SENSORY ADAPTATION OF LEUKOCYTES
TO CHEMOTACTIC PEPTIDES

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ABSTRACT

The morphology and behavior of polymorphonuclear leukocytes (PMNs) were studied after rapid changes in the concentration of a chemotactic factor N-formylnorleucylleucylphenylalanine (f-NorleuLeuPhe) (Schiffmann et al., 1975, *Proc. Natl. Acad. Sci. U. S. A.* **72**:1059-1062). After an increase in peptide concentration, the cells round, form lamellipodia or ruffles over most of their surface, and stop locomotion. These changes are transient. After a delay, the cells, still in the presence of peptide, withdraw most of the ruffles and resume locomotion, forming ruffles only at their front. Cells repeat the transient generalized ruffling upon further increase in peptide concentration. The behavioral changes occur over the same dose range as binding to a saturable receptor. The duration of the transient response after a concentration increase is roughly proportional to the increase in the number of cell receptors occupied as a result of the concentration change. Decreasing the concentration of peptide causes the cells to round transiently and form blebs before they recommence locomotion. The transient nature of these aspects of the cell's responsiveness to chemotactic factors appears to be due to adaptation by the cells. The ability to adapt to the concentration of a chemotactic factor may be important in leukocyte chemotaxis.

KEY WORDS   motility · spreading · chemotaxis · adaptation · polymorphonuclear leukocytes

Chemotaxis, the orientation of movement along a chemical gradient, is an ability exhibited by a number of cells and organisms including bacteria, slime molds, and leukocytes. Thus far, the chemotaxis by bacteria is the best understood. Bacteria have been shown to respond to changes in concentrations of chemotactic factors by transiently altering the frequency of turns in their swimming. If the concentration of a chemotactic factor is increased, the frequency of turns decreases and the bacteria tend to swim with long paths between turns. If the concentration is decreased, the frequency of turns increases and the bacteria swim only a short distance between turns (21). A bacterium senses a chemical gradient by comparing the concentration of the chemical at different times as it swims. This has been termed the "temporal" mechanism of sensing a gradient (6, 21). Since the bacteria make fewer turns when swimming up the gradient than when swimming down the gradient, the net movement of the bacteria is up the gradient. The altered frequency of turns after a change in concentration is a transient response. After a time the bacteria "adapt" to the new concentration and the turning frequency reverts to a normal or baseline level (15, 21). The duration of the altered frequency does not depend merely upon the new concentration but upon the change in concentration that the cells have experienced. Thus bacteria seem to "remember" their previous

environment for a short period of time (15, 19).

Leukocyte chemotaxis differs from that of bacteria. Leukocytes can sense the direction of a chemical gradient without locomoting in the gradient. In addition, they respond to the gradient in a directional manner, turning toward the higher concentration of chemotactic factor rather than depending upon alterations in the frequency or magnitude of their turns to orient their movement in the gradient (4, 12, 23, 28). The accuracy of the leukocyte response, as well as the ability to orient in a shallow gradient, is greatest when the concentration of the chemotactic factor is in the region of the dissociation constant, K_d, of the factor from the cell receptor (29). This is what one would expect if the cell were to detect the direction of the gradient by comparing the number of specific chemotactic receptors that are bound across its surface. This means of sensing a gradient has been called the "spatial" mechanism (21, 28).

It is not clear that either adaptation to the concentration of chemotactic factor or a memory of previous concentrations is required in spatial chemotaxis. Nevertheless, adaptation could contribute to any system which is required to detect small changes in levels of stimulation over a wide range of background stimulation. Such a requirement exists for leukocytes which must be able to detect relatively small changes in the concentration of chemotactic peptide across their dimensions while the mean concentration of peptide to which they are exposed varies over more than two orders of magnitude. Through adaptation, a cell could have an internal messenger that would vary after small rapid changes in external stimulation but that would return to a constant level during long-term exposure to a stimulant.

If leukocytes adapt to the concentration of chemotactic factors, one would expect to see some responses to the factors that decline with time. The stimulation of the rate and direction of locomotion by chemotactic factors is known to be of relatively long duration (1, 20, 30), yet certain other responses to chemotactic factors such as alterations in adhesiveness (8), membrane potentials (14), and ion fluxes (5, 22) are transient, lasting only a few minutes. The relationship of these transient responses to the persistent locomotory behavior and the basis for their limited duration have not been determined.

By performing concentration jump experiments similar to those done on bacteria, we have been able to demonstrate that certain aspects of leuko-

cyte morphology and locomotory behavior are also transiently altered. Rapid increases in the concentration of the chemotactic peptides N-formylnorleucylleucylphenylalanine (f-Norleu-LeuPhe) or N-formylmethionylmethionylmethionine (f-MetMetMet) cause polymorphonuclear leukocytes (PMNs) to stop translocation across the substrate and to form lamellipodia or ruffles over their surface. These responses are transient and the cells soon withdraw most of the ruffles and limit those remaining to a portion of their surface which becomes the cell front as the cells resume locomotion across the substrate. These changes occur even though the peptide is still maintained in solution.

Further experiments demonstrate that the transient nature of this response is not due to either a reduction in the concentration of active peptide or to the exhaustion of a component required for ruffle formation. Rather, the transient nature of the response appears to be due to some feedback control or adaptation by the cell to the peptide concentration which may be mediated at least in part by a reduction in the number of receptors available. Adaptation is defined here as the reversible extinction of a cell's responsiveness to a stimulus caused by an adjustment of the cell's sensitivity (11). As in the case of bacterial adaptation, the duration of the leukocyte transient response depends upon both the absolute concentration and the concentration change that the cells experience. Thus, the leukocyte also exhibits what has been called a short-term memory (21).

