Motility and Adhesiveness in Human Neutrophils

REDISTRIBUTION OF CHEMOTACTIC FACTOR-INDUCED ADHESION SITES

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ABSTRACT Human peripheral blood neutrophils obtained from healthy adults were examined in vitro. We assessed the effects of sequential stepwise increases in the concentration of the chemotactic dipeptide *N*-formyl-L-methionyl-L-phenylalanine (f-Met-Phe) on neutrophil attachment to serum-coated glass, detachment from serum-coated glass and the distribution on the cell surface of binding sites for albumin-coated latex beads. The initial exposure to f-Met-Phe resulted in increased adhesiveness and binding of latex beads in a random pattern over the cell surface. The second exposure to f-Met-Phe resulted in decreased adherence, detachment of neutrophils from serum-coated glass, and movement of binding sites for latex beads to the uropod. Enhanced adhesiveness and redistribution of binding sites were blocked by 0.1 mM *N*-α-*p*-tosyl-L-lysine chloromethyl ketone, a concentration that did not reduce the change in cellular shape caused by f-Met-Phe. Cytochalasin B (5 μg/ml) blocked the redistribution of binding sites as well as the change in shape. The third exposure to f-Met-Phe was given along with the latex beads. The stimulus was stopped after 2 min by fixing cells in suspension with glutaraldehyde. If the third exposure was at a concentration higher than the second, the beads were bound in the region of the lamellipodia in 70% of the cells. If lower, binding to the lamellipodia was found in a significantly smaller proportion of cells (13%). The results support the concept that neutrophils develop a polarized distribution of f-Met-Phe-induced adhesion sites in response to increasing concentrations of f-Met-Phe, and these sites flow from the region of the lamellipodia to the uropod.

INTRODUCTION

Neutrophils attached to a substratum in vitro acknowledge recognition of a gradient of chemotactic factor (CF)[1] by orienting and migrating toward the source of the gradient (1, 2). The cell seems to detect a concentration difference across its diameter, though little information is available on the mechanisms involved (3). An understanding of the cell's ability to discriminate CF concentrations may come from careful investigation of the effects of repeated exposure of neutrophils to CF. Studies using such an approach have shown that the initial exposure of neutrophils to a high concentration of CF alters the cell's response to a second challenge with the same CF. The altered behavior can be detected in the cell's adhesiveness (4, 5), chemotactic and random migration in the Boyden (4–7) and agarose (8) assays, lysosomal enzyme release (9, 10), and chemiluminescence (11), and has been characterized as desensitization or deactivation. The high CF concentration in the initial stimulus induces lysosomal enzyme release (9, 10), apparently irreversible enhancement of adhesiveness (4, 5), and production of highly reactive species of oxygen possibly resulting in autoxidative reactions (12). Low levels of CF can also modify the subsequent response to higher concentrations (9, 10, 13). There seems to be specific desensitization to lysosomal enzyme release and directed migration in the Boyden assay on rechallenge with a CF. Hensen et al. (9) and Showell et al. (10), suggest that this desensitization is not due simply to receptor blockade but may represent a control mechanism operative in the functions of the neutrophil affected by CF.

In this report, we examine the effects of exposure of neutrophils to stepwise increases in the concentration of a CF, *N*-formyl-L-methionyl-L-phenylalanine (f-Met-Phe). We assess changes in cellular shape, attachment to serum-coated glass, detachment from serum-coated glass, and the distribution on the cell surface of binding sites for albumin-coated latex beads.

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[1] *Abbreviations used in this paper*: CB, cytochalasin B; CF, chemotactic factor; HBSS, Hanks' balanced salt solution; TLCK, *N*-α-*p*-tosyl-L-lysine chloromethyl ketone.

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METHODS

Isolation of human neutrophils. Blood samples were obtained from eight healthy adult volunteers (four women and four men). Neutrophils were prepared using the same methodology described in a previous publication (4).

Assessment of neutrophil adhesiveness. Culture chambers were constructed using gaskets for Sykes-Moore chambers and two 25-mm round coverglasses. These were clamped together between two specially milled brass plates. The plates contained injection ports and did not interfere with examinations of either coverglass under oil-immersion phase-contrast microscopy. The adherence assay was performed in these chambers as previously described (4).

Assessment of changes in neutrophil shape. Suspensions of 10^6 cells in 1 ml of Hanks' balanced salt solution (HBSS) were exposed to the various conditions and reagents described. The pH was adjusted using 5% CO_2 in air. At the appropriate time, the cell suspension was added dropwise to 10 ml of cold (4°C) 1% glutaraldehyde in 0.1 M cacodylic acid (Sigma Chemical Co., St. Louis, Mo.). The glutaraldehyde solution was mixed constantly while the cells were being added. After remaining in the cold glutaraldehyde solution for 1 h the cells were washed and resuspended in 0.1 ml of distilled water. Cells were examined using a ×100 phase-contrast objective and classified according to shape (4).

Direct observation of neutrophils responding to chemotactic factors. Microscope slide chambers similar to those reported by Lichtman and Weed (14) were prepared. They held ~30 μl of fluid, could be filled easily by capillary action, and drained by absorption of fluid into a strip of filter paper. The distance between the coverglass and the microscope slide was about 100 μm. The inside of the chamber was pretreated for 2 min with 5% serum in HBSS, and washed twice in HBSS. The cell suspension (5×10^6 neutrophils/ml) was introduced and the chamber was inverted, allowing cells to settle onto the coverglass. After 200 s at room temperature the chamber was placed upright and unattached cells were washed out by exchanging the chamber fluid with HBSS. The chamber was then placed on the stage of a phase-contrast microscope and observed from above. This procedure allowed observation of neutrophils attached to the underside of the coverglass. Observations were made intermittently to avoid continuous exposure of cells to the microscope's illumination.

