Monocyte Responsiveness to Chemotactic Stimuli is a Property of a Subpopulation of Cells that can Respond to Multiple Chemoattractants

GEORGE J. CIANCIOLO and RALPH SNYDERMAN, *Laboratory of Immune Effector Function, Howard Hughes Medical Institute, Division of Rheumatic and Genetic Diseases, Departments of Medicine and Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina 27710*

ABSTRACT The chemotactic migration of leukocytes is preceded by an alteration in the cells' shape from round to a characteristic polar configuration. We have developed an assay that shows that human monocytes, when exposed to chemoattractant in suspension, assume this polarized shape. The three types of chemoattractants studied, a chemotactic lymphokine, complement-activated serum, and the *N*-formylated oligopeptides, all induced polarization in a time, temperature, and dose-dependent fashion. Nonchemotactic agents such as mitogens or phorbol myristate acetate did not induce polarization. At 37°C, polarization was rapid (<1 min) and was inhibitable by cytochalasin B, sodium azide, or low temperature. A series of *N*-formylated oligopeptides were studied and their activity in inducing polarization correlated closely ($r > 0.99$) with their chemotactic activity. Of the entire population of circulating monocytes there is a subpopulation of cells that is capable of polarizing in response to chemotactic stimuli. The maximum percentage of monocytes which polarized to any chemotactic factor was ~60%. Furthermore, the combination of several chemotactic factors could not increase the percentage of polarized monocytes above the maximum obtained with an optimal dose of any single chemoattractant. The data also demonstrate that high doses of a chemoattractant can induce a state of cross-desensitization in monocytes that blocks the response of the cells to other types of chemotactic factors. These results support the concept that the monocytes that do respond to chemotactic stimuli are capable of responding to any of several attractants.

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INTRODUCTION

Chemotaxis, the directed migration of cells along a chemical gradient, appears to be an important mechanism by which phagocytic cells accumulate at sites of inflammation. The development of assays for measuring chemotaxis in vitro has facilitated the study of leukocyte physiology as well as the pathogenesis of diseases, both in humans and animals (1). In particular, an assay for the chemotactic responsiveness of human blood monocytes (2) has enabled the study of defects in monocyte chemotactic response associated with neoplasia (3–8) and other diseases (9–13).

The leukocyte chemotaxis assays currently in general use measure either the number of cells that have migrated through a porous membrane, the distance that cells have migrated into a membrane or under agarose, or the locomotion of individual cells that are adhered to glass (2, 14–19). Whereas these assays often yield a great deal of information, they measure the end result of a complex series of events including the binding of chemotactic factors to the plasma membrane (20–24), the induction of transmembrane signals (25, 26), the reorganization of cytoskeletal elements (27), the assumption of an elongated, polarized configuration (19), and finally, actual cell movement. The measurement of chemotaxis in vitro, particularly by mononuclear cells, requires a lengthy assay period ranging from 60–90 min using even the quickest procedures (2). Elucidation of the physiology as well as the pathology of monocyte chemotaxis would be greatly aided by a rapid, quantitative, reproducible means to measure the response of the cells to chemoattractants.

Recent studies by Smith et al. (28, 29) demonstrated that human blood polymorphonuclear leukocytes in suspension assume a polarized configuration when ex-

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posed to chemotactic stimuli. Since the polarization of human monocytes toward chemoattractants is an early event that precedes their chemotactic response, we developed a visual assay which measures monocyte polarization in suspension. Using this rapid, quantitative assay we sought to determine what percentage of circulating blood monocytes were capable of responding to chemotactic factors, and whether the cells that responded to one chemoattractant were capable of responding to chemically unrelated chemoattractants.

METHODS

Isolation of human mononuclear leukocytes. Heparinized (10 U/ml; Upjohn Co., Kalamazoo, Mich.) venous blood was obtained from healthy volunteers, mixed 1:1 with 3% (wt/vol) dextran (T500; Pharmacia Fine Chemicals, Piscataway, N. J.) and allowed to sediment for 30 min at 22°C. The leukocyte-rich plasma was removed and the mononuclear leukocytes isolated by the Ficoll-Hypaque density gradient method (30). The cells were centrifuged for 20 min at 800 *g* and 4°C with Gey's balanced salt solution (GBSS,[1] pH 7.0) containing 2% bovalbumin and sodium bicarbonate (Flow Laboratories, Inc., Rockville, Md.). The cells were then washed twice at 400 *g* and 4°C for 15 min, resuspended in GBSS, and a cell count was performed. The percentage of peroxidase positive cells was determined from a cytocentrifuge-prepared slide (31). The cell suspension contained ca. 30% (±10%) peroxidase positive cells and this percentage closely approximated the percentage of monocytes as determined by Wright-Giemsa stain.