MATERIALS AND METHODS

Cells

Rabbit peritoneal exudate cells were obtained 4 h after injection of 250 ml of saline containing 0.1 g of shellfish glycogen. For morphological studies, the cells were concentrated 10-fold to 1×10^7 cell/ml and allowed to attach to a cover slip for 3 min. For binding studies the erythrocytes were lysed by treatment with hypotonic saline (0.18%). The cells were washed three times with saline before suspending them at 1 or 2×10^7 cells/ml in Hanks' balanced salt solution (Grand Island Biological Co., Grand Island, N. Y.) buffered to pH 7.0 with 2.40 mg HEPES/ml. In some experiments the medium also contained 1% Knox gelatin.

Human peripheral blood cells were obtained from a finger prick. Blood was allowed to clot on a cover slip in a warm, moist chamber. After the clot had retracted (~45 min), the clot and erythrocytes were washed off with saline. Cells attached to a cover slip were either

observed with an inverted microscope or placed in a plexiglass chamber used for studies on chemotaxis and observed in an upright microscope (29). To allow perfusion of medium in this chamber, cover slip spacers were placed on each side to increase the distance between the cells and the plexiglass barrier and to allow free flow of fluid between the two wells. The fluid can then be removed from both wells by withdrawing it from one well into a syringe. Although both wells are emptied, a layer of fluid remains over the bridge. The wells are refilled by inserting fluid from a Pasteur pipette into one well. The fluid flows over the bridge and into the other well, thus washing the cells on the bridge. The whole process takes ~15 s; the refilling is completed in 2–3 s.

To quantitate the cell response, the time-course of the behavior was analyzed in time-lapse films. The changes in the ~30 cells in the microscope field were followed. Time-lapse movie films were taken with a Sage time-lapse apparatus (Orion Research Inc., Cambridge, Mass.) on Tri-X reversal film. Still photographs were taken on Tri-X film. Studies were done at 23°C unless otherwise noted.

Scanning Electron Microscopy

For scanning electron microscopy, cells on glass cover slips were fixed in 2.5% glutaraldehyde in phosphate-buffered saline, pH 7.4. The cells were dehydrated slowly by adding 20% ethanol dropwise to the fixed cells in buffer solution and then dehydrated stepwise through 30, 50, 70, 95, and 100% ethanol. Cells in 100% ethanol were put directly into liquid CO_2 in a Tousimis Samdri (Rockville, Md.) critical point drying apparatus. After drying, they were shadowed with 50Å of gold/palladium 60/40. The specimens were examined in a Philips 501 field emission scanning microscope at 30 kV.

Binding Studies

Binding studies were carried out on rabbit peritoneal PMNs according to the general procedures of Williams (27) and Aswanikumar (2). For initial studies, the cells were incubated at 4°C for varying periods of time with tritiated f-NorleuLeuPhe (sp Act = 12.5 Ci/mM) (preliminary studies indicated that digestion of the peptides was inhibited at 4°C). At 4°C, plateau binding was reached by 15 min. Thus, in dose-response studies the cells were incubated for 15 or 20 min. After incubation, 1-ml aliquots were placed on glass fiber filters (Whatman GF/A) on a Millipore multifiltration apparatus (Millipore Corp., Bedford, Mass.) and washed with 5 ml of saline. Preliminary experiments indicated that the filters could be efficiently washed with as little as 1 ml; a 5-ml wash took <6 s. The filters were dried and counted in scintillation fluid formula 963 (New England Nuclear, Boston, Mass.) with an efficiency of ~40%. To determine the saturable binding, samples were incubated with tritiated peptide with or without an excess (10^{-6} or 10^{-5} M) of unlabeled f-NorleuLeuPhe. The unlabeled peptide

would be expected to compete for all saturable binding sites. The saturable binding was defined as the difference in counts remaining on the filter in the presence and absence of unlabeled peptide. Additional binding studies were done at 23° and 37°C.

Reversal of saturable binding was examined by incubating the cells in tritiated peptide and then adding an excess of unlabeled peptide for various times before washing. For reversal and rebinding experiments, cells were incubated with tritiated peptide in Eppendorf microcentrifuge tubes. At the time of reversal, the cells were pelleted with a 2-s spin (plus ~15 s for the centrifuge to stop) and resuspended in 1.2 ml of Hanks' medium for 30 s or 1 min. In rebinding experiments, these cells were repelleted and then resuspended in fresh tritiated peptide for various times.

RESULTS

Description of Transient Responses after Addition of Chemotactic Peptide

Human cells in serum and rabbit cells in peritoneal fluid exhibit locomotion (Fig. 1a and d). However, both cell types round and are immobile when all stimulants are removed and the cells are incubated in Hanks' medium or Hanks' medium plus 1% gelatin. When a chemotactic peptide is added to such cells, they begin to ruffle and spread on the substrate (Fig. 1b and e). The spreading and general ruffling is transient. Within a few minutes the cells withdraw most ruffles, develop a polarized morphology with ruffles only at the front, and begin to locomote (Fig. 1c and f). Although these early morphological changes are most dramatic when the peptide concentration is changed from zero to ~1 × 10^{-6} M f-Norleu-LeuPhe or f-MetMetMet, the changes can be observed after addition of concentrations as low as 6 × 10^{-10} M f-NorleuLeuPhe. The changes are not caused by the mechanical stimulation of perfusion since addition of Hanks' medium without peptide has no effect.

