

CHEMOTAXIS BY POLYMORPHONUCLEAR LEUKOCYTES

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The ability of a cell or organism to direct its movement along a chemical gradient has fascinated biologists for over 100 yr. This process of chemotaxis requires transformation of directional information from the environment into a series of cellular responses resulting in directional movement. Many lower organisms including bacteria, protozoa, and slime molds exhibit chemotaxis. This ability helps them find nutrients, avoid noxious stimuli and aggregate at critical times in their development. Reports on higher organisms indicate that primordial germ cells (32, 36), neurons (123), tumor cells (124), and fibroblasts (115) exhibit chemotaxis. However, the leukocytes are the only vertebrate cells in which this ability has been shown definitively. Studies have focused on the chemotaxis exhibited by the neutrophilic polymorphonuclear leukocytes (PMNs), whose chemotaxis presumably facilitates their accumulation at sites of injury or infection.

Chemical gradients are important in morphogenesis (27, 31, 40, 75, 105, 106, 151, 162, 173). A gradient can impart at least two types of information to a given cell, positional and vectorial. Positional information is derived from the mean concentration of a given substance present around the cell. Positional information from gradients of diffusible substances has been implicated in the organization of insect epidermis (90), regeneration in hydra (174), and limb morphogenesis (135, 148, 149). Vectorial information at the cellular level depends on a cell's ability to detect the direction of the chemical gradient and develop a polarity along this direction (31, 76, 90). Most cells have a polarity which corresponds to the overall tissue architecture. Some polarities undoubtedly arise from localized stimuli to which cells respond. For example, local stimuli probably contribute to the differentiation of the luminal

and basal sides of epithelial cells. We know macrophages can selectively ingest opsonized particles, leaving other unopsonized particles attached to their membrane (60). Motile cells, including slime molds (29) and fibroblasts (3), are described as extending exploratory filopodia which, upon attachment to a suitable stimuli, induce cytoplasmic flow and expand into pseudopods. Other polarizations, for example, the direction of hair growth or cell migration, may be due to gradients of fixed or diffusible agents (31, 90). If the steepness of the gradient required for cell detection in these cases is similar to that needed by leukocytes (discussed below), one would expect the size of the gradient fields to be relatively small, probably in the millimeter range.

Since we do not know the chemical nature of the gradient in most developmental systems, it is difficult to study the mechanisms involved in the establishment of cell polarities. In the case of leukocyte chemotaxis, we know some of the chemical signals. Many factors have been shown to be chemotactic for polymorphonuclear leukocytes (118, 163) including: (a) serum factors (85, 158, 169, 172), particularly a fragment of the fifth component of complement (22, 132, 137, 157); (b) bacterial metabolites (86, 152); (c) cell-derived materials from sensitized lymphocytes (159) and from PMNs (18, 30, 111, 181); and (d) denatured proteins (168).

Recently, in an attempt to identify a chemotactic factor derived from bacteria, Schiffmann et al. examined the possibility that *N*-formylmethionyl peptides might be the chemotactic agents since bacteria initiate their protein synthesis with *N*-formylmethionine (127). They discovered that a number of *N*-formylmethionyl peptides are in fact chemotactically active, some at very low concentrations (10^{-10} M) (127, 134). It is not clear

which, if any, of these peptides are the natural chemotactic factors derived from bacteria. However, the potency of their activity and the fact that other small peptides obtained from washings of sensitized lungs are also chemotactic suggest that peptides are natural mediators (56, 57).

Since these peptides are active at low concentrations and can be synthesized in the laboratory, they make excellent tools for studying chemotaxis, the control of cell locomotion and the development of cell polarity. Over the last few years, they have already facilitated major advances in the study of leukocyte chemotaxis.

In this review, I will begin by describing leukocyte locomotion and assays of leukocyte chemotaxis, and then consider PMN chemotaxis as consisting of three parts: a *sensory* mechanism whereby the cell detects the presence of a stimulant and the direction of the gradient, a *transducer* mechanism by which the directional information gets transformed into cellular messengers, and an *effector* mechanism that mediates the mechanical and motile changes which produce cell polarity and locomotion. I make no attempt to be comprehensive but hope to indicate some recent advances in our understanding of PMN chemotaxis. I will focus particularly on studies investigating the cellular responses to defined peptide attractants. Studies on nonmuscle contractile proteins have been reviewed frequently and recently (see references 71, 113, and 141). I will briefly mention some studies specifically on leukocyte proteins. Other interesting topics including chemotactic factors specific for leukocytes other than neutrophilic PMNs, and the role of chemotaxis in the inflammatory response and in various malignant conditions, are beyond the scope of this review.

DESCRIPTION OF LEUKOCYTE LOCOMOTION

Chemotaxis depends upon active cell locomotion. Manipulations which affect locomotion also alter chemotaxis. However, the reverse is not necessarily true since one could alter the ability to orient without affecting the locomotion itself. Polymorphonuclear leukocyte locomotion has been observed directly *in vivo* and *in vitro* and has been analyzed by time-lapse cinematography (63, 92, 94, 116, 117, 175, 176).

The locomotion requires glycolytic energy (25, 180), a temperature between 25° and 40°C (104) and a pH between 6.5 and 7.6 (23). The motile activities of leukocytes, locomotion, and phago-

cytosis, are in general less sensitive to variations in the ionic composition (11, 51, 93, 133, 165) than to the osmotic strength of the medium (93). Locomotion is inhibited by increasing osmotic strengths about 10%. The extent of inhibition does depend upon the molecular species used to increase the tonicity (93). Polymorphonuclear leukocytes tolerate hypotonic media well; the osmotic strength can be reduced to about 50% of the normal levels without decreasing the levels of locomotion (25, 93).