Assessment of binding of latex beads. Latex beads (Sigma Chemical Co.) were washed and dispersed into a solution of 2% human serum albumin in HBSS. After incubation for 2 min at room temperature, the beads were washed and suspended in HBSS. Control cells suspended in HBSS were exposed to a 1% suspension of beads (vol/vol) for 5 min at room temperature. Cells were then fixed in glutaraldehyde as described above and examined with phase-contrast optics. Beads were rarely found associated with cells, either bound to the surface or phagocytized. These observations are consistent with those of Beukers et al. (15) (i.e., albumin coating of latex inhibits binding and phagocytosis). Experiments in this paper used cells whose adhesiveness had been increased by exposure to f-Met-Phe. Cells treated with f-Met-Phe exhibited a high degree of binding of albumin-coated latex beads. However, there was little evidence of phagocytosis. Specific conditions for this procedure will be given for each experiment in Results.

Reagents. Two chemotactic factors (f-Met-Phe and C5a) and cytochalasin B (CB) were used as previously described (4).

TLCK (*N*-α-*p*-tosyl-L-lysine chloromethyl ketone, Sigma Chemical Co.) was prepared at a 1-mM concentration in HBSS and diluted as needed for specific experiments.

Scanning electron microscopy. Cells in suspension or attached to protein-coated glass or aluminum were fixed in cold 1.0% glutaraldehyde in 0.1 M cacodylic acid for 2 h, then dehydrated in a graded ethanol series. Cells were dried using a Bomar critical-point drier (The Bomar Co., Tacoma, Wash.) with CO_2 as the carrier gas. Preparations were then mounted on aluminum stubs, sputter-coated with 20–30 nm of gold, and examined in an ISI Super III scanning electron microscope (International Scientific Instruments, Inc., Santa Clara, Calif.).

Suspensions of glutaraldehyde-fixed cells with bound latex beads were separated from unbound latex beads by centrifugation on Ficoll-Hypaque cushions (sp gr, 1.077 g/ml at 25°C).

Presentation of data. The data are expressed in terms of mean±SEM; n represents the number of separate experiments and in most cases the number of separate donors. Each experiment contained duplicate determinations. Student's t test was used to assess significance.

RESULTS

Neutrophils were exposed in suspension to 10 nM f-Met-Phe, then washed and reexposed to 10 nM f-Met-Phe. Adhesiveness and cell shape were not altered by the second stimulus. The cells seemed simply to retain the enhanced level of adhesiveness induced by the pretreatment (4). However, when cells pretreated with 10 nM f-Met-Phe were exposed to 100 nM f-Met-Phe, significant changes were observed. As with untreated cells, the higher level of CF caused a high percentage (>85) to assume a polarized shape. Yet the adhesiveness was significantly below that of control untreated cells (Fig. 1). In other experiments, exposing cells initially to 100 nM f-Met-Phe followed by washing and reexposure to 500 nM f-Met-Phe gave a level of attachment significantly lower than with a single 100-nM exposure alone (37.2±3.4% on restimulation compared with 64.8±4.2% on a single exposure, n, 4, $P < 0.001$). It is important to note that when the initial stimulus was high (10 μM or greater), restimulation did not reduce adhesiveness (Fig. 1).

Timing of the second stimulus had a significant effect on adhesiveness. Cells were suspended in 10 nM f-Met-Phe for 2, 3, 4, and 5 min at which time the concentration of f-Met-Phe was abruptly increased to 100 nM (HBSS containing 10 nM f-Met-Phe was added to controls). Cells were then incubated for an additional 5 min and adhesiveness was tested. Adhesiveness remained high (81.6±2.1) when the CF concentration was increased after 2 and 3 min. When increased at 4 or 5 min, the adhesiveness was significantly decreased (34.1±2.4, $P < 0.001$, n, 5).

Effects of TLCK. The enhanced adhesiveness caused by the initial f-Met-Phe stimulus was significantly reduced by pretreatment of the cells in 0.2 mM TLCK, an active site inhibitor of trypsin-like enzymes. TLCK blocked enhancement if retained with the cells

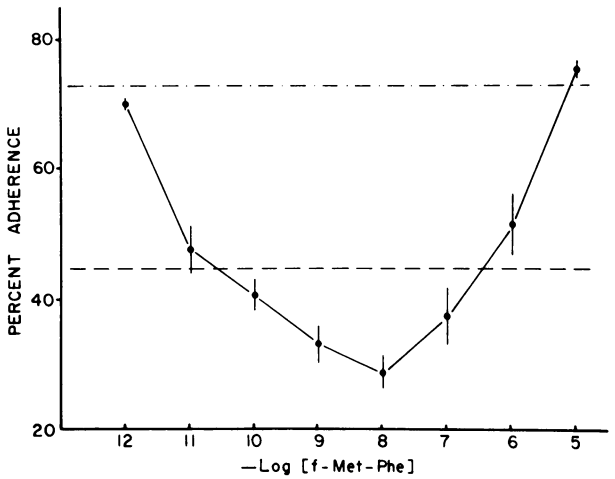

FIGURE 1 Adhesiveness of human neutrophils upon reexposure to f-Met-Phe. Cells were preincubated for 5 min at 22°C in HBSS containing the concentration of f-Met-Phe indicated. The cells were then washed in HBSS and resuspended in HBSS containing 1 μM f-Met-Phe for 5 min. Adhesiveness was then assessed. Mean±SEM, *n*, 4. (– – –), Mean adhesiveness of untreated cells; (– · – · –), mean adhesiveness of cells exposed to 1 μM f-Met-Phe after 5-min preincubation in HBSS.