Depletion or enrichment of peroxidase-positive cells in mononuclear cell suspensions. Mononuclear leukocytes (1.5×10^7 in 10 ml GBSS, 33% peroxidase positive) were incubated for 60 min at 37°C in plastic petri dishes (100 × 15 mm; Fisher Scientific Co., Pittsburgh, Pa.). The supernate was removed, centrifuged for 15 min at 400 *g* and 4°C, and the cells resuspended in GBSS. Approximately 40% of the total cells were recovered in the supernate; ~11% of these cells were peroxidase positive. Percoll density gradients were also used to enrich and deplete the cell suspensions of peroxidase positive cells. Mononuclear cells were washed once in calcium and magnesium-free phosphate-buffered saline (0.7 mM PO_4; 0.2% glucose; 4.0 mM KCl) and resuspended to 7×10^6 cells/ml in 4% Percoll (vol/vol; Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) in calcium and magnesium-free phosphate-buffered saline. 3 ml of this suspension was then layered on top of a gradient consisting of 3 ml each of 5, 6, 7, and 8% Percoll (all vol/vol in calcium and magnesium-free phosphate-buffered saline). The cell suspension was then centrifuged for 40 min at 400 *g* and 4°C. The cells located at the interfaces between the 6 and 7% Percoll (fraction II) and the 7 and 8% Percoll (fraction III) were removed, washed twice with a large excess of GBSS, and resuspended in GBSS. Approximately 23% of the total cells were recovered in fraction II with ~74% of the cells being peroxidase positive. Approximately 36% of the total cells were recovered in fraction III with only ~11% of the cells being peroxidase positive.

Polarization of human mononuclear cells. Mononuclear cells were adjusted to 1×10^6 peroxidase-positive cells per ml of GBSS and incubated for 5 min in a 37°C water bath in a polypropylene tube. 0.9 ml of the cell suspension was added to each of duplicate tubes (12 × 75 mm; Falcon Labware, Div of Becton, Dickinson & Co., Oxnard, Calif.) containing 0.1 ml of either GBSS or GBSS containing the material being tested. The tubes were then incubated for the indicated time in a 37°C water bath. To end the incubation, 1.0 ml of ice-cold, 0.01 M phosphate-buffered formaldehyde (10% vol/vol; pH 7.2) was added. The cell suspensions were kept cold (4°C) until they were examined by phase microscopy. 200 cells were examined from each tube under ×400 magnification and the percentage of total cells that were polarized determined. The percentage of monocytes that were polarized was calculated by: percent monocytes polarized = (percent total cells polarized/ percent peroxidase-positive cells in initial cell suspension) ×100.

Chemotaxis of human mononuclear cells. Mononuclear cells were adjusted to $0.5–1.5 \times 10^6$ peroxidase-positive cells per milliliter. Chemotaxis was performed as previously described (32) using blind well chambers and 5.0-μm pore polycarbonate filters (Neuro Probe, Inc., Bethesda, Md.). The number of cells that had migrated to the bottom of each filter was determined microscopically. 20 fields were examined for each filter and materials were tested in triplicate. The area of the microscopic field was determined with a micrometer and the percentage of cells that had migrated through the filter was calculated by: percent monocytes migrated = (cells/field × No. fields on lower surface of filter)/No. monocytes used in upper compartment × 100.

Chemotactic peptides and other reagents. *N*-formyl-methionyl-leucyl-phenylalanine (FMLP), *N*-formyl-methionyl-phenylalanine (FMP), phorbol 12-myristate 13-acetate, and cytochalasin B were all obtained from Sigma Chemical Co., St. Louis, Mo. *N*-formyl-norleucyl-leucyl-phenylalanine (FNlLP) was obtained from Peninsula Laboratories, Inc., San Carlos, Calif. *N*-formyl-methionyl-methionyl-methionine (FMMM) and methionyl-methionyl-methionine were obtained from Andrulis Research Corp., Bethesda, Md. Concanavalin A (Con A, crystallized three times) was obtained from Miles Laboratories Inc., Elkhart, Ind. Phytohemagglutin P (PHA) was obtained from Burroughs-Wellcome Co., Greenville, N. C. Sodium azide was obtained from Fisher Scientific Co.

Zymosan-activated human serum (AHS) and lymphocyte-derived chemotactic factor (LDCF). AHS was prepared as previously described (32). Briefly, serum was incubated with zymosan (10 mg/ml; Sigma Chemical Co.) for 60 min at 37°C. The particulate zymosan was removed by centrifugation and the serum heated for 30 min at 56°C. LDCF was prepared as described (33). Briefly, human mononuclear cells obtained sterilely from venous blood on Ficoll-Hypaque density gradients (30) were washed twice in RPMI 1640 medium (pH 7.0; Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.) and adjusted to 2.0×10^6 cells/ml. The cells were incubated with Con A (10 μg/ml) at 37°C in a humidified 5% CO_2 incubator. After 48 h, cell-free supernates were stored at −70°C until used.