Cells circulating in the blood stream or incubated in nonstimulatory medium are rounded. Without stimulation, PMNs usually remain rounded and do not extend lamellipodia (181). Most chemotactic factors, when present homogeneously in the medium, stimulate the rate of locomotion in a dose-dependent manner until locomotion is inhibited at high concentrations (85, 165, 177, 181). In early studies, the stimulation of locomotion (chemokinesis) by chemotactic factors was not recognized because the control cells were in a serum-containing medium and were moving rapidly (35, 99, 116). When stimulated by any of a number of agents including chemotactic factors, proteins, e.g., albumin, materials released from cells, and certain substrata, cells begin to extend lamellipodia, spread on a substrate and move randomly. Agents which, when present in a gradient, induce the cells to orient their locomotion are chemotactic.

The general form and coordination of PMN locomotion is similar whether the cells are moving randomly or exhibiting chemotaxis. A moving PMN has a polarized morphology which is characterized by a pseudopod in the front, a midregion containing the nucleus, and a knoblike tail. The pseudopod or lamellipodium is a thin layer of cytoplasm (about 0.2 μm thick) which excludes granules and mitochondria. It frequently forms along the substrate in the direction of movement but also can be projected into the fluid medium (4). In contrast to the leading edge of a moving fibroblast, the front of a moving PMN rarely shows ruffling. Although stationary PMNs can spread and have lamellipodia around their entire circumference, the lamellipodium in a moving PMN is normally limited to the front of the cell or to a side when a cell initiates a turn. Furthermore, in PMNs, lamellipodia rarely form from the tail. The fact that lamellipodia are extended from the front or sides of a moving cell is consistent with the observation that cells usually make turns of

less than 90° relative to their previous direction of movement; the mean angle of turn is about 50° (43, 92, 109, 129). The molecular basis for this polarity and inhibition of pseudopod formation from the tail is unknown.

The midsection of a moving cell contains most of the cytoplasmic granules and mitochondria present in PMNs. The granules are usually concentrated toward the front of the body but, as mentioned above, do not extend into the lamellipodia. The nucleus remains toward the rear of the cell and the centriole is normally between the nuclear lobes and in front of the bulk of the nucleus (17, 43, 96). The distribution and movement of the granules indicate both the physical state of the cytoplasm, a gel or sol, and the direction of cytoplasmic flow. In the region anterior to the nucleus, the granules often exhibit Brownian motion; thus, the cytoplasm in this region is in the form of a sol. In the lamellipodia, and cortex of the body, granules are either excluded or, if present, do not exhibit Brownian motion (33). In these regions, the cytoplasm is believed to be in a gel or solid form (33, 35, 43, 92, 129). The gel-like nature of the cortex is also evident from the stability of its form. Film tracings of cells demonstrate that certain features of the cell outline are conserved as the cell moves. Irregularities in the surface of the cell such as protrusions and concave indentations often remain stationary relative to the substrate while the cytoplasm inside flows forward (see Fig. 1). The concave indentations have been interpreted as signs of cell contraction (92). Alternatively, they may indicate a skeletal rather than (or in addition

to) a contractile function since the indentations do not normally increase in depth or move relative to the substrate until they are included in the constriction at the base of the tail.

The tail of a PMN usually appears as the cell translocates on a substrate. It contains granules, mitochondria and occasionally even a lobe of the nucleus. Granules in the tail do not exhibit Brownian motion (92) but at times flow forward out of the tail. This often occurs as the tail decreases in size, as though a contraction of the tail expelled its cytoplasm (42).

Direct observations indicate that PMNs are primarily adherent in the front (4, 33, 92). With an interference contrast microscope, regions of close adherence to the substrate can be seen under the lamellipodia, cell body, tail and tips of retraction fibers. None of these adhesion sites are as close to the substrate as those seen in fibroblasts (4). On adhesive substrates, cells flatten and retraction fibers are prominent; the tail can be so elongated that it has almost no constriction or knoblike appearance. It is not essential for locomotion that the tail adhere to the substrate. As cells turn, the tail can sometimes be seen to swing around to its new position, indicating that it is not stuck to the substrate (33, 92). Retraction fibers are thin strands of cytoplasm filled with microfilaments which typically extend from the distal side of the tail. These fibers are pulled out as the cell moves past its sites of adhesion. Each retraction fiber ends on a bit of substrate where an attachment site had been previously formed by a lamellipodium or the cell body (117). Eventually, the fibers are pulled up from the substrate or break,

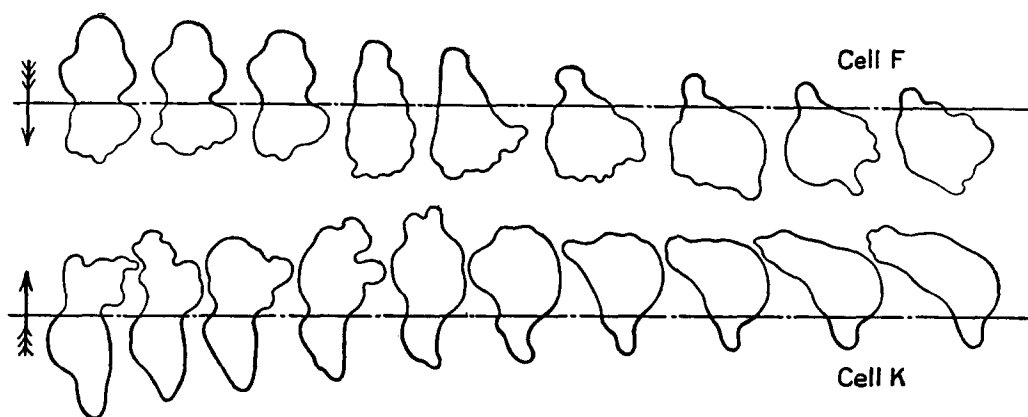


FIGURE 1 Neutrophils K and F at 2.5 s intervals. The lines represent a constant distance from the edge of the field; the constriction rings through which the lines pass remain fixed in space while the cells move forward. Figure by Warren Lewis (92).

and the portion remaining attached to the cell is resorbed into the tail. Retraction fibers can occasionally pull a poorly adherent cell backward.

