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Two Neutrophil Populations in Human Blood with Different Chemotactic Activities: Separation and Chemoattractant Binding

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Normal human blood contains a population of neutrophils that migrates to various chemoattractants and a population that fails to migrate. The percentage of neutrophils migrating to optimal concentrations of chemoattractants was quantified: 20 to 40% migrated to *N*-formylmethionyl-leucyl-phenylalanine, 30 to 50% migrated to human C5a, 25 to 35% migrated to human leukocyte-derived chemotactic factor, 20 to 30% migrated to casein, 15 to 20% migrated to pepstatin, and 1 to 5% migrated to medium alone. Neutrophil migration to the most active chemoattractant was not increased when other chemoattractants were added, indicating that the population of neutrophils migrating to the most active attractant was the same population that was migrating to the other attractants. The percentage of neutrophils migrating to a chemoattractant was not altered by prolonging the assay incubation period or by replacing the attractant with new chemoattractant during the assay, and the percentage was independent of the neutrophil concentration added to the chemotaxis chamber. Nonmigrating neutrophils were isolated with a chemotaxis collection chamber, and they were examined for radiolabeled chemotactic peptide binding. The binding of radiolabeled *N*-formylmethionyl-leucyl-phenylalanine by nonmigrating and migrating neutrophils was identical.

Human peripheral blood neutrophils (PMNs) and monocytes contain subsets of cells which are distinguishable by in vitro assays. Neutrophils have been reported to consist of at least two populations on the basis of their rosette formation with immunoglobulin G (IgG)-coated erythrocytes; 80% form rosettes with IgG-coated erythrocytes, and the remainder do not form rosettes (15). Monocytes comprise two populations with differing responses to chemoattractants (6, 9). We have previously reported (12) that less than 50% of human PMNs migrate in vitro to optimal chemotactic concentrations of human complement-derived C5a and *N*-formylmethionyl-leucyl-phenylalanine (fMet-Leu-Phe). Keller et al. (14) recently reported that less than 50% of human PMNs migrate to C5a and fMet-Leu-Phe, despite the apparent orientation of 90% of the cells in a chemotactic gradient. In the present study, we examined the chemotactic responsiveness of human neutrophils to a variety of chemoattractants and extend our observation that there is a population of neutrophils (nonmigrating PMNs) which fails to migrate in vitro to attractants. Nonmigrating PMNs were separated with a chemotaxis cell collection chamber (described in the accompanying paper [8]), and they were examined for their binding of radiolabeled fMet-Leu-Phe.

MATERIALS AND METHODS

Leukocyte preparation. Human PMNs were isolated from heparinized blood of healthy donors by dextran sedimentation, and residual erythrocytes were hypotonically lysed as previously described (12). PMNs were suspended in Hanks balanced salt solution (HBSS) without albumin for chemotaxis assays and in calcium- and magnesium-free HBSS for radiolabeled peptide binding assays. The suspensions contained 88 to 98% PMNs which were greater than 97% viable (assessed by trypan blue dye exclusion). In some experiments, PMNs were isolated from heparinized blood by Ficoll-Hypaque separation (5) or by gravity sedimentation.

Chemotaxis. PMN chemotaxis was assayed with a multiwell chemotaxis chamber (Neuro Probe, Inc., Bethesda, Md.) and polyvinylpyrrolidone-free polycarbonate filters (10 μm thick with 5-μm pores; Nuclepore Corp., Pleasanton, Calif.) (7, 12). The chambers were incubated for 60 min at 37°C in humidified air, and then the filters were fixed in methanol and stained with Diff-Quick (Harleco, Gibbstown, N.J.). Migrating PMNs were counted with an Optomax Image Analyzer (Optomax, Inc., Hollis, N.H.). All migrating neutrophils were adherent to the lower surface of the polyvinylpyrrolidone-free polycarbonate filters

