DEVELOPMENT OF SPECIFIC RECEPTORS FOR 
N-FORMYLATED CHEMOTACTIC PEPTIDES IN A HUMAN 
MONOCYTE CELL LINE STIMULATED WITH LYMPHOKINES*

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The chemotactic response of leukocytes is a behavioral phenomenon that is the end 
result of a complex series of integrated biochemical events. Chemotaxis appears to 
require recognition by cells of chemical gradients in their surrounding environment, 
transduction of this information from the exterior to the interior of the cell, and 
translation of the information into directed cell movement through alterations in 
cellular metabolism and rearrangement of cytoskeletal elements (1-6). It has recently 
been established that leukocytes contain specific cell-surface receptors for certain 
chemoattractants and it has been surmised that these receptors are important in 
initiating the biological effects of the chemotactic factors (1-3).

Recent studies have demonstrated that a human monocyte-like cell line, U937 (7– 
10), can be stimulated to differentiate into macrophages in vitro in the presence of 
culture supernates obtained from stimulated, but not unstimulated, lymphocytes (9).
We have shown that these cells in the unactivated state lack chemotactic responses to 
three defined chemoattractants, but develop a directed migratory response after 
activation of the cells with lymphokines (11). We now report that the development of two 
N-formylated-peptide-induced biological responses, chemotaxis, and lysosomal 
enzyme release, (12, 13) during differentiation of U937 cells are associated with the 
simultaneous appearance on the cells of specific surface receptors for chemoattractants.

Materials and Methods

Chemicals. N-Formyl-L-methionyl-L-leucyl-L-phenylalanine (fMet-Leu-Phe) and N-formyl-
norleucyl-leucyl-phenylalanine (fNle-Leu-Phe) were obtained from Peninsula Laboratories,

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Abbreviations used in this paper: EC50, one-half maximal inhibition of binding, fMet-Leu, N-formyl-
methionyl-leucine; fMet-Leu-[3H]Phe, tritiated N-formyl-methionyl-leucyl-phenylalanine; fMet-Leu-Phe, 
N-formyl-L-methionyl-L-leucyl-L-phenylalanine; fMet-Met, N-formyl-methionyl-methionyl-methio-
nine; fNle-Leu-Phe, N-formyl-norleucyl-leucyl-phenylalanine; incubation buffer, phosphate-buffered saline 
that contained 1.7 mM KH2PO4, 8 mM Na2HPO4, 0.117 mM NaCl, 0.15 mM CaCl2, and 0.5 mM MgCl2, 
ph 7.2; LDH, lactic acid dehydrogenase; RPMI-FCS, RPMI-1640 medium that contained 10% (vol/vol) 
heat-inactivated (30 min at 56°C) fetal calf serum.

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Inc., San Carlos, Calif. N-formyl-methionyl-methionyl-methionine (fMet-Met-Met) and N-
formyl-methionyl-leucine (fMet-Leu) were purchased from Andrusis Research Corp., Bethesda,
Md. Tritiated fMet-Leu-Phe (fMet-Leu-[H]Phe) (57 Ci/mmol) was from New England
Nuclear, Boston, Mass., and Hepes was obtained from Calbiochem-Behring Corp., American
Hoechst Corp., San Diego, Calif. Phenolphthalein glucuronic acid and dried Micrococcus
lysodeikticus were purchased from Sigma Chemical Co., St. Louis, Mo. The chemotactic
stimulants zymosan, activated human serum (14), and lymphocyte-derived chemotactic factor
(15, 16) were prepared as previously described.

**Cell preparations.** U937 cells were grown in RPMI-1640 medium that contained 10% (vol/
vol) heat-inactivated (30 min at 56°C) fetal calf serum (RPMI-FCS) (B & B Research
Laboratories, Baltimore, Md.). The cells were maintained at 37°C and passaged as previously
described (9). Before use in the functional and binding assays, the cells were washed twice in
phosphate-buffered saline that contained 1.7 mM KH₂PO₄, 8 mM Na₂HPO₄, 0.117 M NaCl,
0.15 mM CaCl₂, and 0.5 mM MgCl₂, pH 7.2 (incubation buffer).

**Preparation of Lymphokine-rich Supernates.** Human mononuclear leukocytes obtained by sepa-
ration of whole human blood on Ficoll (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc.,
Piscataway, N. J.) Hypaque (Winthrop Laboratories, New York) gradients were incubated in
RPMI-FCS for 48 h at 37°C in the presence of 1 btg/ml phytohemagglutinin (Burroughs-
Wellcome Corp., Research Triangle Park, N. C.). After incubation, the cell supernates were
collected after centrifugation and stored at -20°C until used.