RESULTS

Polarization of human monocytes induced by N-formylated chemoattractants. The addition of

[1] *Abbreviations used in this paper*: AHS, zymosan-activated human serum; Con A, concanavalin A; EC_{50}, concentration resulting in half-maximal response; GBSS, Gey's balanced salt solution; FMLP, *N*-formyl-methionyl-leucyl-phenylalanine; FMMM, *N*-formyl-methionyl-methionyl-methionine; FMP, *N*-formyl-methionyl-phenylalanine; FNlLP, *N*-formyl-norleucyl-leucyl-phenylalanine; LDCF, lymphocyte-derived chemotactic factor; PHA, phytohemagglutin P.

FMLP, at concentrations that are chemotactic in vitro, to suspensions of human blood monocytes caused the cells to rapidly assume a polarized configuration as compared to their normal spherical shape (Fig. 1). The polarized morphology closely resembled that of monocytes that have oriented toward a chemotactic gradient. This effect was dose dependent and highly reproducible, as illustrated by Fig. 2 that shows the percentage of monocytes that polarized to varying concentrations of FMLP in 13 different experiments. The maximal percentage of monocytes polarized (58.5±2.6%) was obtained with a concentration of 10 nM FMLP and the concentration giving half-maximal response (EC_{50}) was ~0.1 nM. The maximal response from which EC_{50} was calculated was determined by subtracting the percentage of monocytes that were polarized in buffer alone

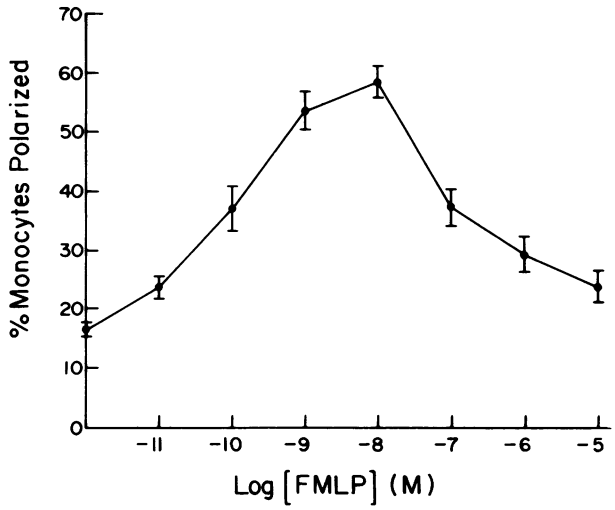

FIGURE 2 Effect of varying concentrations of FMLP on monocyte polarization. Cells were suspended in GBSS at 1×10^6 peroxidase-positive cells/ml, incubated at 37°C for 20 min with the indicated concentrations of FMLP, fixed and the percentage (±SE) of polarized monocytes determined.

(16.3%) from the percentage of monocytes polarized in 10 nM FMLP.

To assess the effects of time and temperature on the polarization of monocytes, 5 nM FMLP was added to cell suspensions that had been equilibrated to 22 or 37°C and, at varying times thereafter, the cells were fixed and the percentage of polarized monocytes calculated (Fig. 3). At 37°C more than 50% of the monocytes were polarized by a 1-min exposure to the peptide. This percentage increased to a maximum (68.5%) after 20 min. At 22°C only 11.5% of the mono-