and did not fall off when protein-free media were used (12). The migrating neutrophils were counted on a 1-mm^2 area of the filter surface. Since the total filter area per well was 8 mm^2, the total number of migrating PMNs was determined by multiplying the cell counts by eight. The percentage of cells that responded was determined by dividing the total number of migrating neutrophils by the input number (25,000). Experiments were performed in which the nonmigrating neutrophils on the upper surface of the filter were also counted. The total number of PMNs quantified on the upper and lower filter surfaces was ≥90% of the input number. The chemoattractants fMet-Leu-Phe, casein hydrolysate, and pepstatin were obtained from Sigma Chemical Co., St. Louis, Mo. Human C5a was prepared as described by Fernandez and Hugli (10) with modifications (12), and human leukocyte-derived chemotactic factor (LDCF) was prepared by the stimulation of mononuclear leukocyte cultures with concanavalin A (Miles Laboratories, Inc., Elkhart, Ind.) as described by Altman et al. (3). Dose-response curves were determined with all attractants, and subsequent experiments were performed with the optimal chemotactic concentration of each attractant. In some experiments, PMNs and chemoattractants were diluted in RPMI 1640 or Eagle minimal essential medium (GIBCO Laboratories, Grand Island, N.Y.). In experiments in which a chemoattractant was replaced with fresh attractant during the assay, the top plate, gasket, and filter were transferred after a 40-min incubation period from one multiwell chemotaxis chamber to a second multiwell chamber containing new attractant. The second chamber was incubated for another 40-min period at 37°C in humidified air. Neutrophil chemotaxis in agarose was performed in some experiments as described by Nelson et al. (18). The center wells of the agarose assay contained 2.5×10^5 PMNs. The responses to HBSS and fMet-Leu-Phe are reported as the chemotactic index (the ratio of the migration distance toward the attractant to the migration distance toward the control medium).

Separation of nonmigrating neutrophils. Nonmigrating PMNs were separated from migrating PMNs with a chemotaxis cell collection chamber (described in the companion paper [8]). An optimal concentration of either LDCF or fMet-Leu-Phe (10^{-6} M) was placed in the lower compartments, and 1.0 ml of HBSS containing 1.5×10^6 PMNs was placed in each upper compartment. A 5-μm-pore-sized, polyvinylpyrrolidone-coated polycarbonate filter was placed between the upper and lower compartments. The chambers were incubated for 40 min at 37°C in humidified air. After incubation, 0.5 ml of HBSS with 2% bovine serum albumin was added to the PMN suspensions in the upper compartments, and the cells were removed by gentle pipetting. The cells were centrifuged at $90 \times g$ for 7 min and resuspended in HBSS. Control (unseparated) PMN suspensions were treated identically except that HBSS alone was placed in the lower compartments of the chamber. Both nonmigrating and control PMNs were tested for chemotaxis in the multiwell and agarose assays. When PMNs were removed from the lower filter surface, they aggregated readily. Because of this, nonmigrating PMNs were compared with unseparated PMNs, which contained the migrating population.

Binding of a radiolabeled chemotactic peptide to neutrophils. The binding of tritiated fMet-Leu-Phe (fMet-Leu-[^{3}H]Phe) to PMNs was assayed by a previously described procedure (21) with modifications. PMNs (5×10^5) were suspended in 1.0 ml of calcium- and magnesium-free HBSS containing 1 mM azide to impair endocytosis (19). Concentrations of fMet-Leu-[^{3}H]Phe (46.4 Ci/mmol; New England Nuclear Corp., Boston, Mass.) ranging from 1×10^{-9} to 5×10^{-7} M were mixed with the PMN suspensions. Before being used in the binding assays, fMet-Leu-[^{3}H]Phe was tested for purity on silica gel thin-layer chromatography plates in a butanol-acetic acid-water solvent system (6:1:2.5). The cells and peptide were incubated for 30 min at 24°C and then filtered through Whatman GFC glass fiber filters. The filters were washed with 10 ml of cold HBSS containing 2% bovine serum albumin and were placed in scintillation vials with 10 ml of Ultrafluor scintillation fluid (National Diagnostics, Sommerville, N.J.). Nonspecific binding, defined as the amount of fMet-Leu-[^{3}H]Phe bound to PMNs when the label was added with a 100-fold excess of unlabeled fMet-Leu-Phe, was less than 15% of the total activity bound. Specific binding refers to the total amount of fMet-Leu-[^{3}H]Phe bound minus the nonspecific binding. The binding of fMet-Leu-[^{3}H]Phe increased linearly with cell concentration over the range of 2×10^5 to 5×10^6 PMNs/ml. Limiting the number of cells to 5×10^5 PMNs/ml in calcium- and magnesium-free medium minimized cell aggregation during the binding assay.