**Chemotaxis Assay.** Chemotaxis was assayed in modified Boyden chambers using a 5.0-μm
polycarbonate filter (Nuclepore Corp., Pleasanton, Calif.) (15, 17). Chemotactic activity of the
various stimulants was measured as the mean number of migrating cells per oil-immersion
microscopic field (magnification: × 1,000) in triplicate samples and is expressed as the
percentage of the maximal response obtained with each chemoattractant.

**fMet-Leu-[3H]Phe-binding Assay.** Five million U937 cells were incubated with the indicated
concentration of fMet-Leu-[H]Phe in a total vol of 0.15 ml in the presence and absence of 10
μM unlabeled fMet-Leu-Phe. After the appropriate incubation time at 25°C, the cell suspensions
were rapidly filtered onto glass fiber discs (Whatman GF/C; Whatman, Inc., Clifton, N.
J.) (1), which were then washed with 24 ml of ice-cold incubation buffer. The filters were
placed into scintillation vials with 10 ml of Aquasol (Imperial Chemical Industries, Inc., Los
Angeles, Calif.) and the radioactivity was measured by liquid scintillation spectroscopy. Specific
binding is defined as total binding minus nonspecific binding which is the amount of fMet-
Leu-[H]Phe bound in the presence of 10 μM unlabeled fMet-Leu-Phe (18, 19).

**Assay of Lysosomal Enzyme Release.** U937 cells (5 × 10⁶ cells/ml) were suspended in phosphate-
buffered saline that contained .138 M NaCl, 2.7 mM KCl, 8.1 mM Na₂HPO₄, 1.5 mM
KH₂PO₄, 0.6 mM CaCl₂, and 1.0 mM MgCl₂, pH 7.4 (20) in the presence and absence of 10⁻⁵
M cytochalasin B. After incubation for 15 min. at 37°C, chemotactic factors or buffer alone
were added to the cells and the reaction was allowed to proceed for 15 min at 37°C. The tubes
were then immersed in an ice bath and centrifuged. The supernates were assayed for lysozyme
(21), β-glucuronidase (22), and lactic acid dehydrogenase (LDH) (23).

**Results**

**Kinetics of the Development of Chemotaxis and Lysosomal Enzyme Secretion in U937 Cells
Treated with Lymphokines.** Our previous studies demonstrated that U937 cells, when
incubated with lymphokines, developed a chemotactic response to fMet-Leu-Phe (11).
To determine whether stimulated U937 cells developed another chemoattractant-
induced function, lysosomal enzyme secretion (13), the cells were incubated with
RPMI-FCS that contained 5% (vol/vol) lymphokine supernates for 24, 48, or 72 h,
washed, and tested for their ability to release β-glucuronidase and lysozyme in response
to 10 μM fMet-Leu-Phe (Fig. 1 A). These cells were also studied for chemotactic
responsiveness to the same agent (Fig. 1 B). Unactivated U937 cells released small but
significant amounts of β-glucuronidase and lysozyme in the presence of peptide and
10 μM cytochalasin B (Fig. 1 A). When the cells were stimulated with lymphokines,
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Fig. 1. Development of lysosomal enzyme secretion (A) and chemotactic responsiveness (B) induced by fMet-Leu-Phe in stimulated U937 cells. U937 cells were incubated in 5% lymphokine supernates (vol/vol) for 24, 48 or 72 h, washed, and resuspended in the appropriate buffer for chemotaxis or secretion assays. Lysosome and $\beta$-glucuronidase activities were measured in supernates of cells (5 x 10^6/ml) that had been incubated with 10 $\mu$M cytochalasin B and 10 $\mu$M fMet-Leu-Phe, and are expressed as the percentage of total enzyme released. Enzyme release obtained in the presence of cytochalasin B alone was subtracted from the values obtained in the presence of the peptide, and each point represents the mean of duplicate determinations. The actual amounts of lysozyme and $\beta$-glucuronidase released at 0, 24, 48, and 72 h were 0.2, 0.5, 0.7, and 1.2 $\mu$g/ml equivalent lysozyme units, respectively, and 1.0, 5.9, 12.3, and 26.5 nmol phenolphthalein formed/h per 10^6 cells, respectively. Chemotaxis is expressed as the percent of maximal chemotactic activity of cells migrating completely through the filter in response to 50 nM fMet-Leu-Phe in triplicate samples. The migration of cells in response to Gey's buffered salt solution alone were subtracted from values obtained in the presence of fMet-Leu-Phe. (O) $\beta$-glucuronidase and (C) lysozyme released; (A) chemotactic response.

