blood, purified by a procedure analagous to that described by Fernandez and Hugli (8); and a synthetic peptide, *N*-formyl-methionyl-leucyl-phenylalanine-methylester. Small peptides are believed to be the attractants released by bacteria (3, 5). The conclusions drawn from this part of the study are that the three attractants are recognized by different receptors and that the great majority of responding cells have all three of these receptors. Evidence for receptor specificity was based on deactivation studies such as the one showing the preincubation of cells with one attractant selectively inhibited the subsequent response to that attractant, whereas the response to a different attractant remained intact (Table 1). These findings are in agreement with results for other migrating cells, like rabbit neutrophils (16), human peripheral blood neutrophils (11), or eosinophils (17).

Evidence for different receptor specificities on the same cell was obtained directly from the back-migration experiment shown in Table 3. The essence of this experiment is that a specified cell population was first selected by its response to one particular attractant. This cell population

was then used for the next step, in which it was determined whether the cells could migrate back upwards toward a different attractant. Since the cells migrated back, we concluded that the same cells that responded to one stimulus could respond to a second or third different stimulus and therefore must have at least three different receptor specificities. The simplest, albeit indirect, experiment to show that there are different receptor specificities on the same cell is shown in Table 4. With all the possible combinations of 3 attractants, one should expect an addition behavior in the case of single receptors on one cell and the same numbers in the case of multiple receptors. Table 4 shows that at least 75% of the migrating cells must have receptors for all 3 attractants.

Restriction of chemotactic responsiveness in vitro to a subpopulation of blood monocytes may also occur in vivo. The ability to migrate may represent one of several maturational stage qualities exhibited by monocytes on their way from the bone marrow to become effector macrophages in normal tissues and in sites of inflammation. Our findings permit us to separate monocytes on a preparative scale into two populations on the basis of their chemotactic response and to determine if there are morphological, biochemical, or functional differences in these two populations.

The defect in chemotaxis of monocytes from cancer patients can be explained either by the formation of subpopulations of migrating cells with missing or blocked receptors, by a change in dose-response, or by a decrease in the responding cell number. A clinical study to answer these questions will require a determination of (i) the number of responding cells, (ii) the distribution of receptors among the cells, and (iii) the dose-responses for various attractants. These studies are in progress.

LITERATURE CITED

1. **Altman, L. C., C. T. Furukawa, and S. J. Klebanoff.** 1977. Depressed mononuclear leukocyte chemotaxis in thermally injured patients. J. Immunol. **119**:199–205.
2. **Altman, L. C., R. Snyderman, J. J. Oppenheim, and S. E. Mergenhagen.** 1973. A human mononuclear leukocyte chemotactic factor: characterization, specificity and kinetics of production by homologous leukocytes. J. Immunol. **110**:801–810.
3. **Asvanikumar, S., E. Schiffman, B. A. Corcoran, C. B. Pert, J. L. Morell, and E. Gross.** 1978. Antibiotics and peptides with agonist and antagonist chemotactic activity. Biochem. Biophys. Res. Commun. **80**:464–471.
4. **Asvanikumar, S., E. Schiffman, B. A. Corcoran, and S. M. Wahl.** 1976. Role of a peptidase in phagocyte chemotaxis. Proc. Natl. Acad. Sci. U.S.A. **73**:2439–2442.
5. **Asvanikumar, S. B. Corcoran, E. Schiffman, A. R. Day, R. J. Freer, H. J. Showell, E. L. Becker, and C. B. Pert.** 1977. Demonstration of a receptor on rabbit neutrophils for chemotactic peptides. Biochem. Biophys. Res. Commun. **74**:810–817.
6. **Boetcher, D. A., and E. J. Leonard.** 1974. Abnormal monocyte chemotactic response in cancer patients. J. Natl. Cancer Inst. **52**:1091–1099.
7. **Boyum, A.** 1962. Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Clin. Lab. Invest. Suppl. **21**:77–89.
8. **Fernandez, H. N., and T. E. Hugli.** 1976. Partial characterization of human C5a anaphylatoxin. I. Chemical description of the carbohydrate and polypeptide portions of human C5a. J. Immunol. **117**:1688–1694.
9. **Ho, P. P., A. L. Young, and G. L. Southard.** 1978. Methyl ester of N-formylmethionyl-leucyl-phenylalanine: chemotactic responses of human blood monocytes and inhibition of gold compounds. Arthritis Rheum. **21**: 133–136.
10. **Leonard, E. J.** 1975. A method for staining multiple chemotaxis filters. J. Immunol. Methods **9**:81–85.
11. **Nelson, R. D., R. T. McCormack, V. D. Fiegel, and R. L. Simmons.** 1978. Chemotactic deactivation of human neutrophils: evidence for nonspecific and specific components. Infect. Immun. **22**:441–444.
12. **Niedel, J., S. Wilkinson, and P. Cuatrecasas.** 1979. Receptor mediated uptake and degradation of ^{125}I-chemotactic peptide by human neutrophils. J. Biol. Chem. **254**:10700–10706.
13. **Snyderman, R., L. Meadows, W. Holder, and S. Wells, Jr.** 1973. Abnormal monocyte chemotaxis in patients with breast cancer: evidence for a tumor-mediated effect. J. Natl. Cancer Inst. **60**:737–740.
14. **Snyderman, R., H. F. Seigler, and L. Meadows.** 1977. Abnormalities of monocyte chemotaxis in patients with melanoma: effects of immunotherapy and tumor removal. J. Natl. Cancer Inst. **58**:37–41.
15. **Territo, M. C., and M. J. Cline.** 1977. Monocyte function in man. J. Immunol. **118**:187–192.
16. **Ward, P. A., and E. L. Becker.** 1968. The deactivation of rabbit neutrophils by chemotactic factor and the nature of the activable esterase. J. Exp. Med. **127**:693–709.
17. **Wasserman, S. I., D. Whitmer, E. J. Goetzl, and K. F. Austen.** 1975. Chemotactic deactivation of human eosinophils by the eosinophil chemotactic factor and anaphylaxis. Proc. Soc. Exp. Biol. Med. **148**:301–306.