Concanavalin A, other lectins and general debris from the culture dish are transported back over the surface of the cell to the tail of a moving cell (125). The molecular mechanism of transporting particles back may be similar to that of transporting the cell forward against the adhesion sites to the substrate (1, 34). Whether the cell or the particle moves would depend on the relative resistances to movement of the two objects (62). The idea that transport of adhesion sites is the basis of cell locomotion is attractive and may be partially true. However, locomotion would seem to involve more than a capping of adhesion sites since it is inhibited by cytochalasin B and enhanced by increased levels of cGMP while the capping of surface-bound concanavalin A occurs in the presence of cytochalasin B and is reduced by increased levels of cGMP (110, 125).

METHODS OF EVALUATING CHEMOTAXIS

To demonstrate chemotaxis, it is not sufficient to show that a given agent can cause cell accumulation or asymmetric spreading of a population of cells since both phenomena could arise from an action of the agent on the rate (chemokinesis) rather than direction (chemotaxis) of locomotion (63, 64). If a substance inhibits or slows locomotion, cells that happen to move near that substance will be trapped and accumulate. If a substance stimulates the rate of locomotion, cells in higher concentrations of this substance will move longer distances over the substrate. The fact that most leukocyte chemotactic factors do increase the rate of cell locomotion at moderate concentrations and inhibit the rate at high concentrations makes careful consideration of assay systems essential (163, 167, 177, 181). Two basic types of systems exist to detect and measure chemotaxis. In the first, one measures *changes in the distribution of a population of cells* after correcting for chemokinetic effects of the test agent (95, 163, 167, 181); in the second, one analyzes the *movement of individual cells* (35, 43, 63, 92, 93, 109, 116, 129, 176).

The most common assay for measuring changes in the *distribution of a population* of cells is a Millipore filter system first described by Boyden and since used to identify a number of chemotactic agents (22). In this system, cells are placed on a

Millipore filter (with 0.65 to 5 μm pores), and a gradient of the agent to be tested is established across the filter. One can then analyze the redistribution of the population by measuring (*a*) the distance that the cells have moved into the filter or (*b*) the number of cells that have moved a set distance into or completely across the filter after a given period (46, 54, 84, 181). If either measure shows an increase when a test agent replaces buffer beneath the filter, the agent has often been considered to be chemotactic. However, this assay is not a definitive test of chemotaxis; it measures a stimulation of cell migration which could be due to either chemotaxis, chemokinesis, or both. The Millipore system can be adapted into a true test of chemotaxis if the chemokinetic effects of a test substance are first determined and then corrected for when evaluating the chemotactic response (95, 163, 167, 177, 181). Similarly, the evaluation of leukocyte chemotaxis in a new under agarose assay (107, 108) is not definitive unless corrections are made for the chemokinetic effects of various concentrations of test agents.

Observing and measuring the *movements of individual cells* with microscope techniques yields detailed information on a variety of parameters of the movement including the variation among the cells of the population or in a given cell at different times (176). One can evaluate aspects such as the rate of locomotion, the frequency or magnitude of turns, and the orientation of movement relative to the gradient. Interpretation of results with this method is straightforward and much of what we know about the behavior of cells that exhibit chemotaxis has come from microscope studies.

SENSORY ASPECTS OF CHEMOTAXIS

Behavioral Studies

A great deal can be learned about the sensory abilities of leukocytes by analyzing their chemotactic behavior under controlled conditions. Studying bacterial chemotaxis, MacNab and Koshland (94) outlined two basic ways in which a cell can sense the direction of a chemical gradient. It can take a concentration reading at one point, move a certain distance, take a second concentration reading, and then compare the two readings. If the second concentration is greater than the first, then the cell could know that it has been moving up a concentration gradient. This has been termed the *temporal* mechanism of sensing a gradient since the cell compares the concentra-

tions at different times (94). Alternatively, a cell can compare the concentration difference across its own dimensions and determine which side is in contact with the higher concentration. It has been argued that a cell or bacterium is too small to use this *spatial* mechanism (94). Studies on bacterial chemotaxis have shown that bacteria are able to exhibit chemotaxis when the gradient across their own dimensions is only 10^{-4} (a 0.01% difference in concentration across their dimensions) and under conditions where the random fluctuation in the instantaneous local concentration of a chemical would seem to make it impossible for a bacterium to detect this difference (94). Indeed, several studies have shown that the bacterium does not sense the gradient across its own dimensions but rather uses the temporal mechanism, comparing the concentrations as it swims. Since a bacterium swims on the order of 100 cell lengths before making a change in direction, the actual concentration difference that it is required to detect may be closer to 10^{-2} (1%) (14, 15, 94).

The question of whether a leukocyte also must move in a gradient in order to detect its direction has been investigated. Stationary leukocytes placed in a gradient of chemotactic factor are able to initiate locomotion in the direction of the higher concentration of attractant. Since they can start off in the correct direction, they must sense the gradient across their own dimensions (178) and are, therefore, using a spatial mechanism.

Another difference between bacterial chemotaxis and leukocyte chemotaxis is illustrated by the behavioral mechanism with which the cells orient their movement in a gradient. The bacterium orients in the gradient by altering the frequency of its turns as a function of whether it is swimming up or down the gradient. There is no preferred direction of turn, but, merely by turning more frequently when swimming down rather than up a gradient of attractant, most cells at any given time move up the gradient (14). This form of orientation was called *phobotaxis* in the early 1900's because it was believed that the cells appeared to turn away from a negative environment (39). This seems to be characteristic of most organisms which use a temporal mechanism of sensing the gradient (39). In contrast, leukocytes orient their movement primarily by turning in the preferred direction, i.e., into the steepest part of the gradient and not by altering the frequency or magnitude of their turns (16, 116, 176). They can turn directly toward a new attractant, such as an

erythrocyte lysed with a laser beam (16) or a pipette containing chemoattractant (116). Thus, leukocytes orient their locomotion toward the chemotactic source, i.e., exhibit *topotaxis* (39). This process involves vectorial or directional responses to the gradient rather than merely quantitative ones. Topotaxis is also exhibited by certain lower organisms, including slime molds (98). The nematode is an example of an organism that appears to use a form of temporal discrimination and yet exhibits topotaxis (160). In theory, the temporal mechanism requires only one chemotactic receptor and a memory system (94) while the spatial mechanism of the leukocyte requires multiple receptors across the cell's dimensions (or one mobile receptor which could be moved across the cell).