Neutrophil phagocytosis of sheep erythrocytes. Neutrophil phagocytosis was evaluated as described by Meltzer and Stevenson (16) with modifications. Rabbit anti-Forssman IgG (generously provided by John Langone and Michael Boyle, National Institutes of Health, Bethesda, Md.) was purified as previously described (4). IgG-coated sheep erythrocytes (EAIgG) were prepared by the incubation of 10^9 erythrocytes with 100 μl of anti-Forssman IgG in 1.0 ml of phosphate-buffered saline for 60 min at 37°C. EAIgG were washed three times with phosphate-buffered saline and adjusted to a final concentration of 10^9 EAIgG/ml. Neutrophils were plated in plastic Costar cluster plates (16-mm well diameter; Costar, Cambridge, Mass.) at a concentration of 2×10^5 PMNs per well and incubated with 10^7 EAIgG for 20 min at 37°C. After incubation, extracellular EAIgG were lysed with ammonium chloride buffer, and PMN monolayers were washed with medium, fixed with methanol, and stained with Diff-Quick. A total of 200 PMNs per sample were examined for internalized EAIgG. The data are presented as the mean percentage of PMNs with internalized erythrocytes ± standard error of the mean for five experiments.

RESULTS

Chemotactic activity of the neutrophil populations. Chemotaxis dose-response experiments were performed in the multiwell assay with all five chemoattractants evaluated in this study. The dose-response data for fMet-Leu-Phe are shown in Fig. 1. Neutrophil chemotaxis occurred over a range of 10^{-10} to 10^{-4} M fMet-Leu-Phe, with the maximum total cell migration occurring at 10^{-6} M. When equivalent concen-

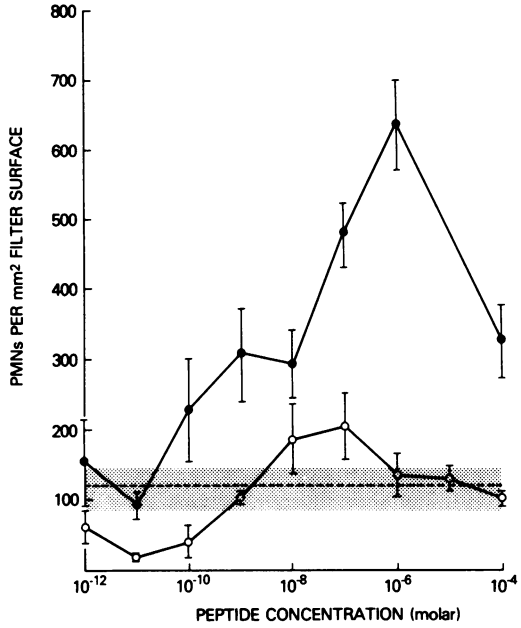

FIG. 1. Dose response of PMNs to fMet-Leu-Phe in the multiwell chemotaxis assay. The values represent the mean responses of five different PMN donors to various concentrations of fMet-Leu-Phe (●). Also shown are the mean responses of three different PMN donors when equal fMet-Leu-Phe concentrations were added to the upper and lower wells of the chamber (○). The broken line and shaded area represent the mean and standard area of the mean of the responses of five different PMN donors to the medium alone. The error bars represent the standard error of the mean.

trations of fMet-Leu-Phe were present in the upper and lower wells of the chamber, PMN migration was significantly suppressed and approached or equalled the response obtained with HBSS alone. Thus, the migration to chemoattractants in this assay represents chemotactic migration.