during CF stimulation (Table I) or if added up to 3 min after the CF stimulus. It did not affect attachment of control cells. The selectivity of this effect is shown by the findings that TLCK (0.2 mM) did not alter spreading of control cells onto an untreated glass surface (72.9±5.1% spread in HBSS, 70.1±5.7% spread in 0.2 mM TLCK, *n*, 6, *P*, NS) or the change in shape caused by a chemokinetic level of f-Met-Phe (Fig. 2). At higher concentrations TLCK also blocked the cellular polarization induced by CF although it did not diminish cell viability (eosin exclusion).

TLCK also inhibited the drop in adhesiveness caused by the second f-Met-Phe stimulus. Cells exposed to TLCK within 3 min after the initial stimulus had a low adhesiveness regardless of whether or not a second 100-nM f-Met-Phe stimulus was applied. If TLCK was added after 4 min, then the cells retained the levels of adhesiveness induced by the initial CF stimulus, and a second CF stimulus did not alter adhesiveness (Fig. 3). TLCK (0.2 mM) did not lower the degree of cell polarization induced by the second stimulus if added 2 or 5 min after the initial CF stimulus (>85% bipolar forms at both times, not significantly different from controls without TLCK, 86.6±3.2%).

Effects of CB. Because CB inhibits the change in cell shape without blocking the enhancement of adhesiveness caused by the initial stimulation with CF (4), we decided to assess its influence on restimulation of cells with CF. As expected, CB-treated cells remained spherical. However, when CB was added

TABLE I
Effect of TLCK on Adhesiveness of Human Neutrophils

First incubation	Second incubation	*n*	Attachment*	*P*
			%	
HBSS‡	HBSS	5	39.3±1.6	NS
0.2 mM TLCK	HBSS	4	37.7±1.6	
HBSS	1 μM f-Met-Phe	5	67.3±2.9	<0.005
0.2 mM TLCK	1 μM f-Met-Phe	5	40.7±1.9	
HBSS	C5a(35 μg protein/ml)	3	76.3±2.2	<0.005
0.2 mM TLCK	C5a(35 μg protein/ml)	3	46.3±2.4	
HBSS§	HBSS	6	42.9±1.5	NS
0.2 mM TLCK	HBSS	4	44.5±2.4	
HBSS	1 μM f-Met-Phe	6	70.9±2.2	<0.001
0.2 mM TLCK	1 μM f-Met-Phe	6	31.5±3.3	
1 μM f-Met-Phe‖	HBSS	5	69.1±2.1	NS
1 μM f-Met-Phe	0.2 mM TLCK	5	68.5±1.3	
10 nM f-Met-Phe	HBSS	4	61.2±2.5	NS
10 nM f-Met-Phe	0.2 mM TLCK	4	60.1±1.5	

* Mean±SEM remaining attached to serum-coated glass.
‡ In these experiments, cells were exposed to HBSS or TLCK for 2 min, then washed twice in HBSS before being stimulated with CF.
§ In these experiments, cells were exposed to HBSS or TLCK for 2 min prior to and throughout the experiment.
‖ In these experiments, TLCK was added to the cell suspension 5 min after the CF, and retained with cells throughout the experiment.

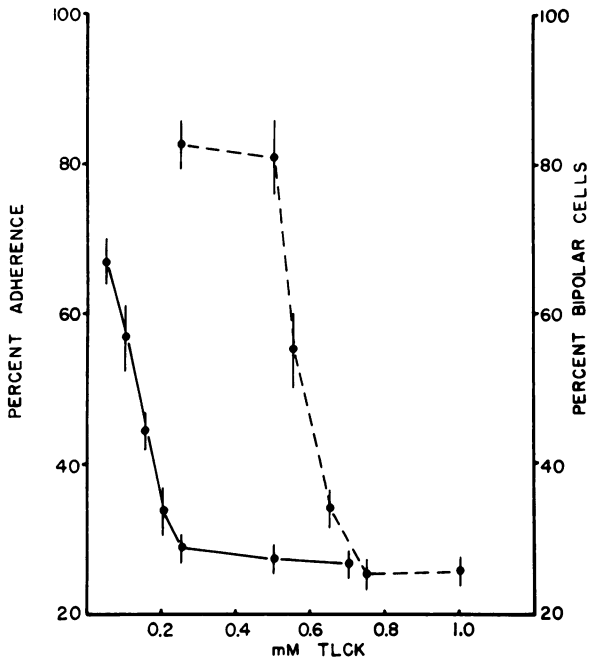

FIGURE 2 Effect of TLCK on the response of human neutrophils to f-Met-Phe. Cells were exposed to 1 μM f-Met-Phe in the presence of the various concentrations of TLCK indicated. After a 5-min incubation at room temperature, the cells' configuration and attachment to serum-coated glass were assessed. n, 3, mean±SEM (——), Percent adherence; (– – –), percent bipolar cells.