The accuracy of leukocyte orientation can be measured by the McCutcheon index: the ratio of the length of a straight line toward a chemotactic source to the length of the path the cell actually took. In some studies, the index has been as high as 0.85 (35, 64, 116, 176). The accuracy of orientation also can be evaluated by determining the direction of movement of each of the cells in a population relative to a direct line to the chemotactic source. In appropriate gradients, at least 60% of the cells orient within 30° of the direct line to the attractant (176, 178, 179).

Many external factors that induce cell changes do so by interacting with specific cell receptors. Stimulation of functions such as locomotion or enzyme release may occur when any given percentage of the receptors are bound. In contrast, for chemotaxis the cell must be able to detect *differences* in the concentration of an agent, not merely its absolute concentration (138). If the chemotactic factor effects a cell response by interacting with a cell receptor which has typical binding characteristics, one would expect that the cell would best be able to sense a difference in the concentration of a factor and exhibit chemotaxis at the concentration of the dissociation constant (Kd) of the receptor when about 50% of the receptors are bound. At this concentration, a given change in the amount of factor present would result in a maximum change in the number of receptors occupied across the cell dimensions. At lower concentrations, most receptors would be unoccupied and at higher concentrations most receptors would be saturated. Thus, by determining the concentration at which the cells exhibit optimal chemotaxis in a standard gradient, one

should be able to predict the binding constant of that factor to the cell (179).

Such studies with peptide chemotactic factors have been done, and the predicted binding constant does correspond to the one measured with radioactive peptides (170, 178, 179, 181). The optimal concentration range can also be used to examine the sensitivity to the cell to detect differences in the concentration of an attractant across its dimensions. In the optimal range, PMNs orient with a 1% concentration difference of a chemotactic peptide across their dimensions (179).

Structural Requirements of Peptide Factors

The discovery by Schiffmann that small synthetic peptides are chemotactic for neutrophils has made possible systematic studies of the structural features of the peptides that account for their activity. More than 20 peptides have been tested to determine the concentration of each which stimulates the migration of cells into a Millipore filter (134). The results are expressed in terms of the concentrations required for a half-maximal response: ED_{50} , the end dose or concentration which stimulates 50% of the maximal cell migration into the filter. The ability of a given peptide to induce a response at low concentrations, low ED_{50} 's, depends on a number of structural features. The *N*-formyl group is important in conferring activity; omitting the formyl group or replacing it with the bulkier *N*-acetyl group increases the concentrations of peptide required to get a response (127). Thus, while F-Met-Leu-Phe has an ED_{50} of 7×10^{-11} M, Met-Leu-Phe has an ED_{50} of 7×10^{-7} M (134). The methionine, although effective as the amino terminal amino acid, is not essential. As long as the second amino acid is neutral and nonpolar, the activities are similar; thus F-Met-Leu, F-Met-Phe, and F-Met-Met all have an ED_{50} of between 4 and 9×10^{-7} M. A peptide with an acidic, F-Met-Glu, or a basic, F-Met-His, second amino acid has a lower activity (increased concentrations are required) (134). Phenylalanine in the third position increases the activity of a peptide. This effect is not merely due to an additional amino acid since F-Met-Met-Met has an ED_{50} of 5×10^{-9} M, and F-Met-Met-Ala has an ED_{50} of 5×10^{-7} M. The order and not merely the hydrophobic character of the amino acids is important; inverting the final two amino acids of F-Met-Leu-Phe to F-Met-Phe-Leu lowers the ED_{50} by threefold to 5×10^{-8} M. The

peptides appear to act at the cell surface since F-Met-Leu-Phe-Lys-Inulin is active (6).

In addition to stimulating cell migration, at increased concentrations (2- to 10-fold), the peptides are able to induce lysosomal enzyme release from cells on a substrate such as a Millipore filter or from cells treated with $10 \mu\text{g/ml}$ cytochalasin B. The concentration of each peptide needed to induce enzyme release is correlated with the concentration required to stimulate migration. This was shown by a coefficient of correlation, $r = 0.98$ for 24 peptides which had peak chemotactic activities at concentrations between 10^{-4} and 10^{-11} M. The parallel doses required for chemotaxis and lysosomal enzyme release indicate that the same peptide-receptor interaction leads to both functional responses (134). In the presence of cytochalasin B, 88 + 19% of the total lysozyme and 53 + 7% of total cell B glucuronidase can be released by peptides. Slightly higher concentrations of peptide are required to induce release of B glucuronidase than lysozyme. The enzyme release requires external calcium (13). The basis for the enhancement by cytochalasin B is not clear. An interesting study has recently demonstrated that cytochalasin B greatly enhances fluxes of calcium and sodium into PMNs treated with F-Met-Leu-Phe (103). These increased fluxes may effect the enzyme release (103). Also unclear is the physiological significance of enzyme release. The released digestive enzymes could aid cell movement through the tissues. In addition, the factors released may play a role in modulating the inflammatory response.

The ability of chemotactic factors to induce lysosomal release to these synthetic peptides but appears to be true of most chemotactic factors, including C5a and denatured proteins (12, 13, 58, 163).