Neutrophils from 10 normal donors were assayed for migration to optimal concentrations of chemoattractants. The percentages migrating ranged from 20 to 40% to fMet-Leu-Phe, 30 to 50% to human C5a, 25 to 35% to human LDCF, 20 to 30% to casein, 15 to 20% to pepstatin, and 1 to 5% to HBSS. This finding suggested that a fraction of the neutrophil population was incapable of responding to chemoattractants. An alternative interpretation was that neutrophil migration was limited in some way by the assay conditions. To evaluate this possibility, we altered several conditions of the chemotaxis assay: (i) the incubation period was extended from 60 min to 4 h; (ii) the chemoattractant was replaced with fresh attractant during the assay; (iii) RPMI 1640 and Eagle minimal essential medium were used instead of HBSS; and (iv) PMNs isolated by gravity sedimentation or by Ficoll-Hypaque separation were tested. The percentage of PMNs that migrated was not increased by these variations of the assay. Experiments were performed in which the PMN concentration in the upper wells of the chamber was varied from 4,000 to 100,000 PMNs per well (Fig. 2). The number of migrating neutrophils increased linearly with an increase in cell input in the multiwell chamber, demonstrating that cell crowding did not impair PMN chemotaxis. Experiments were also performed to determine whether PMNs release substances that inhibit the migration of other neutrophils in the chemotaxis chamber (Table 1). The experimental design was to induce a PMN chemotactic response, add additional neutrophils to the responding cells 40 min later, and then quantify the chemotaxis of the total population. The lower wells of a multiwell chamber were filled with HBSS or with optimal concentrations of fMet-Leu-Phe or LDCF. The upper wells were loaded with 12,000 (group 1) or 24,000 (group 2) neutrophils per well; or with 12,000 neutrophils per well, followed by an additional 12,000 neutrophils after a 40-min incubation period (group 3). The multiwell chamber was incubated for a total of 80 min. When the input number of

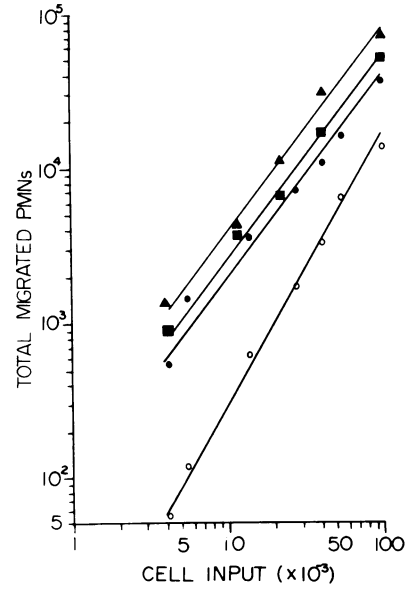

FIG. 2. Effect of cell concentration on neutrophil migration in the multiwell assay. Various concentrations of neutrophils were added to the upper well of the chemotaxis chamber, and the total number of neutrophils migrating to C5a (▲), LDCF (■), fMet-Leu-Phe (●), and medium alone (○) was quantified. Each point represents the mean of triplicate values from one PMN donor.

TABLE 1. Effect of neutrophils on the subsequent migration of other neutrophils[a]

Attractant	Migrating neutrophils per mm² of filter surface		
	Group 1	Group 2	Group 3
HBSS	25 ± 5	70 ± 10	80 ± 10
fMet-Leu-Phe	400 ± 35	890 ± 70	870 ± 90
LDCF	400 ± 90	620 ± 60	730 ± 40

[a] HBSS or optimal chemotactic concentrations of attractants were placed in the lower wells of a multiwell chemotaxis chamber. The upper wells of the chamber were divided into three groups: group 1 contained 12,000 PMNs per well; group 2 contained 24,000 PMNs per well; and group 3 initially received 12,000 PMNs per well and after a 40-min incubation period received another 12,000 PMNs per well. The chamber was incubated for a total period of 80 min at 37°C in humidified air. The data are expressed as the mean ± standard error of the mean of triplicate values from one PMN donor. The experiment was performed with PMNs from three different donors, and similar results were obtained.

neutrophils was doubled, the migrating number doubled (Table 1, group 2 versus group 1). Furthermore, the number of migrating neutrophils was the same whether the cells were all added at the initiation of the assay (group 2) or added sequentially (group 3), which illustrates that migrating PMNs in the assay did not inhibit the subsequent migration of other neutrophils. Experiments were performed in which chemoattractants were combined. The results of a representative experiment are summarized in Table 2. Neutrophil migration to the most active attractant was not significantly increased when other attractants were present, indicating that the population of PMNs migrating to the most active attractant was the same population that was migrating to the other attractants.