The time-course and the dose response of the cell ruffling and spreading were examined in time-lapse films. Ruffling begins within 20 s after addition of peptide, and maximum spreading occurs after ~1 min. By measuring the time between addition of peptide and the time when two or more cells in the microscope field begin to reround, the maximum spreading can be seen to occur between 45 and 80 s (Fig. 2). The longer times tend to be after addition of higher concentrations of peptide; however, the concentration dependence is not dramatic.
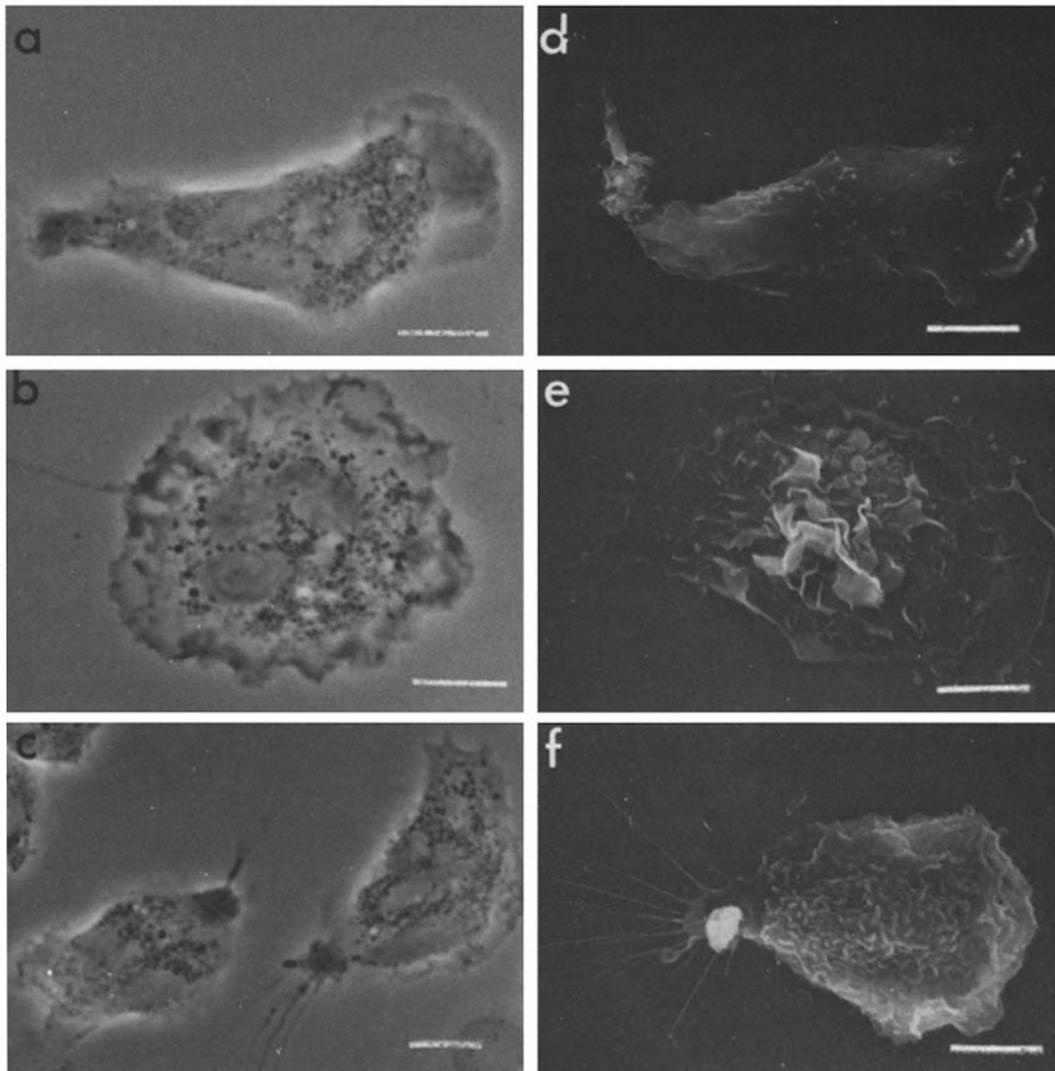
FIGURE 1 Phase and scanning electron micrographs of human PMNs after changes in peptide concentration. (a and d) 10 s after washing off serum; cell still with locomotion morphology. (b and e) 30 s in 10^{-6} M f-MetMetMet; cell flattens on substrate and ruffles from upper surface. (c and f) 3 min in 10^{-6} M f-MetMetMet; cell in transition stage, beginning to locomote. Bars, 5 µm. (a, b, d–f) × 2,400. (c) × 2,000.

The time delay between maximal spreading and ruffling and the time when 30% of the cells commence locomotion across the substrate is clearly concentration dependent. The time increases as the concentration of peptide is increased between 1×10^{-9} and 1×10^{-7} M f-NorleuLeuPhe (Fig. 3). The half-maximal delay of 200 s occurs near 2×10^{-8} M. At concentrations above 1×10^{-7} M, there appears to be a decrease in the time before resumption of locomotion but at these high concen-trations the cells often become vacuolated and locomote poorly.