The functional studies to date would suggest that a single receptor-ligand interaction leads to stimulated locomotion (chemokinesis), chemotaxis, and enzyme release, and that these responses occur in different, but overlapping, dose ranges (134). At low doses, the stimulation of locomotion is the first response observed. At moderate doses, presumably near the K_d , chemotaxis and rates of locomotion are optimal. The dose-response curve for enzyme release in the presence of cytochalasin B is sharp, perhaps indicating that a relatively high percentage of the receptors must be occupied to stimulate release.

Peptide Receptor

The binding of tritiated peptides to PMNs is

specific, saturable, and of high affinity (5, 170). The binding constants of both formylnorleucyl-leucyl-phenylalanine (F-Norleu-Leu-Phe) to rabbit peritoneal PMNs (5) and of F-Met-Leu-Phe to human PMNs (170) are similar to those concentrations of the peptide which gave optimal chemotactic responses (5, 170). The number of F-Norleu-Leu-Phe binding sites on rabbit cells is estimated from a Scatchard plot to be 10^8 while the human cells are estimated to have only 2×10^3 sites/cell for F-Met-Leu-Phe.

The failure of several other tissues, including rat erythrocytes, human platelets, circulating human lymphocytes, and rat brain synaptic membrane, to show high affinity binding is indicative of tissue specificity (5, 170). The functional specificity is demonstrated by the ability of other peptides and a bacterially derived chemotactic factor to compete for the binding at concentrations paralleling their potencies as chemotactic agents (5, 134, 170). The correlation coefficient for concentration of peptide for half-optimal chemotaxis and the concentration which inhibited one half of the binding was 0.998 in the studies on human cells.

The ability of 12 different peptides to compete for binding at concentrations corresponding to those of their optimal chemotactic activity suggests that many of the structural features required for chemotaxis are related to the ability of the peptide to bind to the receptor (5, 134, 170).

In addition to the affinity of a peptide for the binding site, one can examine the efficacy of the binding, that is, the ability of the bound molecule to induce activation. Although the data are not yet sufficiently precise for detailed analysis, the binding of F-Norleu-Leu-Phe to rabbit cells shows some variation in the ratio of the ED_{50} (for the migration assay) and the ID_{50} (dose which inhibits 50% of the F-Norleu-Leu-Phe binding) for different peptides. These ratios varied between 0.11 and 2. Of the peptides examined, F-Met-Leu had the highest $ED_{50}:ID_{50}$ ratio; F-Met-Leu-Glu had the lowest. The two peptides with a carboxy terminal phenylalanine had relatively low $ED_{50}:ID_{50}$ ratios (0.2). These data suggest that the carboxy terminal phenylalanine is particularly important for the efficacy of the interaction while the *N*-formylmethionine gives the peptide a higher affinity for the binding site. In studies on the ability of various inactive tripeptides to inhibit the chemotactic activity of the tetrapeptides, Val-Gly-Ser-Glu or Ala-Gly-Ser-Glu, on eosinophils, Goetzl and Austen concluded that the amino end

of the peptide was critical for the binding of the peptides to the cells while the carboxy terminal was important for activity (57). They also made a distinction between the magnitude of the cell response induced by a certain peptide and its ED_{50} . These two measures of peptide activity are not correlated. Thus, Leu-Gly-Ser-Glu had peak activity at a lower dose than did Ala/Val-Gly-Ser-Glu, but the magnitude of the cell response was never as high as that caused by Ala/Val-Gly-Ser-Glu (57). Information on peptide receptors in eosinophil PMNs has been recently reviewed (19).

In Millipore filter assays of chemotaxis, the peptides require the presence of albumin to be active. The peptides do bind to albumin and can be eluted from a Sephadex column with the albumin peak (166). However, cells orient toward peptides in the absence of protein in a visual assay system (178, 181), and the binding occurs in the absence of albumin or other proteins in the medium (5, 170).

Although various active formyl methionyl peptides compete with one another for binding to the receptor, C5a does not compete for the peptide-binding site even when it is present at concentrations well above those which cause maximal chemotaxis. Thus, there must be more than one type of chemotactic receptor. Oxidized lipids, reported to be chemotactic, also do not compete for binding (153, 170). The number of receptors required to account for the diverse variety of chemotactic agents is not known. It will be of interest if denatured proteins compete for the peptide-binding site since this might suggest that "nonspecific" chemotactic (and phagocytic) agents have specific sequences that signal their denatured form. Alternatively, factors such as denatured proteins and alkylated albumin may have different receptors or interact with the membrane via relatively nonspecific hydrophobic forces (163, 170). The topic of membrane receptors on PMNs has been recently reviewed by Henson (68).

TRANSDUCER MECHANISMS

The binding of chemotactic factors to the cell membrane initiates a series of molecular changes culminating in directed locomotion. These changes control the activation of the mechanical features involved in chemotaxis. Studies of transducer mechanisms are complicated in the case of leukocyte chemotaxis by the fact that the chemotactic factors do not act merely to orient the cells. Most chemotactic factors also stimulate the rate of cell locomotion (are chemokinetic) and, under

certain conditions, induce the release of lysosomal enzymes from cells. These related responses make it difficult to interpret the various molecular changes which include alterations in ion levels, membrane potentials, cyclic nucleotides, and hydrolytic enzymes which occur upon addition of chemotactic factors (9, 20, 44, 45, 47, 48, 51, 55, 66, 69, 70, 73, 74, 88, 101, 102, 155, 156). Certain messages must contain directional information, while others may be activators of the motile processes. An understanding of the transduction process should contribute to an understanding of cell motility. I will discuss only some of the more recent studies on possible transducers. For further information, the reader can refer to several recent reviews (48, 50, 118, 163).

Ionic Requirements for Chemotaxis

Involvement of changes in the internal ionic environment in the regulation of chemotaxis is appealing since this could be controlled by the chemotactic receptor's altering the membrane permeability. Examples of permeability changes induced by a membrane receptor exist, including some which require threshold levels of stimulation followed by a non-excitatory refractory period (82). Such features could contribute to the leukocyte directional response.