Separation and examination of nonmigrating neutrophils. Nonmigrating PMNs were collected in a chemotaxis separation chamber described in the accompanying paper (8). Nonmigrating PMNs were removed from the upper compartments of the chamber after a 40-min incubation period with optimal concentrations of fMet-Leu-Phe or LDCF in the lower compartments. Control PMNs (unseparated) were incubated for an identical period with HBSS alone in the lower compartments. Nonmigrating and control PMNs were tested for chemotaxis to fMet-Leu-Phe in the multiwell and agarose assays. Nonmigrating neutrophils did not demonstrate chemotactic activity in either assay. In the multiwell assay, 2 to 5% of the input PMNs migrated to fMet-Leu-Phe (10^{-8} to 10^{-6} M) and to HBSS; in agarose, the mean chemotactic index was 1.5 to fMet-Leu-Phe and 1.3 to HBSS. Control PMNs retained the migrating population after a 40-min incubation period in the collection chamber. In the multiwell assay, 20 to 40% of the input PMNs migrated to fMet-Leu-Phe, and 2 to 5% migrated to HBSS. The mean chemotactic index in agarose was 3.0 to fMet-Leu-Phe and 1.3 to HBSS. Both the control and the nonmigrating neutrophils were greater than 95% viable, and they were able to adhere and spread on glass and plastic. The phagocytosis of EAIgG was similar for both groups of neutrophils; 73 ± 9% of unseparated and 69 ± 11% of nonmigrating PMNs internalized EAIgG. Both groups contained an average of 2 to 3 EAIgG per neutrophil. Nonmigrating PMNs were compared with control PMNs for their binding of radiolabeled fMet-Leu-Phe. The time course of 5×10^{-8} M fMet-Leu-[^{3}H]Phe binding was identical for both groups of neutrophils (Fig. 3). fMet-Leu-[^{3}H]Phe binding did not increase after 30 min of incubation at 24°C (Fig. 3). In another experiment, 5×10^5 PMNs were exposed to 1×10^{-9} to 5×10^{-7} M fMet-Leu-[^{3}H]Phe for 30 min (Fig. 4). The binding of fMet-Leu-[^{3}H]Phe was identical for the control and the nonmigrating neutrophils. In these experiments, nonmigrating PMNs comprised approximately 60% of the unseparated PMN suspension. Since fMet-Leu-[^{3}H]Phe binding to equal numbers of control and nonmigrating PMNs was identical, the average amount of peptide bound per neutrophil must have been the same for both populations. Peptide binding to nonmigrating PMNs was the same when either fMet-Leu-Phe or LDCF was used to separate the cells.

DISCUSSION

When PMNs were tested for their responses to chemoattractants, 50 to 60% of them did not migrate. An important question is whether this was due to the existence of a nonmigrating

TABLE 2. PMN chemotactic responses to mixed attractants[a]

Attractant(s)	Migrating PMNs per mm² of filter surface
HBSS	85 ± 15
fMet-Leu-Phe	920 ± 70
C5a	780 ± 85
LDCF	700 ± 75
fMet-Leu-Phe + C5a	930 ± 40
fMet-Leu-Phe + LDCF	970 ± 55
LDCF + C5a	660 ± 45
fMet-Leu-Phe + C5a + LDCF	1,000 ± 125

[a] Data are expressed as the mean ± standard error of the mean of triplicate values from one PMN donor. The experiment was performed with PMNs from five different donors, and similar results were obtained.