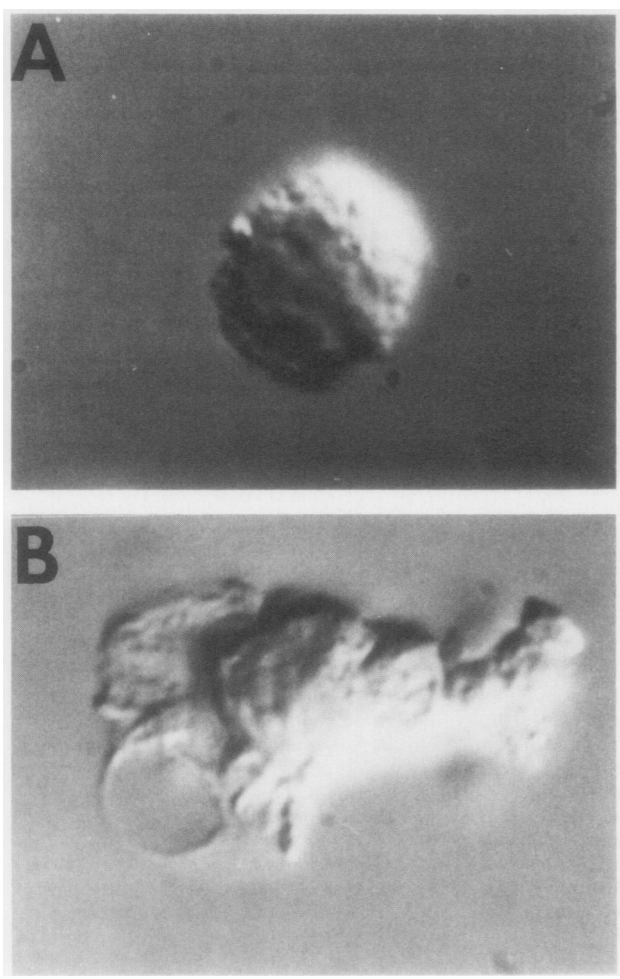

FIGURE 1 Change in shape of human monocytes exposed to FMLP. Cells were suspended in GBSS at 1×10^6 peroxidase-positive cells/ml, incubated at 37°C for 20 min with GBSS (A) or 10 nM FMLP (B), fixed, then examined. Nomarski interference contrast optics. ×1,400.

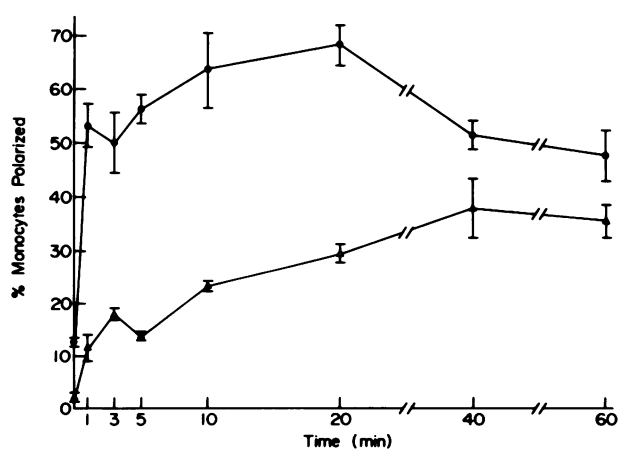

FIGURE 3 Effect of time and temperature on FMLP induced polarization of monocytes. Cells were suspended in GBSS at 1×10^6 peroxidase-positive cells/ml, incubated at 22°C (△) or 37°C (●) for the indicated times with 5 nM FMLP, fixed, and the percentage (±SE) of polarized monocytes determined.

cytes were polarized after 1 min and the peak response was not seen for 40 min. During the 60-min incubation, the percentage of cells that were polarized in buffer alone at 37°C did not change significantly (12.3–13.1%), whereas the percentage of cells polarized in buffer alone at 22°C increased from 1.9 to 9.2%. No significant polarization to FMLP occurred at 0°C.

Preincubation (5 min, 37°C) of monocytes with cytochalasin B (5 μg/10^6 cells) prevented their subsequent polarization (Fig. 4). Cells incubated with dimethyl sulfoxide (7 mM; Fisher Scientific Co.), the solvent used to dissolve cytochalasin B, were not inhibited from polarizing. Preincubation of the cells (30 min, 22°C) with 10 mM sodium azide also prevented polarization (Fig. 4).

Evidence that polarized cells are monocytes. In the experiments described above the percentage of polarized monocytes was calculated by multiplying the percent of total cells polarized by 100 and dividing this number by the percentage of peroxidase-positive cells in the cell suspension. This calculation was used based on the assumption, made from visual observation, that lymphocytes did not polarize in the presence of chemotactic stimuli. To verify this we tested cell suspensions that had been partially depleted of or enriched for peroxidase-positive cells. In both experiments the number of total cells per tube was kept constant. Increasing or decreasing the percentage of peroxidase-positive cells in the initial cell suspension resulted in a parallel increase or decrease in the percentage of total cells polarized (Table I). A plot of the percentages of peroxidase positive cells vs. the percentages of total cells polarized was a straight line with a correlation coefficient of 0.992.