Responses after Increases in the Concentration of Peptide

Cells locomoting in one concentration of peptide can be restimulated to form ruffles over most of their surface by increasing the concentration of peptide (Fig. 4). It is clear from analysis of time-
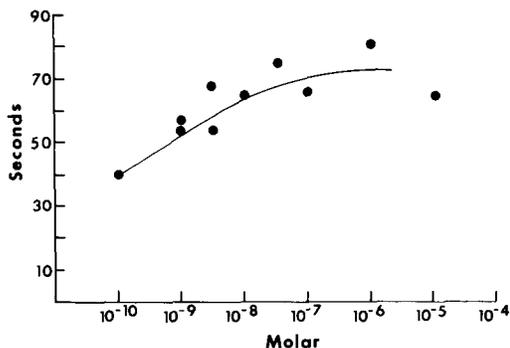
FIGURE 2 Time required for rabbit PMNs to achieve maximal spreading and ruffling after an increase in concentration of f-NoreleuLeuPhe. Peptide was added to cells in Hanks' medium; times were measured from films.

lapse films that individual cells, which have been stimulated to ruffle extensively by addition of peptide and allowed to recover and develop a normal locomoting morphology, are capable of responding again with generalized ruffling when the concentration of peptide is increased (Fig. 5).

The changes observed in cells exhibiting locomotion as the peptide is increased are slightly different from those observed in cells which are round before addition of peptide. The locomoting cells are stimulated to form ruffles over most of their surface, but ruffles do not arise from the knob-like tail. In addition, as the locomoting cells form ruffles, they tend to round rather than flatten onto the substrate. The length of locomoting cells was measured just before and 30 s after an increase in peptide concentration. 10-fold increases in concentration of peptide between 10^{-9} and 10^{-8} M, 10^{-8} and 10^{-7} M, and 10^{-7} and 10^{-6} M cause the mean length of cells to decrease by an average of 9, 16, and 12%, respectively. In contrast, reperfusion with a constant concentration of peptide causes a slight cell elongation (~5%). The change in cell length after increasing the peptide concentration compared to perfusing with the same concentration was significant at the 0.001 level ($n = 139$).

Increasing the concentration of peptide on locomoting cells also results in a transient cessation of locomotion. The increase in concentration required to stop locomotion depended upon the concentration range. While a 50% increased from 1×10^{-8} to 1.5×10^{-8} M was clearly effective, no change could be noted after a 100% increase at the ends of the dose-response curve (3×10^{-10} to $6 \times$
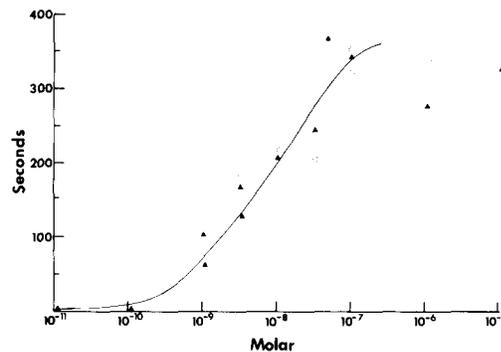

FIGURE 3 Time after a concentration change for rabbit PMNs to recommence locomotion. The time between maximal spreading and the recommencing of translocation across the substrate by 30% of the cells in the microscope field (usually ~30 cells) was determined from films. The concentration changes between 0 and the concentration of f-NorleuLeuPhe indicated on the abscissa were made in one step (▲) or in two sequential steps where the sum of two recovery times is plotted (○).

10^{-10} M and 1×10^{-6} to 2×10^{-6} M). This response correlates with changes in receptor occupancy. With a K_d of binding equal to 2×10^{-8} M (see below), the increase from 1×10^{-8} to 1.5×10^{-8} M peptide would result in a 10% increase in receptors occupied while the changes from 3 to 6 × 10^{-10} or 1 to 2 × 10^{-6} would result in less than a 2% increase in receptors occupied. The time delay before recommencing locomotion after an increase in peptide also shows a concentration dependence. In fact, the sum of recovery times upon increasing the concentration of peptide from zero to some final concentration in two successive steps is equal to the recovery time of cells undergoing the same concentration increase in a single step (Fig. 3, circles).

Cell Responses after a Decrease in Peptide Concentration

Transient changes in cell morphology and behavior are seen when the concentration of peptide is rapidly decreased. After a decrease, the cells stop locomotion, round up, and form small blebs rather than ruffles over their surface (Fig. 6 b and c). After the blebs subside, cells which are still in the presence of some peptide resume locomotion. Again, the time required to start locomotion depends on the concentration change. After drops in concentration from 1×10^{-6}, 1×10^{-7}, 3×10^{-8},
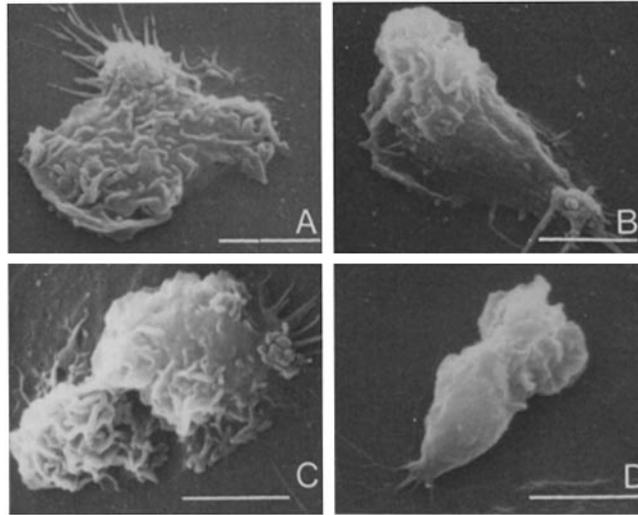
FIGURE 4 Scanning electron micrographs of human PMNs after changes in peptide concentration. (A) 30 s in 10^{-8} M f-NorleuLeuPhe; cell ruffles and shows remains of a tail as small round blebs in the upper left. (B) 3 min in 10^{-8} M f-NorleuLeuPhe; cell ruffles from the front. (C) 30 s after change from 10^{-8} to 10^{-7} M f-NorleuLeuPhe; cell flattens and ruffles. The remains of a tail can be seen on the upper right. (D) 3 min after change from 10^{-8} to 10^{-7} M f-NorleuLeuPhe; cell limits ruffling and exhibits locomotion. Bars, 10 μm. × 1,500.