the release of both enzymes increased markedly with increased time of incubation. Maximal release of lysozyme occurred in cells that had been incubated for 48 h with lymphokines, and was 41% of the total lysozyme content. $\beta$-glucuronidase release was maximal after 72 h, and was 42% of the total amount of this enzyme. The cellular content of both enzymes increased with increasing time of stimulation with lymphokines. Lysozyme content at 0, 24, 48, and 72 h after stimulation was 0.9 ± 0.1, 0.8 ± 0.03, 1.3 ± 0.1, and 1.6 ± 0.06 $\mu$g equivalent lysozyme units/10^6 cells, respectively, and values for total $\beta$-glucuronidase content were 48.6 ± 1.3, 50.8 ± 0.9, 99.2 ± 7.2, 126.6 ± 5.0 $\mu$mol phenolphthalein formed/h per 10^6 cells, respectively. The release of lysosomal enzymes in response to fMet-Leu-Phe was not a result of cell death because there was no observable release of the cytoplasmic enzyme, LDH. The appearance of lysosomal enzyme secretion in U937 cells paralleled the appearance of chemotactic responsiveness to 50 nM fMet-Leu-Phe (Fig. 1 B).

Kinetics of the Appearance of fMet-Leu-[3H]Phe-binding Sites on U937 Cells after Activation
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by Lymphokine-rich Supernates. To determine whether the development of fMet-Leu-Phe induced biologic functions depended on the appearance of a specific cellular binding site for the formylated peptide chemotactic factors, U937 cells were grown in medium alone or in medium that contained 5% (vol/vol) lymphokines for 24, 48, or 72 h. The cells were then washed and tested for the ability to specifically bind fMet-Leu-[3H]Phe. U937 cells grown in the presence of medium alone did not specifically bind fMet-Leu-[3H]Phe in concentrations ranging from 5 to 40 nM (Fig. 2). When cells were incubated in the presence of 5% lymphokine-rich supernate for 24 h, however, specific saturable binding of fMet-Leu-[3H]Phe was detected (Fig. 2). Exposure of U937 cells to lymphokines for longer periods of time in culture resulted in a correspondingly increased amount of specific fMet-Leu-[3H]Phe binding. The numbers of sites per cell calculated using Scatchard analysis (24) were 4,505 ± 1,138, 22,150 ± 4,030, and 37,200 ± 8,000 (n = 2) for cells activated for 24, 48, and 72 h, respectively. The dissociation constants (K_D) for the interaction of fMet-Leu-[3H]Phe with the receptor on U937 cells were approximately the same regardless of the time of activation, and ranged between 15 and 30 nM.

Kinetics and Reversibility of fMet-Leu-[3H]Phe Binding to Activated U937 Cells. U937 cells that had been grown for 48 h in the presence of 5% (vol/vol) lymphokine-rich supernates were incubated with 10 nM fMet-Leu-[3H]Phe, and specific binding was assayed at various times after incubation at 23°C (Fig. 3). The binding of fMet-Leu-[3H]Phe reached equilibrium by 45 min; the time required to reach one-half equilibrium (t_1/2) was ~16 min at 25°C. To determine whether the binding of fMet-Leu-[3H]Phe was reversible, a large excess (10 μM) of unlabeled fMet-Leu-Phe was added to an equilibrated mixture of the labeled ligand and U937 cells, and specific binding was assayed after various times thereafter (Fig. 3). The bound fMet-Leu-[3H]Phe was rapidly displaced by the addition of the unlabeled peptide; >50% of the bound peptide was displaced by 5 min after the addition of unlabeled fMet-Leu-Phe.

Specificity of the N-Formylated Peptide Receptor on U937 Cells. Experiments were performed to determine whether the binding sites for fMet-Leu-[3H]Phe on stimulated U937 cells possessed the specificity expected of a receptor for the N-formylated peptide.
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Fig. 3. Time-course and reversal of fMet-Leu-[3H]Phe binding to activated U937 cells. Cells were cultured for 48 h in the presence of 5% lymphokines (vol/vol), washed, and incubated with 10 nM fMet-Leu-[3H]Phe at 23°C for various periods of time, after which specific binding was calculated (○). A large excess (10 μM) of unlabeled fMet-Leu-Phe was added to some mixtures (●) after 45 min at 23°C and the specific binding of the ligand was determined at subsequent time intervals. Each point represents the mean of duplicate determinations.

