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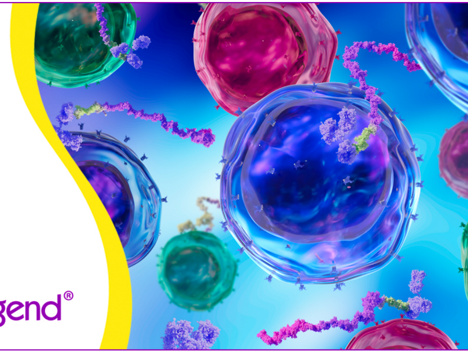

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Chemotactically Responsive and Nonresponsive Forms of a Continuous Human Monocyte Cell Line[1]

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Leukocyte accumulation at sites of antigen is essential for immunologically mediated host defense, and all types of inflammatory cells thus far studied have been found to be capable of directed migration along chemical gradients *in vitro* (1, 2). This type of cellular behavior, termed chemotaxis, appears to be an important mechanism for the accumulation of leukocytes *in vivo* (3-5). Although much information has been gathered regarding the biochemical nature of chemoattractants, little is known about the mechanisms by which the interaction of chemotactic factors with cells results in their directed locomotion. Studies of the biochemical aspects of chemotaxis would be greatly facilitated by the availability of cell lines that could be manipulated in such a way as to be obtained in chemotactically responsive and unresponsive states. Recently, a human monocyte-like cell line termed U937 has been described (6-8). We have demonstrated that when these cells are incubated with culture supernatants of lectin-stimulated but not unstimulated lymphocytes, they develop an increase propensity to destroy antibody-coated tumor or erythroid cells (8). In the present report, we demonstrate that continuously grown U937 cells lack the ability to migrate directionally but develop chemotactic responsiveness to three different stimuli after incubation of the cells with supernatants of lectin-stimulated lymphocyte cultures. Furthermore, the acquisition of responsiveness to one well defined chemotactic factor *N*-formyl-methionyl-leucyl-phenylalanine (fMet-Leu-Phe)[3] (9, 10) is associated with the appearance of a high affinity-binding site for this peptide on the surface of the U937 cells.

U937 cells were cultured in RPMI 1640 medium supplemented with 10% heated fetal calf serum (FCS) and 2 mM L-glutamine, 1 mM pyruvate, 1× essential amino acids (B & B Research Laboratories, Inc., Baltimore, Md.) and 50 μg/ml gentamicin (Schering Corp., Kenilworth, N. J.) (RPMI-FCS).

Lymphokine-rich supernatants were obtained from Ficoll-hypaque separated human mononuclear leukocytes (1×10^6 cells/ml) consisting of approximately 80% lymphocytes and 20% monocytes that had been incubated in RPMI-FCS with 1 μg/ml phytohemagglutinin (PHA) for 48 hr.

Chemotaxis was assayed *in vitro* in modified Boyden chambers as previously described (11). Briefly, washed U937 cells were suspended in Gey's balanced salt solution (Flow Laboratories, Rockville, Md.) containing 2% bovine serum albumin and 0.01 M HEPES buffer, pH 7.0 (Gey's BSS) to a concentration of 2×10^6 cells/ml. Two-tenths milliliter of the cell suspension was placed in the upper compartment of a modified Boyden chamber and separated from the chemotactic stimulant in the lower compartment by a 5.0-μ polycarbonate (Nuclepore, Pleasanton, Calif.) or a 8.0-μ nitrocellulose filter (Millipore Corp., Bedford, Mass.). After incubation at 37°C for 2 hr the filters were removed, fixed in ethanol, and stained in hematoxylin. When using polycarbonate filters, chemotaxis was scored by counting and averaging the number of cells that migrated completely through the filter in 20 microscopic fields. The distance to which the leading front of cells had migrated into the filter was used to measure chemotaxis in nitrocellulose filters (12). All assays were performed in triplicate, and each experiment was done at least three times.