1×10^{-8}, and 1×10^{-9} M each to 6×10^{-10} M, f-NorleuLeuPhe, cells resumed locomotion after delays of 380, 370, 210, 180, and 150 s, respectively.

Both increasing and decreasing the concentration of peptide caused a decrease in the length of a locomoting cell. However, the mode of shortening or rounding differs in the two cases. By tracing cell profiles in films, the maximum cell length could be determined immediately before and 30 s after a change in peptide concentration. In addition, one could determine what movements contributed to the change in length or position during this 30-s period. As illustrated in Fig. 7, the movement of the tail was evaluated by measuring the distance the back of the tail had moved during the change. The movement of the pseudopods was determined by measuring the distance between lines tangent to the direction of movement at the farthest extension of the pseudopods before and after peptide change. When the concentration of peptide is increased, the tail usually moves forward as though a contraction brought it toward the body of the cell (Fig. 7 A). Upon decreasing the concentration of peptide, the pseudopods often collapse back; the tail normally does not move forward and, instead, often swells (Fig. 7 b). 90 human cells were traced from films, and the distance moved by the tail was analyzed after increasing or de-

creasing the concentration of peptide. This analysis indicates that the greater tail movement which occurs when the concentration is increased is significantly different $(P = 0.01)$ from that which occurs when the concentration is decreased.

Examination of the Transient Nature of the Responses

The termination of the generalized ruffling response was examined in several experiments. It is possible that the ruffling continues until the concentration of peptide is decreased by cell-associated proteases (3). This could explain the increased duration of the ruffling after a large increase in peptide concentration. However, this possibility is ruled out since reperfusion of cells with the same concentration of fresh peptide does not restimulate ruffling or rounding of the cells. The cells do not appear to have depleted an energy source or some essential component since they are capable of responding again to the same concentration if they are washed briefly (30 s) in Hanks' medium before readdition of the peptide. Furthermore, as mentioned above, cells in one concentration of peptide can be stimulated to respond again if the peptide concentration is increased (Fig. 4). Since cells already locomoting can be stimulated to exhibit
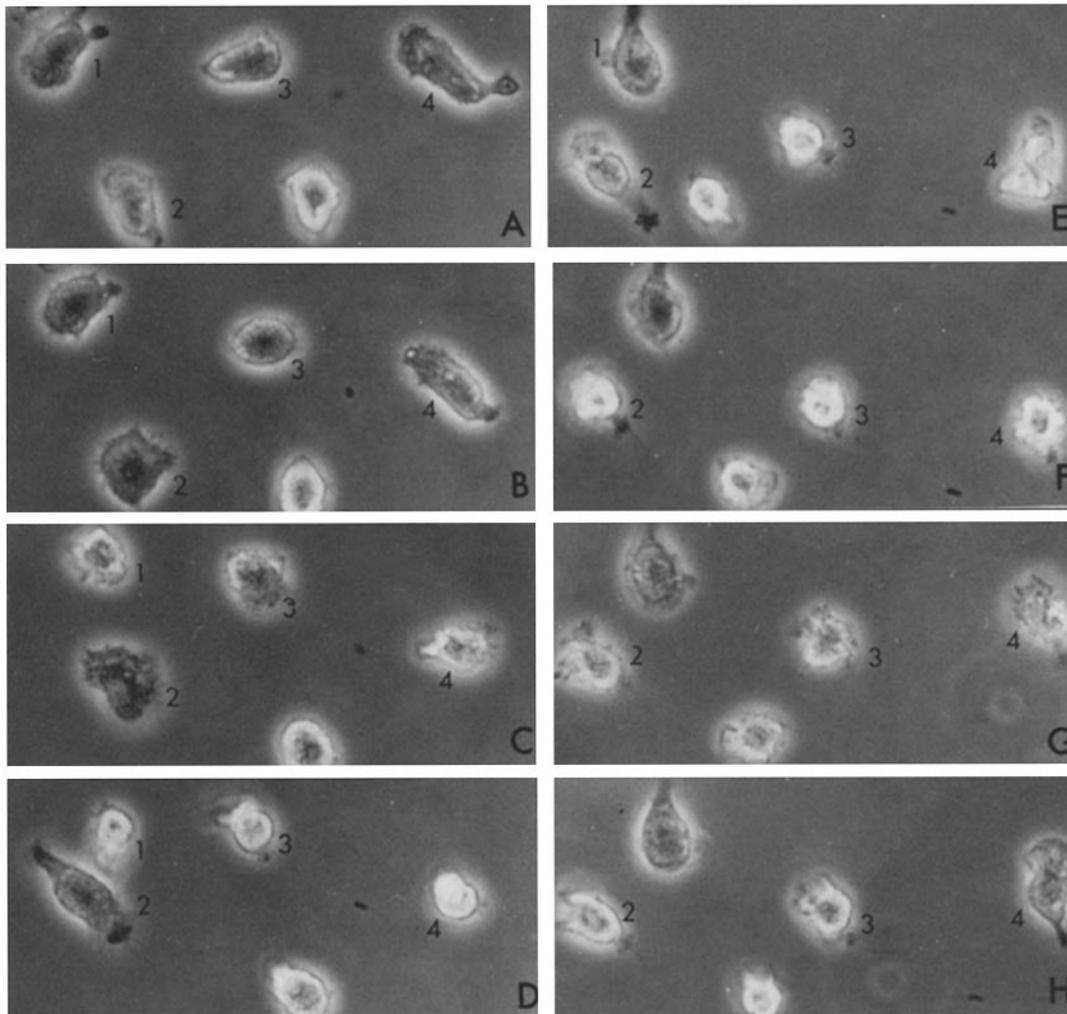
FIGURE 5 Phase micrographs of individual human PMNs during peptide concentration changes. Cells followed in sequential pictures are numbered *1* thru *4*. (*A*) 10 s after washing off serum; cells are locomoting with ruffles in the front and a knob-like tail in the rear. (*B*) 30 s in Hanks' medium containing 1% gelatin; cells round. (*C*) 20 s in 10^{-8} M f-NorleuLeuPhe; cells form ruffles over their surface. (*D*) 150 s in 10^{-9} M f-NorleuLeuPhe; many ruffles are withdrawn, leaving regions of the surface smooth, and cells are beginning to locomote again. (*E*) 60 s in fresh 10^{-8} M f-NorleuLeuPhe; possible some increased ruffling but cells continue to locomote. (*F*) 30 s in 10^{-7} M f-NorleuLeuPhe; cells round and ruffle over most of their surface. (*G*) 60 s in 10^{-7} M f-NorleuLeuPhe; cells still have many ruffles over their surface. The plane of focus was lowered to demonstrate the ruffles more clearly. (*H*) 210 s in 10^{-7} M f-NorleuLeuPhe: Cells limit the ruffling on their surface and exhibit locomotion. All, × 625.