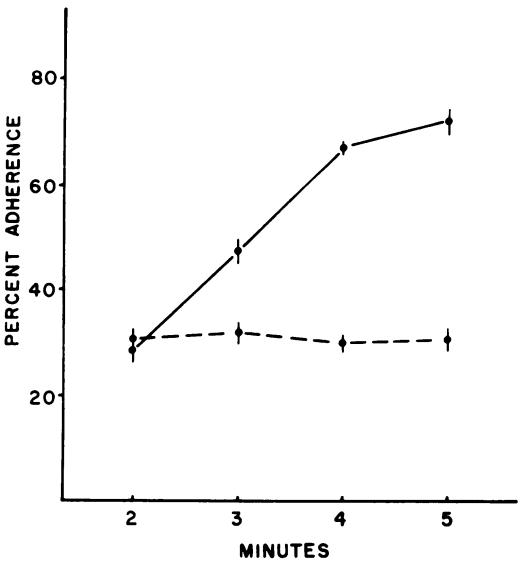

FIGURE 3 Effect of TLCK on cellular adhesiveness. Cells were preincubated in 10 nM f-Met-Phe. At the times indicated TLCK was rapidly introduced in the suspension to a final concentration of 0.2 mM (equivalent volume of HBSS added to control). After an additional 2-min incubation the cells were restimulated with f-Met-Phe (100 nM) for 5 min. Adhesiveness was then assessed. "Percent adherence" refers to cells remaining attached to serum-treated glass. (——), TLCK; (– – –), control; n, 4; differences in two experimental groups at 4 and 5 min were significant ($P < 0.001$).

before or after the initial stimulus, a second f-Met-Phe stimulus at a higher concentration enhanced adhesiveness in a dose-dependent fashion (data not shown).

Direct observations of neutrophils responding to restimulation with f-Met-Phe. Cells pretreated with 10 nM f-Met-Phe (5 min, 22°C) or HBSS were allowed to adhere to the upper coverglass of the slide chamber. Adherent cells were washed, incubated in HBSS or TLCK (0.1 mM) for 2 min, and then exposed to 100 nM, 1 μM, or 10 μM f-Met-Phe. Cell-spreading occurred rapidly after infusion of f-Met-Phe into the chamber. This reaction seemed unaffected by pretreatment in f-Met-Phe or TLCK (>92% spread cells at 100–150 s in each pretreatment group on exposure to 1 μM f-Met-Phe). In chambers with 100 nM or 1 μM f-Met-Phe cells gradually assumed a polarized shape typical of motile neutrophils, i.e., lamellipodia at one end and a distinct tail or uropod at the other (3). This occurred maximally between 300 and 350 s and was also unaffected by pretreatment in f-Met-Phe or TLCK (>89% bipolar cells in each pretreatment group on exposure to either 100 nM or 1 μM f-Met-Phe). However, cells pretreated in f-Met-Phe became detached from the coverglass in greater numbers than control cells (Table II). Detachment occurred first at the anterior end of the cells (i.e., region of the lamellipodia, Fig. 4) resulting in a high percentage of cells hanging by the uropod. Many of these cells eventually dropped off the glass surface and did not attach to the bottom glass surface of the slide chamber. Essentially all of these cells were washed out of the chamber by a very gentle flow of HBSS inadequate to dislodge attached cells. This enhanced detachment was prevented by exposing pretreated cells to 0.2 mM TLCK (Table II). Retraction fibers formed at the uropod as cells migrated across the substratum and were seen frequently in control and TLCK-treated cells (~70% of cells in both groups). Retraction fibers were less frequently observed in f-Met-Phe pretreated cells (~18%).

In contrast to these results, addition of 10 μM f-Met-Phe caused cell spreading which was retained throughout the observation period.

Binding of albumin-coated latex beads to neutrophils: effects of restimulation with f-Met-Phe. Experiments described in the above section were repeated with the following modification. Cells were preincubated for 5 min in 10 nM f-Met-Phe, attached to the upper coverglass of the slide chamber, and exposed to a high concentration of latex beads for 4 min at 22°C. They were then washed twice in HBSS and observed over a period of 2,600 s. Initially the cells were spherical with latex beads attached randomly on

TABLE II

Direct Observations of Neutrophils on Restimulation with an Increased Dose of f-Met-Phe

Pretreatment*	Restimulation‡	*n*	Hanging§	*P*	Detached‖	*P*
			%		%	
HBSS	100 nM f-Met-Phe	7	18.8±1.1		19.5±1.9	
10 nM f-Met-Phe	100 nM f-Met-Phe	8	77.3±2.2	<0.001	56.7±3.4	<0.001
10 nM f-Met-Phe	100 nM f-Met-Phe 0.1 mM TLCK	7	22.8±3.9		21.6±2.1	

* Cells pretreated in suspension for 5 min, 22°C, washed, then allowed to adhere to the upper coverglass in a slide chamber.

‡ Adherent cells in the slide chamber were exposed at 22°C over an observation period of 2,600 s.

§ Refers to cells attached to the upper coverglass only by the uropod. Determined between 1,000 and 1,500 s after infusion of the restimulating CF.