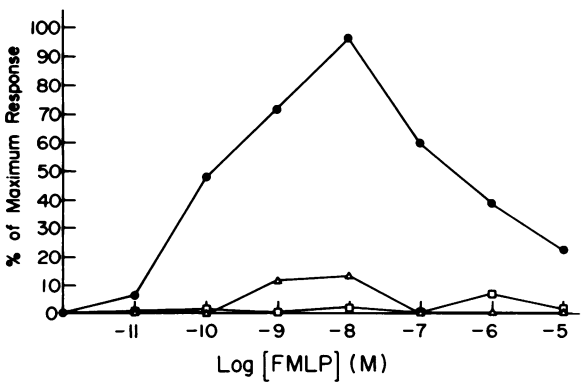

FIGURE 4 Effect of DMSO (●), Cytochalasin B (□), and sodium azide (△) on polarization of monocytes. Cells were suspended in GBSS at 1 × 10^6 peroxidase-positive cells/ml and incubated at 37°C for 5 min with GBSS, DMSO (7 mM), or cytochalasin B (5 μg/10^6 cells) or they were incubated for 30 min at 22°C with GBSS or 10 mM sodium azide in GBSS. The cells were then incubated at 37°C for 20 min with GBSS or varying concentrations of FMLP in GBSS, fixed and the percentage of polarized cells determined. Since the agents were tested on different days the responses are plotted as a percentage of the response obtained using FMLP (10 nM) and cells incubated in GBSS alone.

TABLE I
Effect of Monocyte Depletion or Enrichment on Percentage of Total Cells Polarized

Treatment after Ficoll-Hypaque*	Peroxidase positive cells	Polarization‡	
		Buffer alone	10 nM FMLP
	%	% of total cells	
None	33	6.2±1.8	20.0±1.5
Plastic adherence	11	3.5±0.5	5.7±1.3
None	41	7.2±1.3	21.2±1.8
Percoll density gradient fraction III	11	2.8±1.3	6.5±0.5
Percoll density gradient fraction II	74	6.2±1.2	35.8±1.2

* Mononuclear cells obtained after Ficoll-Hypaque density gradient centrifugation were either suspended in GBSS to a concentration of 10^6 peroxidase-positive cells/ml and then tested as such or further separated by adherence to plastic petri dishes, or fractionation on a Percoll density gradient. The nonadhered cells from the petri dishes and the cells from two Percoll fractions were washed in GBSS and resuspended to the same number of total cells per milliliter as the cells that were fractionated by Ficoll-Hypaque only.
‡ Cells were incubated with GBSS or 10 nM FMLP (in GBSS) for 20 min at 37°C and then the percentage of total cells polarized determined.

Comparison between monocyte polarization and chemotaxis. We had previously observed that under the most optimal conditions the maximum number of monocytes that migrate in the chemotaxis assay using polycarbonate filters was 30–40% (unpublished observations). We therefore performed simultaneous polarization and chemotaxis assays on the same preparations of cells to compare the percentage of responding cells in either assay. Cells were tested for the ability to polarize in response to and migrate towards FMLP at concentrations from 10 pM to 10 μM. In three separate experiments the maximum percentage of monocytes polarized was 55.6, 54.3, and 61.8 at concentrations of 1, 1, and 10 nM, respectively. The maximum percentage of monocytes that had migrated was 30.4, 17.7, and 28.7, all obtained with a concentration of 10 nM FMLP. These latter results were obtained using 2 × 10^5 monocytes per chamber and incubating the chambers for 90 min. Increasing or decreasing the number of monocytes per chamber to 3 × 10^5 or 1 × 10^5 did not increase the percentage of monocytes migrating through the filter nor did increasing the incubation time to 150 min. The bottom compartments of the chambers were examined for cells that might have fallen off the filter; this number was always <4% of the monocytes used.

To test if the specificity of chemoattractants for inducing monocyte polarization paralleled their ability to induce chemotaxis, cells were incubated for 20 min at 37°C with varying concentrations of FMLP, FMMM, FNlLP, or FMP. The results (Fig. 5) demonstrate that FMMM, FNlLP, or FMP are all capable of inducing monocytes to polarize in suspension but they are substantially less potent than FMLP. The potency of these peptides for inducing polarization closely parallels their potency for inducing chemotaxis with a correlation coefficient of $r > 0.99$ (Fig. 6). The unformylated peptide MMM, which is not chemotactic, did not induce polarization.

Ability of other chemoattractants and other membrane-binding agents to induce polarization. To see if other chemotactic factors could cause monocytes to polarize, AHS and LDCF were used. Cells were incubated with varying concentrations of AHS or LDCF, fixed, and the percentage of monocytes polarized was calculated (Fig. 7). As little as 0.01% AHS induced polarization, whereas the peak response was obtained at ~5%. Significant polarization was also induced by as little as 5.0% LDCF with the maximum response occurring at 10%.

The effects on polarization of other membrane-binding but nonchemotactic agents such as phorbol 12-myristate 13-acetate, Con A, or PHA were also tested. The results (Table II) indicate that none of these agents induced monocyte polarization after exposure for 20 min at 37°C. Shortening the period of incubation to 1, 5, or 10 min did not produce any polarization.