generalized ruffling, the response does not appear to be solely an intermediate stage in the development of the locomotion morphology.

Binding Studies

Studies of the binding of tritium-labeled f-NorleuLeuPhe were carried out to examine whether the transient responses were a result of various receptors or receptor modulation. If the cell had a series of receptors with different affinities, then the transient responses observed might be due to sequential saturation of different receptors each resulting in an "all or nothing" response by the cell. Although this possibility cannot be
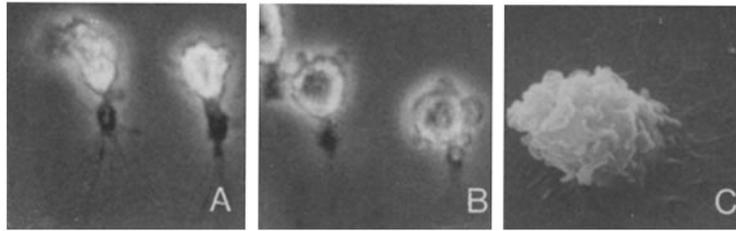
FIGURE 6 Blebbing by human PMNs after removal of peptide. × 1,000. (A) cell in 10^{-7} M f-Norleu-LeuPhe. × 1,300. (B) Cells 1 min after change to Hanks' medium. × 1,300. (C) Scanning micrograph of cell 30 s after change from 10^{-7} M f-NorleuLeuPhe to Hanks' medium. × 1,500.
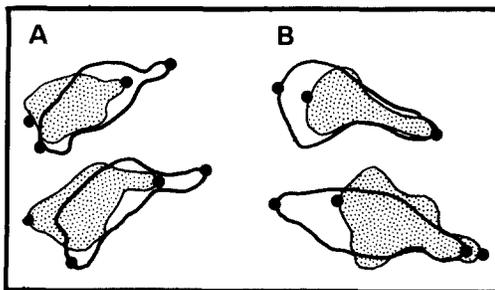

FIGURE 7 Tracings of human cells immediately before (heavy outline, no stipples) and 30 s after (thin outline, stippled) a change in peptide concentration. In Fig. 7 A, the peptide concentration was changed from 10^{-8} to 10^{-7} M f-NorleuLeuPhe. In Fig. 7 B, the peptide concentration was from 10^{-7} M f-NorleuLeuPhe to Hanks' medium. The dots mark the points on the cell used for measurement of cell length and the changes in tail and pseudopod position that occurred during the change.