Fig. 4. Specificity of the chemotactic response of activated U937 cells to a series of N-formylated peptides. U937 cells cultured for 48 h in lymphokines were tested for chemotactic responsiveness to the indicated concentrations of peptides. Chemotactic activity was determined as the average number of cells migrating completely through the filter in 20 microscopic fields and is expressed as the percent of the maximal response obtained for each peptide. The maximal responses were (cells/microscopic field, magnification: × 1,000): (○) fMet-Leu-Phe (57.7); (*) fMet-Met-Met (206.0); (Δ) fNle-Leu-Phe (81.7); and (O) fMet-Leu (160.0). Results given are the mean of triplicate determinations.

peptides. U937 cells were cultured for 48 h with lymphokines and tested for their ability to respond chemotactically to various concentrations of a series of N-formylated peptides. Fig. 4 indicates that the peptides, fMet-Leu-Phe, fNle-Leu-Phe, and fMet-Met-Met were approximately equipotent for eliciting a chemotactic response in stimulated U937 cells, whereas the peptide fMet-Leu was less potent by approximately three orders of magnitude. These results are in good agreement with the potencies of these same peptides for producing a chemotactic response in guinea pig macrophages (25).

The same peptides were also tested for their relative potency for inducing lysosomal enzyme secretion by U937 cells. Cytochalasin B-treated cells, which had been stimulated with lymphokines for 48 h, were incubated with various concentrations of the peptides for 15 min at 37°C, after which time the amount of lysozyme and β-
glucuronidase activities in the supernate fluid was measured. The peptides exhibited the same order of potency for releasing lysozyme activity from the U937 cells (Fig. 5) as was noted for the induction of the chemotactic response, fMet-Leu-Phe = fNle-Leu-Phe = fMet-Met-Met > fMet-Leu. Identical results were obtained when β-glucuronidase activity was measured in the cell supernates. The concentrations of peptides necessary to induce lysosomal enzyme release were 10–15 times greater than the concentrations necessary for eliciting a chemotactic response, a phenomenon which has also been noted in studies using rabbit polymorphonuclear leukocytes (13).

If the binding of fMet-Leu-[3H]Phe to activated U937 cells is in fact representative of binding to a site that mediates the biological responses of these cells to N-formylated peptides, then the affinity of various peptides for the binding site should reflect their biological potency. The ability of the various peptides to compete with fMet-Leu-[3H]Phe for cellular binding was therefore tested. Each of the peptides was added in several concentrations to incubation mixtures containing fMet-Leu-[3H]Phe (10 nM) and stimulated U937 cells, and the percent of fMet-Leu-[3H]Phe binding inhibited in the presence of the unlabeled peptides was determined (Fig. 6). The order of potencies of these peptides in competing for fMet-Leu-[3H]Phe-binding sites exactly paralleled their order of potencies for producing chemotactic responses and lysosomal enzyme release. Excellent correlation between the doses of peptides which produced one-half maximal inhibition of binding (EC50) and half maximal lysosomal enzyme release (r = 0.99) and chemotaxis (r = 0.99) were obtained.

Discussion

The monocyte-like cell line U937, established from a human histiocytic lymphoma (7) was adapted to rapid growth in vitro (9). U937 cells have low levels of antibody-dependent cellular cytotoxicity, immune phagocytic activity, and Fc receptors in the unstimulated state, but develop increased killer and phagocytic activity as well as Fc receptors upon incubation with lymphokines (9). Our studies demonstrate that other

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Fig. 6. Inhibition of fMet-Leu-[\(^{3}\)H]Phe binding to activated U937 cells by a series of N-formylated peptides. U937 cells cultured for 48 h with lymphokines were washed and incubated at 23°C with 10 nM fMet-Leu-[\(^{3}\)H]Phe in the presence or absence of the indicated concentration of peptide for 1 h, after which the specific binding was calculated. Results are expressed as percent inhibition of fMet-Leu-[\(^{3}\)H]Phe binding, and each point represents the mean of duplicate incubation conditions.

important functions of leukocytes, chemotaxis and lysosomal enzyme secretion, develop in the U937 cells upon differentiation in the presence of lymphokines. Furthermore, the appearance of these biological functions is correlated with the development of specific cell-surface receptors for an agent which induces these same functions. The incubation of U937 cells with culture supernates from stimulated lymphocytes induces the appearance of specific cell-surface receptors for N-formylated peptides which are potent chemoattractants and secretagogues for leukocytes. Cells incubated with lymphocyte supernates for increasing periods of time developed increasing numbers of receptors. The numbers of sites per cell, assuming cellular homogeneity were 4,505 ± 1,138, 22,150 ± 4,030, and 37,200 ± 8,000 for cells stimulated for 24, 48, or 72 h, respectively. The development of greater chemotactic responsiveness in the U937 cells paralleled the appearance of larger numbers of receptors for the N-formylated peptides. The affinity of the receptor did not appear to differ during the various times after stimulation with lymphokines and consistently ranged between 15 and 30 nM. The binding of fMet-Leu-[\(^{3}\)H]Phe to the receptor sites was rapid and reversible in the presence of a large excess of unlabeled peptide.