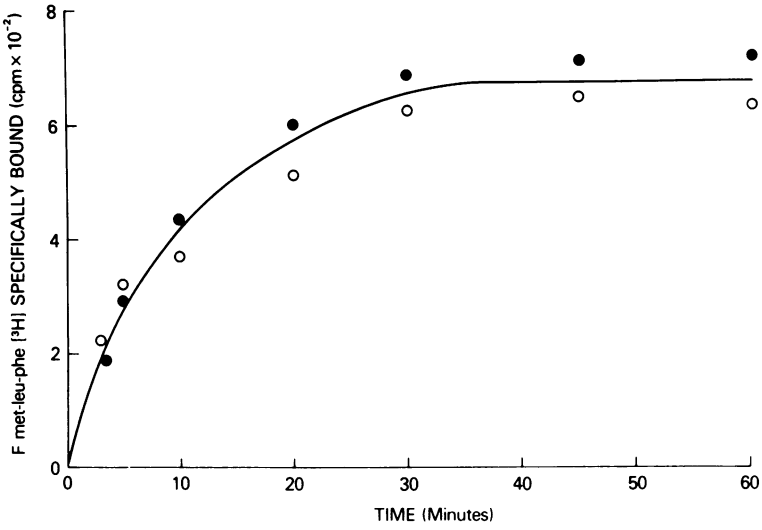

FIG. 3. Time course of fMet-Leu-[^{3}H]Phe binding to control and nonmigrating human neutrophils. A concentration of 5×10^5 nonmigrating (○) or control (●) neutrophils in a volume of 1.0 ml of medium with 1 mM azide containing 5×10^{-8} M fMet-Leu-[^{3}H]Phe (2×10^6 cpm) was incubated for various time intervals at 24°C. The experiment was performed with PMNs from three different donors, and the same results were obtained.

subpopulation of neutrophils or to the conditions of the assay. After testing several assay variables, we found that the lack of migration was not due to: (i) insufficient incubation time, since migration was not improved by increasing the incubation period by several hours; (ii) disappearance of the chemotactic gradient, since no additional migration was observed when the cells were exposed to a new gradient; or (iii) loss of cell viability, since recovered nonmigrating PMNs excluded trypan blue, were adherent, and ingested EAIgG. When the input neutrophil concentration was increased over a 25-fold range, there was a linear increase in neutrophil migration. Therefore, the nonmigrating population could not be explained by cell crowding or by competition of neutrophils for pores in the polycarbonate filter. Goetzl and Austen have reported that leukocytes release a factor (neutrophil inhibitory factor) that inhibits neutrophil chemotaxis (11). We could not attribute the nonmigrating neutrophil population of the present study to an inhibitory factor released by the cells in the assay. When PMNs were added to a chamber in which other PMNs had been incubated, the migration of the second group of cells was not impaired. The chemotaxis chamber did not appear to limit cell migration, since Aksamit et al. have demonstrated 100% migration with some macrophage cell lines in the chamber (2). These findings support the concept of a nonmigrating population of human neutrophils.

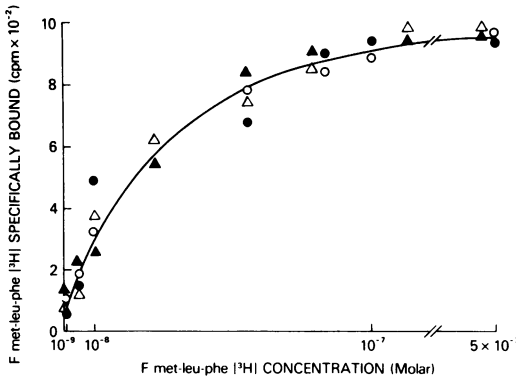

FIG. 4. Binding of fMet-Leu-[^{3}H]Phe to control and nonmigrating human neutrophils. Nonmigrating (△, ○) and control (▲, ●) PMNs were incubated at a concentration of 5×10^5 PMNs in 1.0 ml of medium for 30 min at 24°C with various concentrations of fMet-Leu-[^{3}H]Phe. The data presented are the results from two PMN donors in which nonmigrating PMNs were separated in the collection chamber with LDCF as the attractant. Four PMN donors were tested, and the binding by the control and the nonmigrating neutrophils was identical. A straight line was obtained by Scatchard analysis of the data, resulting in an approximation of 25,000 receptors per neutrophil for fMet-Leu-[^{3}H]Phe and a binding affinity of 13 nM.