‖ Determined between 2,500 and 2,600 s after infusion of restimulating CF.

their surfaces. Infusion of 100 nM f-Met-Phe into the chamber caused the transient spreading reaction noted above and the cells assumed a polarized shape. The beads then began to move toward the uropod. By 1,000 s after infusion of 100 nM f-Met-Phe, 74.2±3.8% (*n*, 5) of cells had latex beads clustered on the

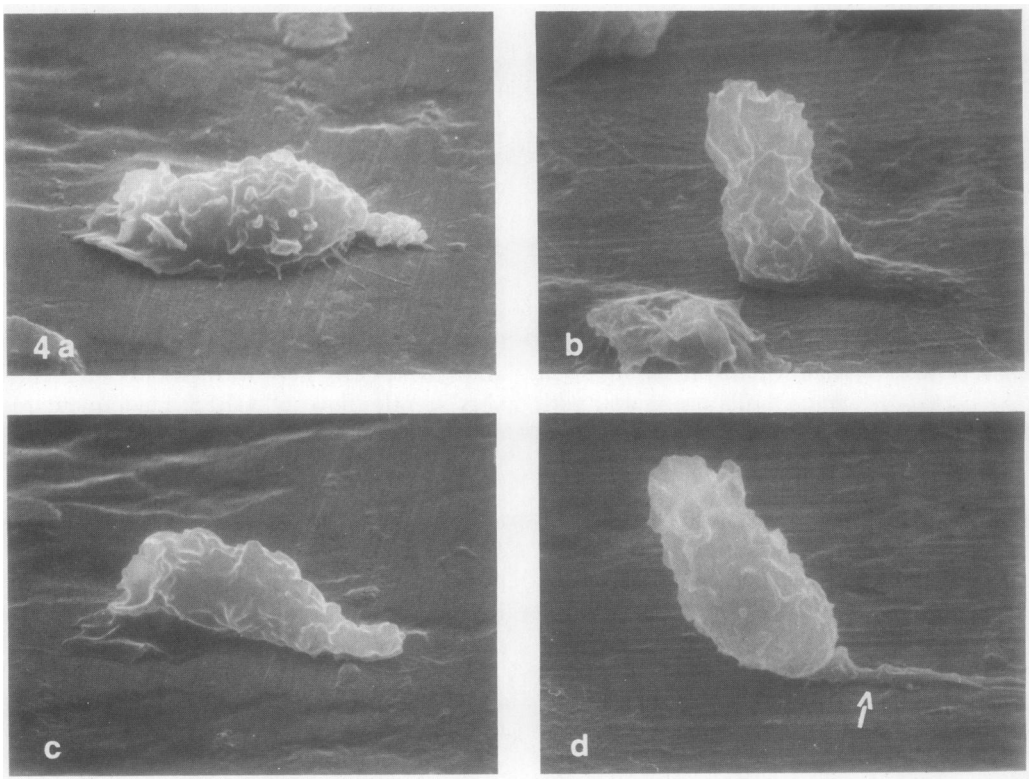

FIGURE 4 Detachment of human neutrophils after restimulation with f-Met-Phe. Cells were preincubated in HBSS or 10 nM f-Met-Phe for 5 min, then allowed to adhere to a serum-coated aluminum surface (aluminum exposed to 40% serum for 2 min then rinsed in HBSS). The substratum was then inverted and placed in a slide chamber. The chamber was filled with 100 nM f-Met-Phe and allowed to incubate for up to 1,500 s. At the appropriate time the chamber fluid was exchanged with 1% glutaraldehyde and processed for SEM. (*a* and *c*) Typical appearance of cells preincubated in HBSS 800 and 1,300 s after addition of 100 nM f-Met-Phe; (*b* and *d*) cells preincubated in 10 nM f-Met-Phe, 800 and 1,300 s after addition of 100 nM f-Met-Phe. (*b*) Cell partially detached; (*d*), cell remaining attached only by the uropod (arrow). Scanning electron microscopy, ×3,500, 55° tilt.

uropod. The opposite pole of these cells was free of beads. This movement of beads corresponded in time to detachment of cells (described above). These cells were hanging from the upper coverglass or had become detached. When TLCK (0.2 mM) was present in the 100-nM f-Met-Phe solution, 17.0±4.7% of cells clustered beads at the uropod over the same time period (n, 5; $P < 0.001$).

The observed progress of detachment (i.e., from lamellipodia to tail) and the movement of latex beads to the tail raised the possibility that the diminished adhesiveness of detached cells was the result of a restricted distribution of adhesion sites. The following experiments were designed to assess further the effects of the CF stimulus on the distribution of binding sites for latex beads.

Cells were examined in suspension. Neutrophils were preincubated for 5 min, 22°C, in 10 nM f-Met-Phe or HBSS. Cells were centrifuged, the supernate was discarded, and the cells were resuspended in 100 nM f-Met-Phe for 15 min. These cells were then exposed to latex beads for 2 min and fixed in suspension with glutaraldehyde. Cells pretreated in f-Met-Phe had 75.8±4.1% (n, 5) with beads clustered on the uropod (Fig. 5*a*), a finding not significantly different from that of restimulated cells attached to a coverglass. Fixing cells at 30 s after adding the beads gave essentially the same results (73.2±2.9%, n, 3, with beads bound only at the uropod). In contrast, cells pretreated in HBSS had 18.3±4.6% (n, 5; $P < 0.001$) with beads on the uropod. Cells exposed to 0.1 mM TLCK before restimulation with f-Met-Phe had 11.5±6.8% (n, 6; $P < 0.001$) with beads clustered at the uropod. Most cells in the latter preparation had beads distributed over the entire cell (Fig. 5*b*).