Although we could detect no consistent specific binding of fMet-Leu-[\(^{3}\)H]Phe or chemotactic activity by unstimulated cells, some release of lysozyme and \(\beta\)-glucuronidase was detected when these cells were exposed to high doses (10 \(\mu\)M) of fMet-Leu-Phe. The unactivated U937 cells could contain small numbers of low-affinity binding sites for fMet-Leu-Phe on their surfaces. The radioligand-binding assay is insufficient to detect low-affinity binding sites because the high concentrations of fMet-Leu-[\(^{3}\)H]Phe needed for such analyses results in large amounts of nonspecific binding to the cells. The release of enzymes in unstimulated cells cannot be attributed to an increase in receptor number as a result of incubation with cytochalasin B because this compound did not affect the amount of fMet-Leu-[\(^{3}\)H]Phe bound to these cells.

The affinity of a series of N-formylated peptides for the receptor on stimulated U937 cells paralleled the activity of the peptides for producing chemotaxis and lysosomal enzyme release, thereby demonstrating the specificity of the receptor as well as its biological relevance. The correlation coefficients (\(r\)) calculated from the EC\(_{50}\) for
inhibition of fMet-Leu-[3H]Phe binding and the EC50 for chemotaxis and lysosomal enzyme secretion were both 0.99. The specificity of the receptor on activated U937 cells was similar to that found on guinea pig macrophages (25, 26), but may be slightly different from that present on human peripheral blood monocytes. The potencies of fMet-Met-Met and fNle-Leu-Phe for human monocyte chemotaxis are less than that of fMet-Leu-Phe (M. C. Pike and R. Snyderman. Unpublished observation), whereas the potencies of all three peptides for chemotaxis by stimulated U937 cells are identical. The affinity of fMet-Leu-Phe for the receptor on stimulated U937 cells (15–30 nM) is similar to the affinity of fMet-Leu-Phe for the receptors on guinea pig macrophages (8–12 nM) and human polymorphonuclear leukocytes (10–15 nM) (1). The affinity of fMet-Leu-Phe for human monocytes has not yet been determined by direct binding assay.

The availability of a human monocyte-like cell line that, in one state, lacks detectable amounts of the N-formylated chemotactic peptide receptor, but possesses it in another state, will greatly facilitate both the biochemical characterization of the receptor and provide information concerning the regulation of the biological activities of leukocytes during differentiation processes. This cell line should also be useful for further delineating the transductional events that occur after receptor binding and that are required for chemotaxis and lysosomal enzyme release.

Summary

A human monocyte-like cell line, U937, when grown in continuous culture, does not secrete lysosomal enzymes or migrate towards chemotactic factors. When the cells are stimulated by lymphokines, however, they develop the ability both to migrate directionally and to secrete enzymes in response to several types of chemoattractants. The development, by stimulated cells, of chemotactic and secretory responses to one class of chemoattractants, the N-formylated peptides, is accompanied by the appearance on the cells of specific binding sites for these substances. Using tritiated N-formyl-methionyl-leucyl-phenylalanine (fMet-Leu-[3H]Phe) as a ligand, it was determined that unstimulated U937 cells possess no detectable binding sites. However, after stimulation with lymphocyte culture supernates for 24, 48, and 72 h, they developed 4,505 ± 1,138, 22,150 ± 4,030, and 37,200 ± 8,000 sites/cell, respectively. The dissociation constants for the interaction of fMet-Leu-[3H]Phe with the binding sites were approximately the same regardless of stimulation time and ranged between 15 and 30 nM. The binding of fMet-Leu-[3H]Phe by stimulated U937 cells was rapid and readily reversed by the addition of a large excess of unlabeled peptide. The affinity of a series of N-formylated peptides for binding to U937 cells exactly reflected the potency of the peptides in inducing lysosomal enzyme secretion and chemotaxis. The availability of a continuous human monocytic cell line that can be induced to express receptors for N-formylated peptides will provide a useful tool not only for the characterization of such receptors but also for the delineation of regulatory mechanisms involved in cellular differentiation and the chemotactic response.

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