The chemotaxis assay used in this study quantified neutrophil migration through the pores of a 10-μm-thick, polyvinylpyrrolidone-free polycarbonate filter. All of the responding cells were in one optical plane on the lower surface of the filter, and the total number of migrating cells could be readily determined. In contrast, neutrophils in agarose or on cellulosic filters (100 to 150 μm thick) migrate farther than 10 μm. A deter-

mination of the total number of migrating cells in cellulosic filters is difficult, since counts must be done in a series of optical planes through the 100- to 150-μm thickness of the filter. Therefore, in most studies with cellulosic filters, the total number of migrating cells is not determined. Instead, chemotactic responses are quantified at the leading front (22), at a specified depth of the filter, or by a count of the cells that reach the bottom of the filter plus those that fall off and are trapped by a second filter (13). The range of fMet-Leu-Phe concentrations that induce a maximal human neutrophil chemotactic response in cellulosic filters is 1×10^{-9} to 5×10^{-8} M (14, 20, 21). In contrast, the concentration that induced the maximal number of migrating neutrophils in our assay was 10^{-6} M. An inspection of the dose-response curve (Fig. 1) shows that fMet-Leu-Phe induced significant chemotactic responses from 10^{-10} to 10^{-4} M. There is a distinct shoulder in the dose-response curve at 10^{-8} M, which is caused by the migration of about 15% of the neutrophils added to the chamber. This percentage is not greatly different from that reported by Keller et al. for the optimal migration to fMet-Leu-Phe in the cellulosic filter assay (14). It is thus possible that there are two subpopulations of responding neutrophils: one group of cells migrates to approximately 10^{-8} M fMet-Leu-Phe, and the other group of cells responds to 10^{-6} M fMet-Leu-Phe. The group migrating to 10^{-6} M peptide may migrate a relatively short distance (10 to 20 μm) because of peptide-induced adherence or deactivation; thus, this group would be unable to migrate to the leading front of cells in the cellulosic filter. The migration of this group of cells would, therefore, be detected in the thin polycarbonate filter assay but not in the thicker cellulosic filter assay.

The migrating population of neutrophils responded to several chemoattractants. Since PMN migration to the most active attractant was not significantly increased when other attractants were present, the cells with receptors for other attractants were among the cells that responded to the most active attractant. Therefore, individual PMNs have receptors for a variety of attractants. Pepstatin (1), which has recently been reported to bind to the fMet-Leu-Phe receptor (17), attracted 15 to 20% of the input neutrophils, compared with 20 to 40% for fMet-Leu-Phe. These differences have not been explored in this investigation; however, it is possible that the cells responding to pepstatin are a subset of the cells with receptors for fMet-Leu-Phe.

When we isolated the nonmigrating cells and examined their fMet-Leu-[^{3}H]Phe binding, we found that the binding was identical to that of the control neutrophils. Despite fMet-Leu-[^{3}H]Phe binding to isolated nonmigrating neutrophils, these cells did not demonstrate chemotactic activity to the peptide in either the agarose or the multiwell assay. Thus, the difference between the migrating and nonmigrating populations must lie in events subsequent to receptor-ligand binding. These findings are in striking contrast to the data in the accompanying paper (8), which demonstrate a marked deficiency in fMet-Leu-[^{3}H]Phe binding to the nonmigrating human monocyte subpopulation.

At present it is not known why 50 to 60% of human neutrophils do not migrate in vitro to chemoattractants. One possibility is that the nonmigrating population is extremely sensitive to chemoattractants and is desensitized during the assay. Another possibility is that there is a maturational difference between the two populations. Such a difference could be reflected in the chemoattractant processing and receptor turnover of these cells. The separation of neutrophil populations on the basis of their response to chemoattractants provides a new experimental tool for studying the mechanisms of the chemotactic response.