Leukocyte locomotion and chemotaxis are remarkably persistent in media of varied ionic composition. The absence of potassium in the external medium has little or no effect on cell locomotion (133, 165) although Showell and Becker (133) report a decreased sensitivity to chemotactic factors by cells incubated in the absence of potassium. The presence of ouabain, an inhibitor of the sodium-potassium-ATPase, also decreases the sensitivity to chemotactic factors but has little effect on maximal rates of locomotion (133, 165). Cells can move in the absence of external sodium, and in fact, the levels of random locomotion are stimulated when all of the sodium is replaced by potassium, choline, or even glucose. Even in the absence of calcium, cells can move at about 50% of their normal levels (11, 51, 93, 165). However, in the presence of 10^{-3} M LaCl_3 which blocks transmembrane calcium movements, locomotion is inhibited (20). In a visual assay system, cell polarization in a chemotactic gradient can develop in the absence of calcium and in the presence of 1 mM EDTA or ethylene glycol-bis(β -aminoethyl ether) N,N,N,N' -tetraacetate (EGTA) (178, 179).

Although the decreased locomotion in the absence of calcium could be due to effects on cell-substrate adhesions or to nonspecific effects, such as effects on membrane stability, calcium levels probably are critical to some aspect of the motile apparatus (71).

Ionic Fluxes Associated with Addition of Chemotactic Factors

In spite of normal locomotion in various ionic media, chemotactic factors do induce changes in the fluxes of ^{22}Na , ^{42}K , and ^{45}Ca across PMN membranes (20, 44, 48, 51, 101, 102, 103). Using these radioactive elements, one can look at a change in the uptake from the medium (influx), a change in the rate of loss from a cell previously equilibrated with the element (efflux), or a change in the amount of cell-associated label (intracellular) under equilibrium conditions. *N*-formylmethionyl peptides increase the rate of ^{22}Na influx into rabbit peritoneal exudate PMNs up to fourfold. The initial rate of influx, measured after 5 min, is proportional to the concentration of F-Met-Leu-Phe between 1×10^{-10} and 3×10^{-9} M. The optimal concentration of peptide for sodium influx is higher than that which maximally stimulates locomotion (101, 102). After a 5- to 7-min lag, an increase in sodium efflux and ^{42}K influx (20% increase) is observed. These later fluxes can be inhibited by 10^{-5} M ouabain, suggesting that the Na^+ , K^+ -ATPase has been stimulated (10). The peak peptide concentration for this K^+ influx is about 3×10^{-10} M F-Met-Leu-Phe, close to that of maximal migration. No change in potassium efflux is observed.

The efflux, influx, and internal pool of calcium are increased in polymorphonuclear leukocytes treated with chemotactic factors. A marked efflux was noted in human cells treated with C5a (20, 51) or in rabbit cells treated with 10^{-9} M F-Met-Leu-Phe (101, 102). In experiments which used higher concentrations of chemotactic agents or included LaCl_3 washes, a net influx of calcium is also seen (20, 101, 102). This influx requires at least 0.25 mM calcium in the external medium and can be blocked by 1 mM La^{+3} (20, 101, 102). The calcium uptake results in an increase in intracellular calcium comparable in magnitude to the increase that stimulates contraction in skeletal actomyosin (20). Increased pyroantimonate precipitate in human neutrophils treated with acti-

vated serum may be an indication of the increased calcium levels (48).

Further information regarding the ionic changes occurring upon addition of the chemotactic factors comes from studies on changes in the membrane potentials. Using a fluorescent dye, Seligmann et al. (128) have obtained evidence for a change in membrane potential of PMNs that were exposed to F-Met-Leu-Phe. Thus far, there have been no microelectrode studies measuring potential changes in PMNs, but Gallin and Gallin (44) and Gallin et al. (48) have recorded them from cultured human macrophages stimulated by various chemotactic factors including kallikrein, C5a, and F-Met-Leu-Phe (44, 48). Although the responses are variable, 80% of the cells examined show some change in membrane potential. About 20% of the cells have a brief, 3-s depolarization which is followed by a more sustained, 20-s or longer hyperpolarization -10 to -50 mV in amplitude. In nearly 60% of the cells, the hyperpolarization is not preceded by a measurable depolarization. A second potential change can be stimulated by removing the source of chemotactic factor and reintroducing it; however, several repeated stimulations result in desensitization. The evoked potential changes are inhibited by 2.5 mM Mg^{++} with 5.0 mM EGTA.

This hyperpolarization is associated with a decrease in membrane resistance as monitored by the decreased electrotonic potential produced by constant current pulses (44, 48). The membrane potential at which the depolarizing response disappeared (the reversal potential) was 0 mV. These authors postulate that a reversal potential of 0 mV is compatible with depolarization resulting from a concomitant increase in the permeability of Ca^{++} or Na^{+} (flowing in) and K^{+} (flowing out). The reversal potential for the hyperpolarizing response was -70 mV which could arise from increased potassium permeability (44, 48). Gallin and Gallin and Gallin et al. suggest that a small transient Ca^{++} or Na^{+} influx is followed by a larger, prolonged potassium efflux causing the hyperpolarization (44, 48). The emphasis on the hyperpolarization's being due to a potassium efflux puts the recording data at odds with the flux studies where the major change noted was the large sodium influx (101, 102). Such comparisons are probably premature since the cell type, the time-courses of the events being examined and the doses of chemotactic factors used are not the

same. The possibility remains that some or all of these changes are more relevant to enzyme release induced by the chemotactic factors than to the chemotactic response. Recent evidence from Becker's lab supports this possibility (103).