CB-treated cells were spherical with beads distributed over the surface. Restimulation of f-Met-Phe-pretreated cells in the presence of CB had little effect on the distribution of latex beads binding to the cell surface. There was some indication of beads shifting to one hemisphere of these spherical cells (Fig. 5*c*).

Effects of a third exposure to f-Met-Phe. For these experiments, albumin-coated latex beads were added to the cell suspension simultaneously with the third f-Met-Phe challenge, and the cells were fixed in suspension with glutaraldehyde 30 s–2 min later. When the third stimulus was at a higher concentration than the second, beads were frequently observed clustered on both poles of the cell (Table III, Fig. 6*a* and *b*). In ~20% of the cells, beads were clustered only in the region of the lamellipodia (Fig. 6*c*). In contrast, when the third stimulus was at a lower concentration than the second, a low percentage of cells had clusters of beads on both poles and <3% had binding only in the region of the lamellipodia. The pattern of binding in this group was not significantly different than on cells not receiving a third CF stimulus (i.e., 68±4.2% had beads bound only to the uropod).

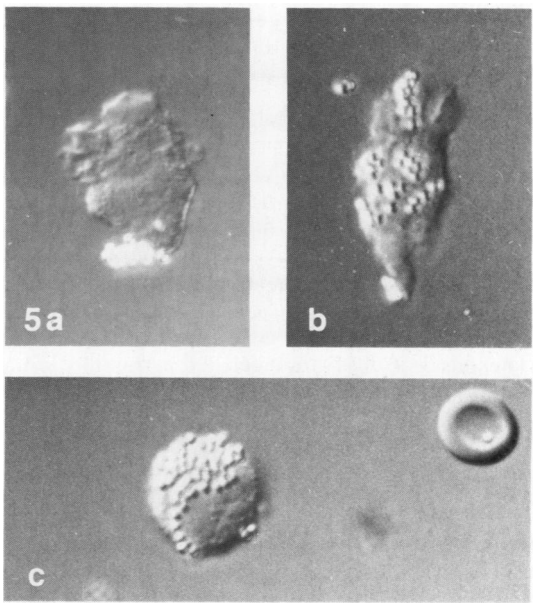

FIGURE 5 Effects of TLCK and CB on the distribution of bound albumin-coated latex beads on human neutrophils in suspension. This illustration depicts the predominant result of the following experimental manipulations: (*a*) Neutrophils were exposed to 10 nM f-Met-Phe for 5 min, washed, exposed to 100 nM f-Met-Phe for 10 min, and then exposed to beads for 2 min without changing the concentration of the second CF stimulus. (*b*) Neutrophils were exposed to 10 nM f-Met-Phe for 5 min, washed, then exposed to 100 nM f-Met-Phe in the presence of 0.1 mM TLCK for 10 min and then exposed to beads for 2 min without changing the concentration of the second CF stimulus. (*c*) Neutrophils were exposed to 10 nM f-Met-Phe for 5 min, washed, exposed to 100 nM f-Met-Phe in the presence of 5 μg/ml CB, and then exposed to latex beads for 2 min without changing the concentration of the second CF stimulus. Nomarski optics; ×1,900; glutaraldehyde fixation.

In a variation of the above experiment, cells were exposed to 0.6-μm beads 10 min after the second f-Met-Phe stimulus, then washed to remove excess beads. Larger beads (1.1 μm Diam) were included with the third f-Met-Phe stimulus. Fig. 6*d* and Fig. 7 illustrate the results. Small beads were clustered at the tail of the cells and large beads were attached most frequently to the lamellipodia when the third stimulus was at a higher concentration than the second stimulus. When cells were allowed to incubate an additional 5 min, most of the larger beads were found adjacent to small beads on the uropod. Large beads were rarely attached at any site on the cells when the third stimulus was at a lower concentration than the second.

DISCUSSION

The results in this and a previous report (4) support the idea that a single exposure of previously un-

TABLE III

Effect of a Third f-Met-Phe Stimulus on Binding of Albumin-Coated Latex Beads to Human Neutrophils

Stimulus*	n	Uropod binding‡	P	Bipolar binding§	P	Lamellipodia binding‖	P
		%		%		%	
1 μM f-Met-Phe	5	12.8±2.5		53.6±1.2		17.6±3.2	
100 nM f-Met-Phe	5	68.4±3.3	<0.001	9.8±1.4	<0.001	3.2±1.2	<0.01

* The third exposure of neutrophils to f-Met-Phe. Cells were incubated at 22°C, suspended in HBSS first with 100 nM f-Met-Phe for 5 min, then centrifuged and resuspended in 500 nM f-Met-Phe for 15 min. Cells were then centrifuged and resuspended in the concentration of f-Met-Phe given above. This solution also contained the albumin-coated latex beads. Cells were incubated in the third stimulus for 2 min, then fixed in suspension with glutaraldehyde.

‡ Cells with beads clustered only at the uropod (see Fig. 5a).

§ Cells with beads clustered at both poles of the cell. If any beads were in the midregion of the cell, then the cell was not included in this category (see Fig. 6a).