LITERATURE CITED

1. **Ackerman, S. K., and S. D. Douglas.** 1979. Pepstatin A: a human leukocyte chemoattractant. Clin. Immunol. Immunopathol. **14**:244–250.
2. **Aksamit, R. R., W. Falk, and E. J. Leonard.** 1981. Chemotaxis by mouse macrophage cell lines. J. Immunol. **126**:2194–2199.
3. **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.
4. **Boyle, M. D. P., and J. J. Langone.** 1980. A simple procedure to use whole serum as a source of either IgG- or IgM-specific antibody J. Immunol. Methods **32**:51–58.
5. **Boyum, A.** 1962. Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Clin. Lab. Invest. Suppl. **21**:77–89.
6. **Cianciolo, G. J., and R. Snyderman.** 1981. Monocyte responsiveness to chemotactic stimuli is a property of a subpopulation of cells that can respond to multiple attractants. J. Clin. Invest. **67**:60–68.
7. **Falk, W., R. H. Goodwin, and E. J. Leonard.** 1980. A 48-well microchemotaxis assembly for rapid and accurate measurement of leukocyte migration. J. Immunol. Methods **33**:239–247.
8. **Falk, W., L. Harvath, and E. J. Leonard.** 1982. Only the chemotactic subpopulation of human blood monocytes expresses receptors for the chemotactic peptide formyl-methionyl-leucyl-phenylalanine. Infect. Immun. **36**:450–454.
9. **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.
10. **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.
11. **Goetzl, E. J., and K. F. Austen.** 1972. A neutrophil-immobilizing factor from human leukocytes. I. Genera-

tion and partial characterization. J. Exp. Med. **136**:1564–1580.

12. **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.
13. **Keller, H. U., J. F. Borel, P. C. Wilkinson, M. Hess, and H. Cottier.** 1972. Reassessment of Boyden's technique for measuring chemotaxis. J. Immunol. Methods **1**:165–168.
14. **Keller, H. U., J. H. Wissler, and B. Damerau.** 1981. Diverging effects of chemotactic serum peptides and synthetic F-met-leu-phe on neutrophil locomotion and adhesion. Immunology **42**:379–383.
15. **Klempner, M. S., and J. I. Gallin.** 1978. Separation and functional characterization of human neutrophil subpopulations. Blood **51**:659–669.
16. **Meltzer, M. S., and M. M. Stevenson.** 1978. Macrophage function in tumor-bearing mice: dissociation of phagocytic and chemotactic responsiveness. Cell. Immunol. **35**:99–111.
17. **Nelson, R. D., S. K. Ackerman, V. D. Fiegel, M. P. Bauman, and S. D. Douglas.** 1979. Cytotaxin receptors of neutrophils: evidence that f-methionyl peptides and pepstatin share a common receptor. Infect. Immun. **26**:996–999.
18. **Nelson, R. D., P. G. Quie, and R. L. Simmons.** 1975. Chemotaxis under agarose: a new and simple method for measuring chemotaxis and spontaneous migration of human polymorphonuclear leukocytes and monocytes. J. Immunol. **115**:1650–1656.
19. **Snyderman, R., and E. J. Fudman.** 1980. Demonstration of a chemotactic factor receptor on macrophages. J. Immunol. **124**:2754–2757.
20. **Wilkinson, P. C.** 1979. Synthetic peptide chemotactic factors for neutrophils: the range of active peptides, their efficacy and inhibitory activity, and susceptibility of cellular response to enzymes and bacterial toxins. Immunology **36**:579–588.
21. **Williams, L. T., R. Snyderman, M. C. Pike, and R. J. Lefkowitz.** 1977. Specific receptor sites for chemotactic peptides on human polymorphonuclear leukocytes. Proc. Natl. Acad. Sci. U.S.A. **74**:1204–1208.
22. **Zigmond, S. H., and J. C. Hirsch.** 1973. Leukocyte locomotion and chemotaxis: new methods for evaluation and demonstration of cell-derived chemotactic factor. J. Exp. Med. **137**:387–410.