Digestive Enzymes Associated with Leukocyte Chemotaxis

A number of studies have focused on defining the role of various proteolytic activities in the leukocyte chemotactic response. Cell proteases may have a role in the production (157, 171) and destruction (6) of active peptides. Hydrolytic activities have also been implicated in permitting (53, 150, 156, 171) and inhibiting the cell response (53, 136). In addition, as mentioned above, under appropriate conditions chemotactic factors stimulate release of lysosomal enzymes. The abundance of hydrolytic enzymes that may be secreted or leak from damaged cells and the use, in some studies, of enzyme inhibitors that have toxic or nearly toxic effects on the cells confuse the interpretation of the results.

Several independent lines of evidence have implicated a hydrolytic enzymatic activity in leukocyte locomotion (8, 9, 154, 156, 163). Studies by Becker and co-workers demonstrate that the interaction of certain chemotactic factors with rabbit blood PMNs results in 10-20% increase of a leukocyte chymotrypsin-like esterase activity which can cleave acetyl-DL-phenylalanine B naphylester. The parallel inhibition of this enzymatic activity and PMN chemotaxis by various phosphate esters and aromatic amino acid derivatives suggested to Ward and Becker that this activity is essential for the chemotactic response (154, 156;¹ see reviews, references 8 and 163).

Recently, it has been demonstrated that rabbit leukocytes can digest the peptide chemotactic factors (6). The digestive enzyme appears chymotrypsin-like and can be inhibited with 0.1 mM L-1-(tosylamido 2-phenyl)ethyl chloromethyl ketone (TPCK) or 0.1 mM benzoyltyrosine ethyl ester (BTEE). Since these inhibitors also prevent peptide stimulation of leukocyte locomotion and lysosomal enzyme release, Aswanikumar et al. have

¹ It is of interest that two chemotactic factors, kallikrein and plasminogen activator, have enzymatic activities which seem essential for their chemotactic activity (49, 80, 81).

suggested that digestion of the peptides is essential to the functional interaction of the peptide with the cell (6). Inhibitors with broad specificity such as macroglobulin (10 $\mu\text{g}/\text{ml}$) and human antitrypsin (1 $\mu\text{g}/\text{ml}$) are also reported to inhibit human PMN chemotaxis to C5a; most trypsin specific inhibitors do not block the response (53, 171).

One can imagine the digestion (or energy derived from the digestion) of the chemotactic agent as being critical for a transduction process in the membrane; however, there is no conclusive evidence that such a protease is essential for locomotion. The effective inhibitors are often irreversible and have nonspecific actions which can damage the cell (40). The digestive enzymes associated with leukocyte chemotaxis may be important in destroying chemotactic factors and digesting and loosening intercellular spaces to permit PMN infiltration rather than in activating the motile response (77, 78).

Incubation of cells with high concentrations of certain chemotactic factors (C5a, activated serum, and casein) (155) but not others (bacterially derived factors) (9, 86, 155) leads to an irreversible inhibition of the cell's ability to respond to any chemotactic factor. This chemotactic factor-induced inhibition has been termed *deactivation*. It is most pronounced when cells are incubated in concentrations of chemotactic factors above those which optimally stimulate cell locomotion. The inhibition does not appear to be factor-specific since deactivation with C5a inhibits the subsequent cell response to bacterial factors as well as to C5a. Addition of certain phosphonate esters or aromatic amino acid derivatives to the incubation mixture prevents deactivation. Thus, deactivation may involve an esterase which is inhibited by the phosphonate esters (155).

The requirement for high doses of chemotactic factors and the presence of calcium for deactivation and the protection against deactivation by protease inhibitors suggest that the phenomenon may be mediated at least in part by released lysosomal enzymes (122). Gallin has correlated an inhibition of locomotion with the incubation conditions which induce lysosomal enzyme release (personal communication). Although the cells are perhaps damaged by the enzymes, the ability of deactivated cells to show increased hexose monophosphate shunt activity when treated with chemotactic peptides suggests that the cells are still viable and retain some receptor for the chemotactic interaction (55).

Role of Cyclic Nucleotides

Small increases (usually less than twofold) in the levels of cGMP have been noted in both PMNs and monocytes after the addition of chemotactic factors (66, 69, 70). Although cGMP itself does not appear to be chemotactic, drugs which increase the intercellular GMP levels, such as acetyl choline, carbonyl choline and imidole, enhance the chemotactic responses (38, 66, 126, 182). On the other hand, the leukocyte response can be depressed somewhat by incubating with 10^{-2} – 10^{-4} M dibutryl cAMP or agents which increase cAMP levels, such as prostaglandin E1 or epinephrine or theophylline (38, 121, 122). Temporal and quantitative discrepancies between the increases in intercellular cyclic nucleotides and the changes in chemotaxis and motility make interpretation of this data difficult (121, 122). In addition, there are conflicting reports regarding the chemotactic activity of cAMP. When assayed in a Millipore system, its activity is weak if present at all (79, 91, 121, 145, 161, 163). However, several workers report directed locomotion toward cyclic AMP when assayed by direct microscope observation (51, 61, 116). The basis for this discrepancy is unknown.

A number of molecules are probably involved in the transduction process. A change in one parameter may lead to a series of changes which eventually control features of the motile apparatus. The chemotactic response requires that at least one of these molecules have an asymmetric distribution along the direction of the gradient. The degree of asymmetry might not directly reflect the steepness of the gradient since the cell response to the gradient is not a linear response to the absolute concentration of stimulant in contact with the cell surface. With a 10% difference in concentration across its dimensions, a given cell does not merely form 10% more pseudopods on the high side, nor is there a mere 10% increase in the number of cells moving up the gradient. Rather, there is a 900% increase in the number of cells moving up the gradient (178, 179). The molecular basis for this amplification and coordination probably involves a competition across the cell dimensions. A region of the cell surface with a certain percentage of receptors bound could undergo a local change in membrane permeability. This change could then inhibit permeability changes in the rest of the cell for a certain time, and thus allow a directional response. Alterna-

tively, a difference in the number of receptors bound could cause the asymmetric distribution of a substance required for a particular function such as pseudopod formation. Further knowledge of the transducers which carry vectorial information will increase our understanding of the development of cell polarity.