Effects of multiple chemoattractants on monocyte polarization. To test whether cell populations that were polarized by one chemoattractant could be induced to further polarize in the presence of additional chemoattractants, monocytes were incubated with several concentrations of FMLP, AHS, or LDCF, either alone or in various combinations. All three chemoattractants induced approximately the same maximal percentage of polarized cells (62.9–66.9%) and any combination of two or three of these chemoattractants did not result in any increase in percentage of cells polarized (Table III). When concentrations of chemoattractants that induced less than the maximal response were combined, the percentage of polarized cells increased but did not exceed the maximum.

Evidence for cross-desensitization of monocyte polarization by FMLP. High concentrations (0.1–10 μM) of FMLP desensitized the monocytes' ability to polarize (Fig. 2). This effect was time dependent in that after 1 min of incubation with 10 μM FMLP the percentage of cells polarized was approximately the same as obtained after 20 min of incubation with 10 nM FMLP; this percentage, however, decreased rapidly, reaching background levels within 10–15 min (data not shown). To test whether cells that were desensitized by a high concentration of FMLP could respond to other chemoattractants, monocytes were incubated either in the presence or absence of 10 μM FMLP for 20 min with 10 nM FMLP, 20% LDCF, 1% AHS, 0.1 μM FNlLP, and 0.1 μM FMMM. The results (Table IV) indicate that the cells made unresponsive by the high concentration of FMLP did not respond to a second chemotactic stimulus.

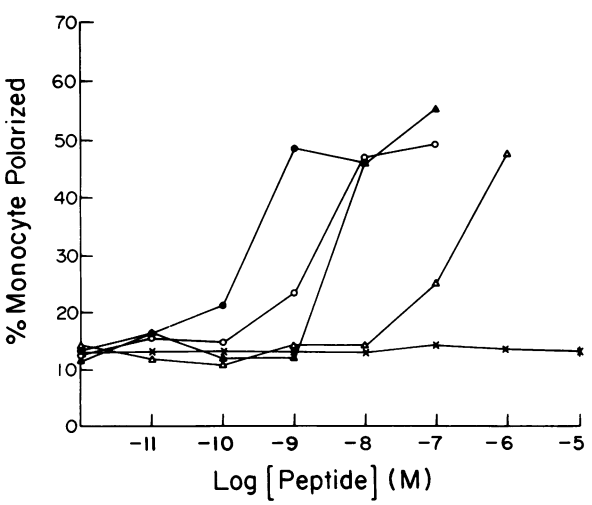

FIGURE 5 Ability of various synthetic peptides to induce monocyte polarization. Cells were suspended in GBSS at 1 × 10^6 peroxidase-positive cells/ml, incubated at 37°C for 20 min with the indicated concentrations of FMLP (●), FMMM (○), FNlLP (▲), FMP (△), or MMM (×), fixed and the percentage (±SE) of polarized monocytes determined.

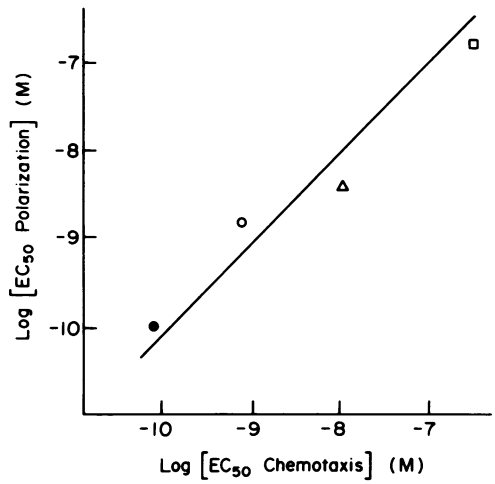

FIGURE 6 Correlation between the ability of FMLP (●), FMMM (○), FNlLP (△), and FMP (□) to induce polarization and chemotaxis. The EC_{50} values of each peptide were determined to be those concentrations that gave half-maximal responses in the respective assays.