FIGURE 8 Scatchard plot of binding of f-Norleu-LeuPhe to rabbit PMNs. Cells were incubated for 20 min at 4°C in various concentrations of [^{3}H]f-Norleu-LeuPhe. The peptide bound was determined from the cell-associated counts which remain on the glass filter; the free peptide was determined by counting an aliquot of the measured filtrate plus wash. The line was drawn by a linear regression equation, omitting the two points with the highest binding. The coefficient r for the line is −0.96.

ruled out, the binding of peptide at concentrations $<3 \times 10^{-8}$ appeared to be to a simple receptor with no apparent discontinuities (Fig. 8). Although the Scatchard plot could have been drawn as a smooth curve indicating negative cooperativity, it was interpreted to be the sum of two curves. A Hill plot of the binding data between 2×10^{-9} and 3 $\times 10^{-8}$ M at 4°C had a slope of 0.9; a Hill plot of similar binding studies done at 37°C had a slope of 0.98. Both indicate a lack of cooperativity. The K_d determined from the Scatchard plot is ~2 × 10^{-8} M. The dose range of the binding is the same as that of the behavior and morphology reported above. These binding studies, done on the same cell populations and under conditions similar to those of the behavioral studies, show a lower affinity binding site than that reported previously (2). We do see a high capacity, low affinity (non-saturable) binding site as reported previously (Fig. 7) (2, 27).
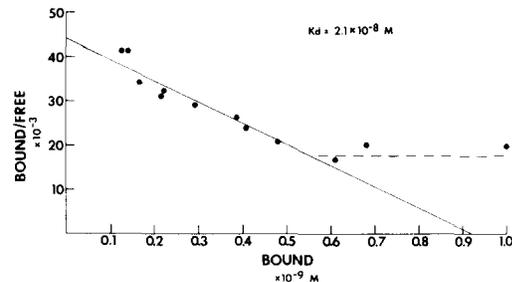
Both the binding and dissociation of peptide from the receptor are rapid, as would be expected from the rapid behavioral changes. At 23° and 37°C, the cell-associated counts do not reach a plateau within 15 min but continue to increase at a reduced rate for at least 40 min. The increase over this time is not readily reversible and thus may be due to interiorization of the peptide.

The reversibility of binding and the ability to rebind peptide were examined to determine whether a decrease in available receptors was involved in the transient nature of the response. Reversal was accomplished by washing the cells in Hanks' medium or by competition for the binding sites by adding 100-fold excess of unlabeled peptide (the off rates measured in both cases were similar, again suggesting that the binding is not cooperative). There was no detectable change in the amount of binding of 2 × 10^{-8} M f-Norleu-
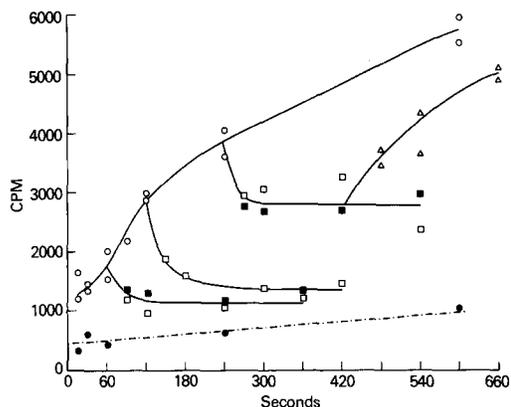
FIGURE 9 The time-course of f-NorleuLeuPhe binding in terms of cell-associated $cpm/10^7$ cells at 23°C. (O) Cells were incubated in 2×10^{-8} M tritiated f-Norleu-LeuPhe. (●) Cells were incubated in 2×10^{-8} M tritiated f-NorleuLeuPhe plus 2×10^{-6} M unlabeled f-Norleu-LeuPhe. (□) Cells were incubated for 60, 120, and 240 s in tritiated f-NorleuLeuPhe and then 2×10^{-6} M unlabeled peptide was added for 30, 60, 180, and 300 s. (■) Cells were incubated for 60 and 240 s in tritiated peptide and then washed in Hanks' medium for 30, 60, 180, and 300 s. (△) Cells were incubated for 240 s in 2×10^{-8} M tritiated peptide, washed for 180 s in Hanks' medium, and then fresh 2×10^{-8} M tritiated peptide was added for 60, 120, and 240 s.

LeuPhe which was reversible over the 10-min time-course (Fig. 9). Furthermore, these washed cells are able to rebind an amount of fresh tritiated peptide comparable to that removed by the wash.

A nonformylated analogue of f-NorleuLeuPhe, norleucylleucylphenylalanine, which does not bind to the cell receptor (2) does not induce any of these cell responses even at 10^{-5} M.

DISCUSSION

The behavioral responses of leukocytes to most chemotactic factors have long been known to include a stimulation of the rate of locomotion and the orientation of locomotion if the chemotactic factor is present in a gradient (17, 27). Both of these responses occur over several hours. We have demonstrated that leukocytes also exhibit transient alterations in their morphology and behavior after a rapid increase in the concentration of chemotactic peptides. These alterations include generalized ruffling over the cell surface. This ruffling is concomitant with a spreading of round cells and a lengthwise shortening and cessation of translocation by locomoting cells. Transient changes are

also observed after a decrease in peptide concentration. These changes differ from those seen after a peptide increase. A decrease induces cell blebbing, cell rounding primarily by the withdrawal of pseudopods, and a cessation of locomotion. The dose-response range for these changes parallels that of binding of the peptide to a saturable cell receptor.

The duration of these transient responses depends upon the particular concentration change that the cells experience. The half-maximal delay occurs at 2×10^{-8} M f-NorleuLeuPhe, the same concentration as the half-maximal binding, i.e., the K_d, to the cell receptors. The time required for the resumption of locomotion roughly correlates with the change in receptor occupancy that would be expected to occur in a given concentration change.