EFFECTOR MECHANISM

Work on the effector mechanisms has focused on defining the structures and proteins present in the leukocyte and the factors controlling their form and function *in vitro*.

Microtubules

Microtubules are involved in the maintenance of a polarized form in a number of systems (114). Thus, it is not surprising that the role of microtubules in leukocyte locomotion and chemotaxis has been of interest to a number of investigators. Most studies have used a pharmacological approach and examined cell responses in the presence of various antimetabolic drugs, particularly colchicine and vinblastine. These drugs do cause microtubule depolymerization in PMNs (7, 51, 58, 96, 97, 178, 179). Leukocytes are capable of active and even stimulated locomotion in the absence of polymerized microtubules (7, 13, 26, 37, 72, 96, 97, 119). Several studies show that the chemotactic response can be decreased by colchicine and vinblastine (24, 96, 97, 162, 163). Other studies report little or no inhibition of PMN chemotaxis by colchicine (24, 178, 179). Wilkinson showed that colchicine inhibited the directional response of lymphoblasts migrating through a Millipore filter even while stimulating their random locomotion (164).

Since most cells have one nucleus and one centriole, an acentric location of either, or the alignment of these two organelles, gives the cell a polarity. In a typical moving PMN, the lobes of the nucleus are located toward the rear of the cell and the centriole is normally in front of the nucleus (18, 86). The cause and consequences of this asymmetry are unknown, but microtubules are involved in its maintenance. In the presence of colchicine, the position of the nucleus in cells exposed to a chemotactic gradient is irregular (96, 97). The relevance of this disorganization to chemotaxis is uncertain since cell orientation in a chemotactic gradient, as determined by a vector drawn from the tail to the center of the lamellipodium, is only slightly decreased even in high (100

$\mu\text{g/ml}$) levels of colchicine (178, 179). In addition, Keller and Bessis report oriented locomotion by cell fragments which lack both a centriole and a nucleus (83).

Drug studies are of course limited by the possibility that the effects are not specifically on the microtubules. Both colchicine and vinblastine are known to affect membrane transport (100), and these drugs have been shown to affect PMN adhesion (89) and lysosomal enzyme release (12, 13, 58, 67, 72, 111, 161). However, the involvement of microtubules in some aspect of the chemotactic response is further suggested by studies showing an increased number of polymerized tubules in cells treated with chemotactic factors (51, 58). Cells exposed to phagocytic stimuli and agents which increase the intercellular levels of GMP are also reported to have increased numbers of tubules (72, 161). The modulation of the chemotactic response by cyclic nucleotides may be mediated by alterations in the number of polymerized tubules.

Motile Apparatus

In the leukocyte, as in other cells, workers are beginning to examine the molecular basis of motile functions (28, 112, 113). Already there is documentation for the presence of actin, myosin, a cofactor that allows actin activation of the myosin ATPase, as well as an actin-binding protein that combines with actin to form a gel which is sensitive to temperature and cytochalasin B (65, 130, 131, 141-144, 146). Other proteins must contribute to the motile process. These may include other proteins prominent in muscle such as tropomyosin, troponin, and α -actinin. This field is rapidly growing and has been recently reviewed (71, 113, 141).

To understand the molecular basis of the leukocyte chemotactic response, we will want to know the distribution in the moving cell of each of these proteins and the factors controlling their activity and structural states. The organization must result in the coordinated locomotion described above including: (a) the extension of pseudopodia; (b) the presence of a cortical gel in the pseudopods and cortex and the constriction at the base of the tail; (c) the flow of cytoplasm into advancing pseudopods and out of the tail; (d) the formation, backward movement and breaking of attachments to the substrate; and (e) the polarization of the cell in the chemical gradient.

Although PMNs are the most rapidly moving of

all mammalian cells and contain about 10% of their total protein as actin, the fibrous elements seen by electron microscopy of moving PMNs are not impressive in their organization or quantity. They contain no prominent stress fibers of actin filaments such as those seen in fibroblasts. Rather, a filamentous network is visible particularly in the pseudopods, tail, and cortex. Perhaps the amorphous structure permits the rapid flexible movements characteristic of PMNs. With immunologic techniques, myosin can be seen to be distributed throughout the cytoplasm (41, 131). There is some indication that lamellipodia are enriched in actin-binding protein (21, 141). Actin filaments, 100-Å filaments and microtubules align parallel to the cell surface in regions where the cell contacts a substrate. This organization is particularly prominent in macrophages (120). The activation of the motile apparatus must be mediated by the transduction process. Although there are indications of ionic and nucleotide changes, the parameter(s) which regulates the motile apparatus remains unknown. Calcium is reported to alter the superprecipitation of a leukocyte extract containing actomyosin (130), but it does not alter the activity of the actin and cofactor activated myosin ATPase from leukocytes (142, 143). The calcium may be affecting an actin-binding protein gel since EGTA is required for the extraction of this protein from the cells (142).

In other systems, protein modifications including phosphorylations and methylations have been shown to alter the myosin ATPase (2) or alter the properties of membrane receptors (59, 147, 153). Thus far, no analysis of leukocytes at this level has been made. The control of coordinated movement of a leukocyte is undoubtedly a complicated process. Genetic complementation experiments in bacteria indicate that at least nine gene products are required for the relatively simple chemotactic response exhibited by these organisms (87). The leukocyte response, both more flexible and more complicated than that of the bacterial form, must involve control over a large number of parameters. Progress will undoubtedly involve simplifying the system by examining the behavior of purified components in vitro and analyzing particular features of the whole cell behavior. The ability to manipulate the cell orientation and rates of locomotion with defined agents makes the leukocyte system useful for investigating the molecular basis of cell orientation and locomotion.

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