U937 cells cultured in RPMI-FCS (Time 0) lacked chemotactic responses to three types of chemoattractants: a lymphocyte-derived chemotactic factor (LDCF) (13), activated human serum (AHS) (14), and fMet-Leu-Phe (9) (Fig. 1). When the cells were cultured in the presence of 5% (v/v) lymphokine-rich supernatants, however, they developed chemotactic responses to all three attractants (Fig. 1). The response to LDCF and AHS reached approximately 80% of the maximal value after a 24-hr incubation with lymphokines and peaked at 48 hr. In contrast, the chemotactic response to fMet-Leu-Phe was only 20% of maximum at 24 hr and peaked at 72 hr. When cultured in the presence of supernatants from unstimulated lymphocytes (5% v/v) U937 cells did not develop the ability to respond chemotactically.

The differences in the responsive and nonresponsive states of the U937 cells could not be explained by differential adherence of the cells to the underside of the polycarbonate filters, since we did not observe increased numbers of cells in the bottom compartment of the chemotaxis chambers containing the unresponsive cells. In addition, the migration of the cells was tested by using nitrocellulose filters, which, unlike polycarbonate filters, are thick and structured so that nonadherent cells are also trapped in their pores. Chemotaxis was quantified by measuring the distance that the leading front of cells had migrated into the filter during a 2-hr incubation time. The cells cultured in the absence of lymphokines migrated 22.5 ± 3.5μ into the filter regardless of whether the chemoattractant AHS was or was not present in the lower compartment of the chemotaxis chamber. The U937 cells, which were cultured in

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[3] *Abbreviations used in this paper*: AHS, zymosan-activated human serum; fMet-Leu-Phe, *N*-formyl-methionyl-leucyl-phenylalanine; Gey's BSS, Gey's balanced salt solution containing 2% bovine albumin serum, 0.01 M HEPES buffer, pH 7.0; LDCF, lymphocyte derived chemotactic factor; RPMI-FCS, medium RPMI 1640 containing 10% FCS; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

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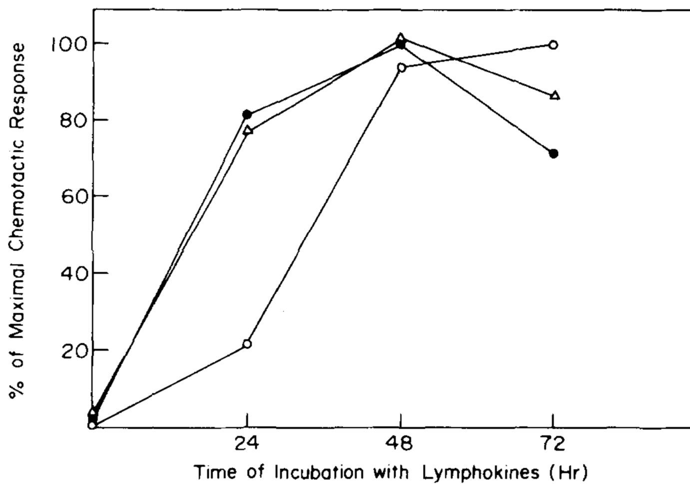

Figure 1. Kinetics of development of chemotactic response in U937 cells incubated with lymphokines. U937 cells were incubated in RPMI supplemented with 10% FCS and 5% lymphokine-containing supernatants. After the indicated incubation time the cells were washed and tested for their *in vitro* chemotactic response to a gradient of activated human serum (AHS) (●), LDCF (△), or the chemotactic peptide, fMet-Leu-Phe (○). Chemotaxis is expressed as the percent of the maximal responses obtained for each factor which were (number of cells per oil immersion field ± S.D. × 1000); 42.9 ± 5.2 for AHS; 39.5 ± 8.3 for LDCF, 21.1 ± 3.1 for fMet-Leu-Phe. Each point represents the mean of triplicate samples from two to six experiments.