Transient responses to stimulation can occur for a number of reasons: (a) The stimulation may be transient. (b) The response may result in the depletion of a required component. (c) A temporal control or feedback mechanism could limit the response. This could occur through receptor inactivation (9) or through some internal mechanism. In the data presented here, the transient response does not seem to be due to either of the first two possibilities. Reperfusion with fresh peptide does not cause a second generalized ruffling response. Thus, the cessation of ruffling is not due to digestion or destruction of the peptide. The cessation of overall ruffling does not appear to be due to the depletion of some required component since stimulation with a higher concentration of peptide or with the same concentration after a brief wash in Hanks' medium can initiate a second response. Rather, the transient nature of the response appears to be due to some temporal control on its duration. The amount of peptide that is able to bind reversibly to the cell receptors is not significantly decreased over the time-course of these experiments. However, recent studies indicate that cells treated briefly with unlabeled peptide and then washed bind less tritiated peptide than untreated cells. This suggests receptor modulation may be playing a role in the transient response. The control also could be mediated through any of a number of cell parameters including ionic, metabolic, adhesive and/or cytoskeletal elements. Whatever the molecular mechanism, the cells do exhibit adaptation: the reversible extinction of certain responses to the chemotactic peptides.

In a phenomenon involving adaptation, a single

cell can undergo repeated transient responses to external changes in concentration of a stimulant but does not continue to respond to a constant concentration. The reversibility of the phenomenon described in this paper sets it apart from desensitization, a process in which leukocytes reportedly become irreversibly inactivated after incubation in high concentrations of chemotactic factors (26).

The transient response can be observed as cells initiate chemotaxis but is not apparent in cells locomoting in a gradient. Cells observed in a visual chemotaxis assay system (29) during the initiation of a chemotactic response to 10^{-8} M f-Norleu-LeuPhe spread and flatten on the substrate soon after the addition of peptide. Within a few minutes the cells begin to locomote, and soon nearly 80% of them orient and move toward the well containing the f-NorleuLeuPhe. As they move up the gradient, they maintain a locomotion morphology, and ruffle only at their front. Thus, the concentration changes incurred during movement in this gradient are not sufficiently abrupt to reinitiate a detectable ruffling response. This is not surprising since the magnitude and duration of the response appear to depend upon a rapid change in the number of receptors occupied. Detection of the spreading response requires changes in concentration that would result in a change in receptor occupancy of ~5% (e.g. 0 to 10^{-9} M f-Norleu-LeuPhe). The change in receptor occupancy estimated for cells moving in the visual assay chamber, even at rapid rates of locomotion (10 μm/min), would be only ~1% after 30 s of movement up the gradient.

The possible relevance of these transient responses to the behavioral and molecular basis of the inflammatory response is worth considering. The transient response may be important in situations where the cells are exposed to a rapid increase in the concentration of a chemotactic factor. This might occur to cells just entering an inflammatory region. An increase in cell ruffling and cell adhesion could contribute to the local margination of cells in the blood stream and to retention of cells in this region (8). When the cells have adapted to the concentration, they could move into the tissue and toward the focus of the lesion. It is possible that the various transient molecular changes observed after addition of chemotactic factors, including altered membrane potentials (14), ion fluxes (5, 22), cyclic nucleotide levels (17), and chemiluminescence (16), contrib-

ute to this transient behavior.

The transient nature of these molecular responses has made it difficult to assign them a role in chemotaxis which continues over an extended time-course. However, it is possible that they are part of an adapting response such as the behavioral changes described here. If so, they may be detectable only after a rapid concentration change but nevertheless may be involved in chemotaxis. To examine this possibility, it is helpful to consider a general model of adaptation. The model was designed to describe the phenomenon in bacteria but it also describes the leukocyte response (19, 21). In this model, the binding of peptide would rapidly activate some component(s) or enzyme(s) E_1 which leads to the increase of a signal "b." The factor b can be imagined as important in stimulating an activity such as pseudopod formation or cell adhesion. The peptide binding also would activate, with a slower time-course, a second component(s) or enzyme(s) E_2 which in some manner leads to decrease in b. Shortly after stimulation by peptide binding, the concentration of b would be expected to rise due to the rapid stimulation of E_1, but it would then return to its baseline level as E_2 became active. Thus, an increase in b might be undetectable except after a large concentration jump. However, the turnover of b might remain increased as long as the peptide binding was maintained. No modifications of this very general model are required to describe the phenomenon presented in this report.

The possibility of adaptation suggests a novel mechanism for gradient detection. In a gradient of chemotactic factor, if the cell could adapt to the mean concentration it experiences, the side of a cell exposed to concentrations above the mean could continue to be stimulated to form pseudopods and the cell would move up the gradient (7, 13). In this way the magnitude of signal for pseudopod formation would be independent of the mean concentration of chemotactic factor and depend only on the concentration difference. Using the model of adaptation, this might occur if E_1 and its product b were localized near the chemotactic receptors and thus activate pseudopod formation locally, while E_2, or its active product, was free to distribute itself throughout the cell. A cell in a gradient would thus have an increased level of b on the side of the cell with greater receptor occupancy. Amplification of this internal signal might be required and could occur via a futile cycle mechanism, such as suggested for the control of

glycolysis, if component b were turning over rapidly (18).

In an elegant series of experiments, Devreotes and Steck have demonstrated a role for sensory adaptation in the relay signaling of the slime mold *Dictyostelium discoideum* (11). They have shown that the secretion response elicited by perfusion with constant levels of cyclic AMP is a transient, adapting response (10, 11). Cyclic AMP is also a chemotactic factor in this species. The possibility that adapting responses are characteristic of chemotactic stimuli in a number of diverse organisms might indicate a functional role of adaptation in the detection of chemical gradients in both temporal and spatial chemotaxis.

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