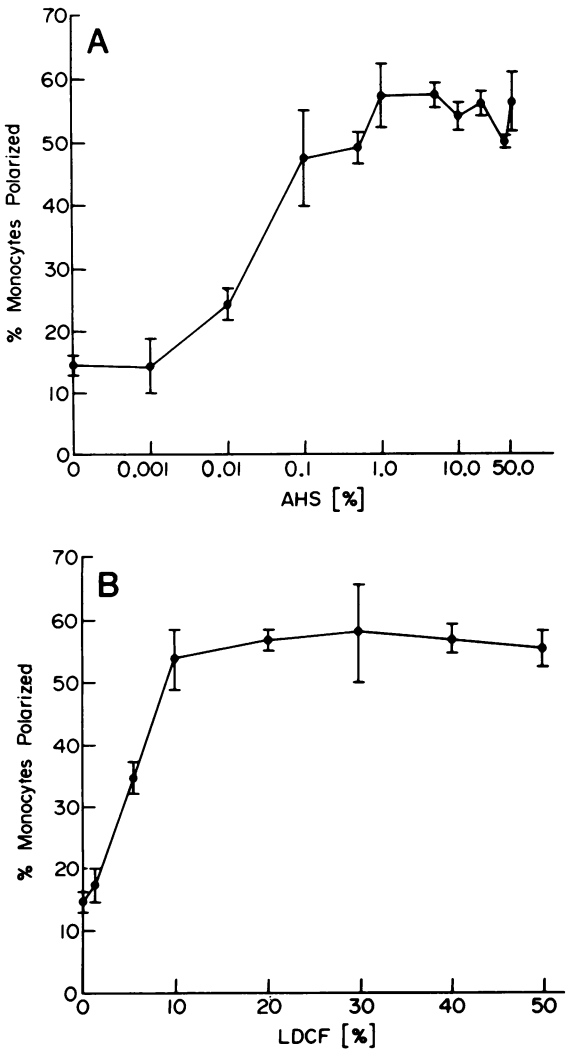

FIGURE 7 (A) Ability of AHS to induce monocyte polarization. Cells were suspended in GBSS at 1×10^6 peroxidase-positive cells/ml, incubated at 37°C for 20 min with the indicated concentrations of AHS, fixed and the percentage (±SE) of polarized monocytes determined. (B) Ability of LDCF to induce monocyte polarization. Cells were suspended in GBSS at 1×10^6 peroxidase-positive cells/ml, incubated at 37°C for 20 min with the indicated concentrations of LDCF, fixed and the percentage (±SE) of oriented monocytes determined.

TABLE II
Effect of Various Membrane-binding Agents on Monocyte Polarization

Agent tested*		Monocytes polarized‡
		%
Phorbol myristate acetate	10 μM	21.4±5.4
	1 μM	17.0±0.7
	0.1 μM	21.4±1.8
Concanavalin A	100 μg/ml	25.0±1.8
	10 μg/ml	17.8±0.7
	1 μg/ml	23.2±1.8
Phytohemagglutinin P	100 μg/ml	18.8±4.6
	10 μg/ml	17.8±1.8
	1 μg/ml	20.5±0.7
FMLP	10 nM	59.8±10.0
Buffer alone		19.6±7.0

* Cells were incubated for 20 min at 37°C with the various agents at the concentrations indicated and polarization was then determined.

‡ The percentage of monocytes polarized was calculated by: percent monocytes polarized = (percentage of total cells polarized/percentage of peroxidase-positive cells in initial cell suspension) × 100. Values are ±SE.

DISCUSSION

The migration of leukocytes to gradients of chemoattractants in vitro is initiated by the cells' assuming a polarized oriented position towards the direction of movement. Polarized cells can be identified by their triangular shape characterized by a flattened lamellapod at the leading edge and pointed uropod at the tail (19).

Our data demonstrate that human blood monocytes in suspension assume a polarized configuration when exposed to chemotactic stimuli. This effect is both time and temperature dependent but extremely rapid at 37°C. The time required for polarization of half the number of cells that can polarize was <1 min. Exposure of monocytes to chemoattractants at 22°C resulted in fewer cells being polarized, even after longer periods of exposure. The ability to polarize appears to require intact microfilaments since cytochalasin B completely blocked this response. Furthermore, the effect is dependent on oxidative metabolism since pretreatment of the cells with sodium azide also prevented polarization.

In addition to the formylated chemotactic peptides, other chemoattractants such as AHS and LDCF also induced monocyte polarization. The excellent correlation ($r > 0.99$) between the EC_{50} values of polarization and chemotaxis obtained with a series of synthetic peptides suggests that measuring polarization can serve as an effective tool for examining an early event involved in the chemotaxis of human monocytes. Moreover, the specificity of the *N*-formylated peptides for inducing polarization suggests that their response is mediated by the binding of the peptides to the *N*-formylated peptide chemotactic factor receptor (34). The polarization of monocytes does not result from nonspecific binding of substances to the cells' surfaces since neither phorbol 12-myristate 13 acetate, Con A, nor PHA induced the response.