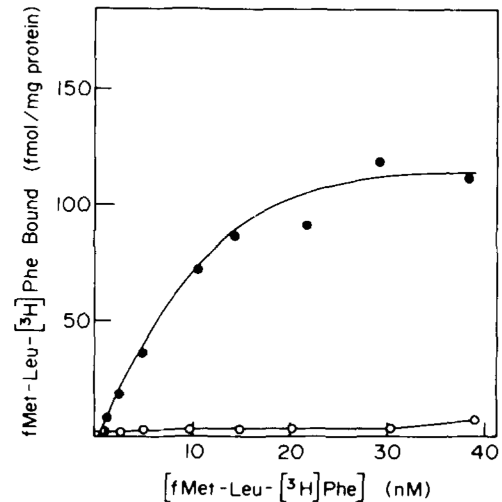

Figure 2. Binding of fMet-Leu-[^{3}H]Phe to unstimulated and stimulated U937 cells. Cells were cultured in RPMI-FCS alone or containing 5% lymphokine supernatants for 48 hr, washed, and specific binding was determined. Specific binding is total fMet-Leu-[^{3}H]Phe bound minus the nonspecific binding. Nonspecific binding is defined as the amount of fMet-Leu-[^{3}H]Phe bound in the presence of 10 μM unlabeled fMet-Leu-Phe. Results shown are the mean of duplicate samples from one representative experiment. Standard deviations were <10% and too small to include in the graph. Similar results were obtained in three additional experiments. ○, Unstimulated; ●, stimulated.

5% lymphokines for 24 hr migrated 33.1 ± 1.3μ into the filter in the absence of AHS and 54.3 ± 1.3μ in its presence.

A checkerboard experiment (12) was performed to determine whether the cells were exhibiting a true chemotactic response or solely increased random migration (chemokinesis) in response to the attractants. U937 cells that had been incubated in lymphokine supernatants for 48 hr, then washed, were placed in the chemotaxis chambers along with various concentrations of LDCF in the cell and/or chemotactic factor compartments. When equal concentrations of stimulant were added to the upper and the lower chamber the response was higher than that of cells incubated with medium alone (chemokinesis). With increasing concentration of LDCF in the lower chamber, the cells exhibited increasing migration. These results are consistent with the previously described characteristics of a true chemotactic response (12, 15, 16).

The existence of cell-surface receptors for the *N*-formylated chemotactic peptides on human and rabbit polymorphonuclear leukocytes and on a human promyelocytic cell line (HL-60) has been previously described (17–20). In order to determine if the development of chemotactic responsiveness of the U937 cells was associated with the appearance of receptors for chemotactic factors on the cells' surface, we investigated the binding characteristics of the radiolabeled chemoattractant fMet-Leu-[^{3}H]Phe (17) to lymphokine-treated and untreated U937 cells. U937 cells incubated for 48 hr in medium with 5% lymphokines, or medium alone were suspended in phosphate-buffered saline (PBS) and five million cells were incubated with various concentrations of fMet-Leu-[^{3}H]Phe in the presence and absence of 10 μM unlabeled peptide for 60 min at 22°C. After incubation, the cell suspensions were rapidly vacuum filtered onto glass fiber filters (Whatman GFC 3), which were then washed with 24 ml of ice cold PBS (17). Specific binding of fMet-Leu-[^{3}H]-Phe was then calculated, and is defined as the total amount of fMet-Leu-[^{3}H]Phe bound minus the nonspecific binding (11). The binding of fMet-Leu-[^{3}H]Phe to the chemotactically responsive U937 cells was saturable approaching a concentration of 120 fmoles/mg protein (Fig. 2). This value corresponded to approximately 19,000 sites per cell as determined by Scatchard analysis (21) (N = 4). The equilibrium dissociation constant (K_D) for the interaction of fMet-Leu-[^{3}H]Phe was 15 to 30 nM. The binding of fMet-Leu-[^{3}H]Phe was rapid and readily reversible after the addition of a 1000-fold excess of unlabeled peptide (data not shown). In contrast, the chemotactically unresponsive cells did not specifically bind fMet-Leu-[^{3}H]Phe at concentrations of the ligand ranging from 1 to 40 nM.

These studies demonstrate that a human monocyte cell line, U937, which lacks directed migratory responses to three different chemoattractants, develops the ability to respond chemotactically when cultured in the presence of lymphokines. The directional migratory response to AHS and LDCF develops earlier than does the response to fMet-Leu-Phe. The differences in the migratory responses between the responsive and unresponsive cells could not be explained by differential adhesiveness to the polycarbonate filter, since similar results were obtained by using a nitrocellulose filter in which the depth of migration into the filter was measured. The development of the chemotactic response to fMet-Leu-Phe in the lymphokine-treated U937 cells is associated with the appearance of cell-surface-binding sites for the peptide.