‖ Cells with beads attached only in the region of the lamellipodia (see Fig. 6c).

stimulated human neutrophils to f-Met-Phe causes a sustained enhancement of adhesiveness. At high concentrations the cells are typically flattened onto the substratum (5). However, under carefully controlled conditions, restimulation of neutrophils with f-Met-Phe causes a significant drop in adhesiveness. One hypothesis to account for this effect is that the decreased adhesiveness is the result of movement of adhesion sites to a small area of the cell's tail. Support for this possible mechanism comes from the following observations: (*a*) Detachment of cells from the coverglass in the slide chamber occurred in a consistent fashion, proceeding from the region of the lamellipodia and progressing to the tail. (*b*) Albumin-coated latex beads bound to the cell surface migrated toward the tail upon exposure of the cells to a second and higher concentration of f-Met-Phe. (*c*) TLCK (0.2 mM) inhibited the detachment of cells from the coverglass and the movement of latex beads to the uropod.

Further support for this hypothesis comes from the study of the distribution of latex beads on the surface of cells in suspension. Here we assume that the behavior of binding sites for albumin-coated latex beads is analogous to the behavior of adhesion sites for protein-coated glass. If this assumption proves to be true then the following observations are pertinent to our hypothesis: (*a*) Cells exposed to a second chemo-

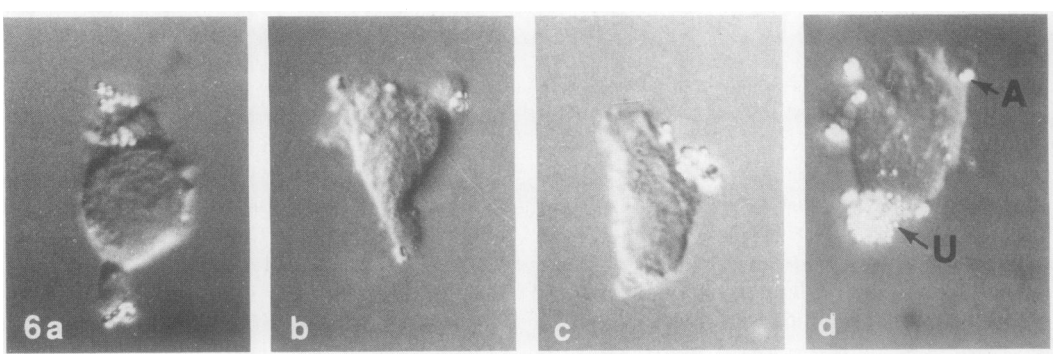

FIGURE 6 Effect of a third f-Met-Phe stimulus on binding of albumin-coated latex beads to human neutrophils in suspension. This illustration depicts the predominant result of the following experimental manipulations: (*a*, *b*, and *c*) neutrophils were exposed to 100 nM f-Met-Phe for 5 min, washed, then exposed to 500 nM f-Met-Phe for 15 min. Cells were then collected by centrifugation, exposed to a 1-μM f-Met-Phe solution containing beads (0.8 μm Diam) for 2 min and then fixed in glutaraldehyde; (*d*) neutrophils were exposed to 100 nM f-Met-Phe for 5 min, washed, exposed to 500 nM f-Met-Phe for 10 min, and then exposed to beads (0.6 μm Diam) for 2 min without changing the concentration of the second CF stimulus. This allowed binding of these beads to the uropod (arrow, U). The cells were then washed in HBSS containing 500 nM f-Met-Phe to remove excess beads and prevent cells from rounding up. Pelleted cells were then resuspended in HBSS containing 1 μM f-Met-Phe and 1.1-μm Diam beads for 2 min. The binding of the larger beads is depicted (arrow, A). Nomarski optics; ×1,900; glutaraldehyde fixation.