Our previous observation that <40% of human mono-

TABLE III
Effect of Multiple Chemotactic Stimuli on Monocyte Polarization

Stimulus*		Monocytes polarized‡
		%
5.0% AHS	(A)	63.7±0.6
30.0% LDCF	(B)	66.9±3.9
10 nM FMLP	(C)	62.9±1.6
A + B		65.3±9.0
A + C		66.1±4.8
B + C		64.5±0.2
A + B + C		66.1±1.6
0.015% AHS	(D)	34.7±2.6
3.0% LDCF	(E)	37.1±1.6
30 pM FMLP	(F)	42.7±2.6
D + E		53.2±3.2
D + F		50.0±1.6
E + F		49.2±7.1
D + E + F		59.7±1.6
Buffer alone		21.3±1.9

* Cells were incubated for 20 min at 37°C with the indicated concentrations of the chemotactic stimuli, either singly or in combination, and polarization was then determined.
‡ The percentage of monocytes polarized was calculated by: percent monocytes polarized = (percentage of total cells polarized/percentage of peroxidase-positive cells in initial cell suspension) × 100. Values are ±SE.

cytes are capable of migrating in chemotaxis assays led us to investigate various possible explanations. One explanation was that only a certain percentage of blood monocytes have the capability to respond chemotactically. This was suggested by Arenson et al. (35) in studies in which they separated human monocytes into volumetrically distinct subpopulations, one of which comprised >90% of the chemotactically responsive cells as measured by a modification of the agarose plate method. The concept of functionally distinct subpopulations of monocytes has also been advanced by Norris et al. (36) who reported the isolation by counterflow centrifugation of a monocyte population relatively deficient in antibody dependent cellular cytotoxicity.

TABLE IV
Effect of High Concentration of FMLP on the Polarization of Monocytes by Chemotactic Stimuli

Chemotactic stimulus*	Simultaneous addition of	
	Buffer	10 μM FMLP
	% of monocytes polarized‡	
None	22.4±1.3	16.4±3.4
FMLP, 10 nM	53.3±0.5	22.4±6.6
AHS, 1%	47.4±3.9	25.0±3.9
LDCF, 20%	52.6±2.6	25.0±2.6
FN1LP, 0.1 μM	47.4±1.3	25.6±0.5
FMMM, 0.1 μM	40.1±3.4	25.0±1.3

* Cells were incubated for 20 min at 37°C with the indicated concentrations of the chemotactic stimuli, either in the presence or absence of a high concentration of FMLP, and polarization was then determined.
‡ The percentage of monocytes polarized was calculated by: percent monocytes polarized = (percentage of total cells polarized/percentage of peroxidase-positive cells in initial cell suspension) × 100. Values are ±SE.

The existence of subpopulations of monocytes with differing responsiveness to chemotactic factors is difficult to verify using unfractionated mononuclear leukocytes and currently available chemotaxis assays. The development of the polarization assay allowed us to directly examine one early response of the monocytes to chemotactic factors. Our results indicate that, on the average, a maximum of ~60% of blood monocytes are able to assume a polarized configuration in the presence of a chemotactic stimulus. Because some of these cells may be polarized nonspecifically, i.e. to factors present in the media, etc., the maximum number of human monocytes that polarize to chemotactic stimuli is probably about 50%. Thus the fact that only up to 40% of the monocytes put on top of a chemotaxis filter migrate through the filter is not surprising since not all cells capable of sensing chemoattractants and polarizing would necessarily be capable of migrating as well.

The data further indicate that the subpopulation of human monocytes that responds by polarization to a chemotactic stimulus is capable of responding to several different chemotactic stimuli. Support for this contention comes from our results, which show that a population of cells maximally polarized by one stimulus cannot respond further to another stimulus; however, a population of cells that have less than maximally responded to one stimulus can indeed be further polarized to the maximal value by a second stimulus. The finding that combinations of less than maximal concentrations does not result in strictly additive increases in polarization is consistent with the concept that the responsive cells share receptors for the different chemotactic stimuli. The results that show that polarization of monocytes to five different chemotactic factors can be blocked by a desensitizing concentration of one of those factors further supports the concept of a common, responsive, monocyte subpopulation.

Why only a subpopulation of monocytes polarizes to chemotactic factors has not yet been determined. The defect in the nonresponsive cells may be related to deficiencies in the binding of chemotactic factors to the cells or to defects in the subsequent transmembrane events that follow binding and are required to initiate polarization and migration. It is also not known whether the ability of monocytes to polarize to chemotactic stimuli is related to the level of cellular differen-

tiation or whether distinct monocyte cell lines exist. These possibilities should provide fruitful areas for investigation.

In any case the data indicate that human blood monocytes in suspension assume polarized configurations in response to various chemotactic stimuli. This response appears to be characteristic of a distinct subpopulation of these cells. The assay for this activity is rapid and quantitative and may thus be extremely useful for studies evaluating the function of monocytes in individuals with neoplasia, immune deficiency, or inflammatory disorders.

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