These studies demonstrate that the U937 monocyte cell line when grown under normal conditions is not chemotactically responsive but can be made to differentiate into responsive cells and develop high affinity binding sites for chemotactic factors. The availability of this cell line will not only facilitate the characterization of the binding sites, but will also aid in the further delineation of other biochemical events specifically associated with the chemotactic response.

REFERENCES

1. Wilkinson, P. C. 1974. Chemotaxis and Inflammation. Churchill-Livingstone, Edinburgh.

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2. Gallin, J. I. and P. G. Quie, eds. 1978. Leukocyte Chemotaxis: Methods, Physiology and Clinical Implications. Raven Press, New York.
3. Snyderman, R., J. K. Phillips, and S. E. Mergenhagen. 1971. Biological activity of complement *in vivo*: role of C5 in the accumulation of polymorphonuclear leukocytes in inflammatory exudates. J. Exp. Med. 134:1131.
4. Cohen, S., P. A. Ward, T. Toshida, and C. L. Bucek. 1973. Biologic activities of extracts of delayed hypersensitivity skin reaction sites. Cell Immunol. 9:363.
5. Postlethwaite, A. E., and R. Snyderman. 1975. Characterization of chemotactic activity produced *in vivo* by a cell mediated immune reaction in the guinea pig. J. Immunol. 114:274.
6. Sundstrom, C., and K. Nilsson. 1976. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). Int. J. Cancer. 17:565.
7. Lachman, L. B., J. O. Moore, and R. S. Metzgar. 1978. Preparation and characterization of lymphocyte-activating factor (LAF) from acute monocytic and myelomonocytic leukemia cells. Cell Immunol. 41:199.
8. Koren, H. S., S. J. Anderson, and J. W. Larrick. 1979. *In vitro* activation of a human macrophage-like cell line. Nature 379:328.
9. Schiffmann, E., B. A. Corcoran, and S. M. Wahl. 1975. N-formylmethionyl peptides as chemoattractants for leukocytes. Proc. Natl. Acad. Sci. 72:1059.
10. Showell, H. J., R. J. Freer, S. H. Zigmond, E. Schiffmann, S. Aswanikumar, B. A. Corcoran, and E. L. Becker. 1976. The structure activity relations of synthetic peptides as chemotactic factors and inducers of lysosomal enzyme secretion for neutrophils. J. Exp. Med. 143:1154.
11. Snyderman, R., L. C. Altman, M. S. Hausman, and S. E. Mergenhagen. 1972. Human mononuclear leukocyte chemotaxis: a quantitative assay for mediators of humoral and cellular chemotactic factors. J. Immunol. 108:857.
12. Zigmond, S. H., and J. G. Hirsch. 1973. Leukocyte locomotion and chemotaxis: new methods for evaluation and demonstration of cell derived chemotactic factor. J. Exp. Med. 137:387.
13. 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 lymphocytes. J. Immunol. 110:801.
14. Snyderman, R., and S. E. Mergenhagen. 1972. Characterization of polymorphonuclear leukocyte chemotactic activity in serums activated by various inflammatory agents. *In* Biological Activities of Complement. Edited by D. G. Ingram. Karger, Basel. P. 117.
15. Keller, H. U., and E. Sorkin. 1966. Studies on chemotaxis. IV. The influence of serum factors on granulocyte locomotion. Immunology 10:409.
16. Snyderman, R., and M. C. Pike. 1978. Pathophysiological aspects of leukocyte chemotaxis: Identification of a specific chemotactic factor binding site on human granulocytes and defects of macrophage function associated with neoplasia. *In* Leukocyte Chemotaxis: Methods, Physiology and Clinical Implications. Edited by J. I. Gallin and P. G. Quie. Raven Press, New York. P. 357.
17. 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. 74:1204.
18. Aswanikumar, S., B. A. Corcoran, E. Schiffmann, A. R. Day, R. J. Freer, H. J. Showell, and C. B. Pert. 1977. Demonstration of a receptor on rabbit neutrophils for chemotactic peptides. Biochem. Biophys. Res. Commun. 74:810.
19. 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.
20. Niedel, J., I. Kahane, L. Lachman, and P. Cuatrecasas. 1980. A subpopulation of cultured human promyelocytic leukemia cells (HL-60) displays the formyl peptide chemotactic receptor. Proc. Natl. Acad. Sci. 77:1000.
21. Scatchard, G. 1949. The attraction of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. 51:660.

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