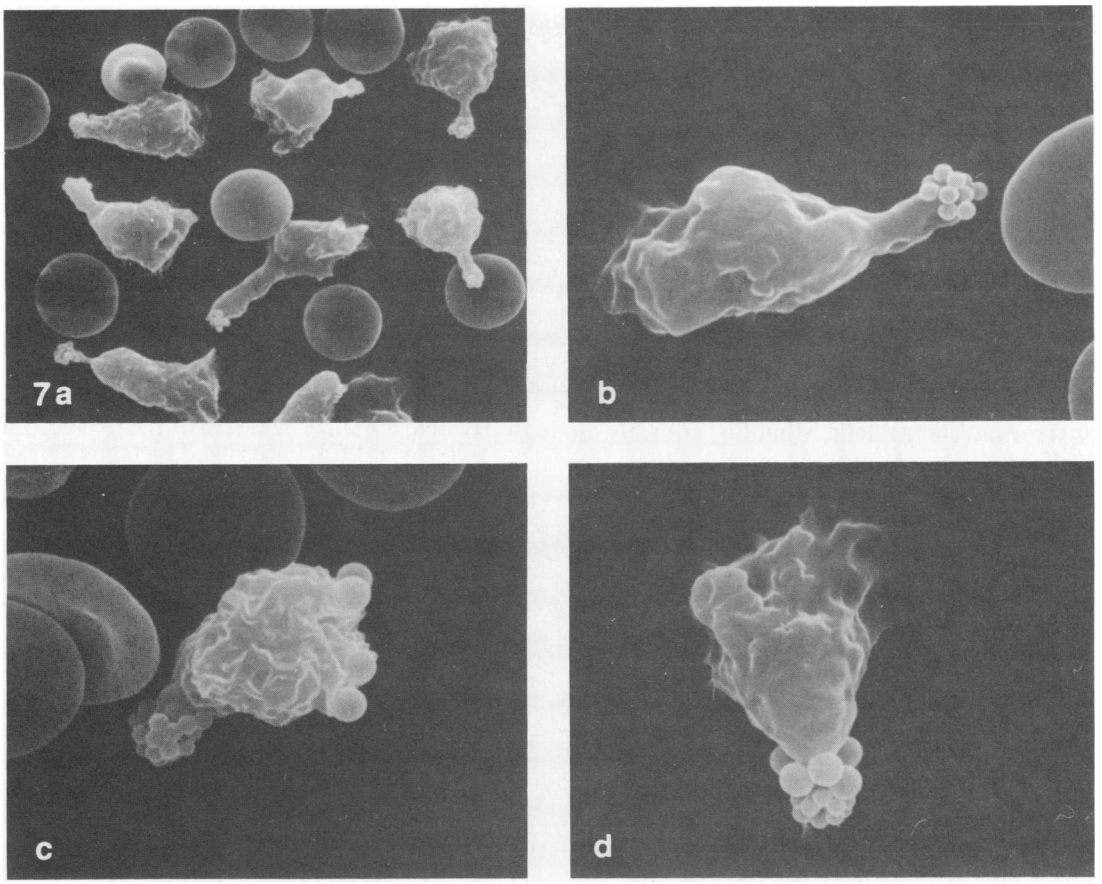

FIGURE 7 Effect of a third exposure to f-Met-Phe on the binding of albumin-coated latex beads to human neutrophils in suspension. This illustration depicts the predominant result of the following experimental manipulations: (*a* and *b*) cells were exposed to 100 nM f-Met-Phe for 5 min, washed, then exposed to 500 nM f-Met-Phe for 10 min. Cells were then collected by centrifugation and exposed to a 500-nM f-Met-Phe solution containing 0.6-μm Diam latex beads for 3 min. Beads were found exclusively on the tails of 90% of the cells; (*c* and *d*) neutrophils were exposed to 100 nM f-Met-Phe for 5 min, washed and then exposed to 500 nM f-Met-Phe for 10 min. Cells were collected and exposed for 3 min to a solution of 500 nM f-Met-Phe containing latex beads (0.6 μm Diam). This allowed binding of beads to the uropod. Cells were then washed in HBSS containing 500 nM f-Met-Phe to remove excess beads and to prevent cells from rounding up. Pelleted cells were then resuspended in HBSS containing 1 μM f-Met-Phe and 1.1-μm Diam beads for 30 s (*c*) and 5 min (*d*) before fixing in glutaraldehyde. Scanning electron microscopy, (*a*) ×1,600; (*b*, *c*, and *d*) ×3,800.

tactic stimulus for 10 min bound beads on the tail of the cell, often only on a small region at the tip. In some experiments cells were exposed to the beads for only 30 s before being suspended in fixative. If significant movement of bound beads did not occur after suspension of cells in the fixative, then it appears that the binding sites were essentially restricted to the tail. (*b*) In contrast to controls, CB-treated cells exhibited increased adhesiveness on exposure to a second f-Met-Phe stimulus. CB-treated cells bound beads over a large area of their surface. (*c*) TLCK, when added after the first CF stimulus and before the second stimulus, blocked the decrease in adhesiveness. Cells treated in this way bound beads generally over their surface and were indistinguishable from cells receiving a single CF stimulus.

The transport of surface-bound substances to the tail of the cell has been observed in neutrophils migrating in a chemotactic gradient (16) and in cells treated with colchicine (17). Our results support the concept that in human neutrophils a similar transport is activated by an increasing chemotactic stimulus and that the process can occur without binding to a substratum or particle. Our data also indicate that the development of a polarized cellular configuration is separable from this transport process. An initial stimulus of 100 nM f-Met-Phe or greater produced a polarized shape, but the cells exhibited increased

adhesiveness, detached minimally from a protein-coated coverglass, and bound latex beads in a generally random distribution on their surface. The cells migrated across the substratum as evidenced by the development of retraction fibers and showed little tendency to transport surface-bound latex beads to the uropod. Furthermore, TLCK (0.2 mM) treatment of cells after the initial stimulation with 10 nM f-Met-Phe did not inhibit the development of a bipolar configuration upon restimulation with 100 nM f-Met-Phe though it blocked the polarized distribution of adhesion sites for latex beads, the detachment of cells from the coverglass in the slide chamber, and the expected drop in adhesiveness.

The increasing chemotactic stimulus not only induces redistribution of surface binding sites, but appears to promote additional binding. A 30-s exposure to a third and higher f-Met-Phe concentration led to binding of latex beads in the region of the lamellipodia. Continued incubation in the higher stimulus led to predominant binding of beads on the uropod. Cells exposed to a decreased concentration of f-Met-Phe showed little evidence of binding on the lamellipodia. These results seem to indicate that increasing the concentration of f-Met-Phe in a stepwise manner induces discrete cycles of appearance and migration of binding sites for albumin-coated latex beads on the cell surface. If adhesion sites exhibit analogous behavior, they may play a role in orientation of cells in a gradient of CF. It seems plausible that cells orient toward a higher concentration because it stimulates a regional expression of adhesion sites.

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