Cell Polarity:

An Examination of Its Behavioral Expression

and Its Consequences for

Polymorphonuclear Leukocyte Chemotaxis

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ABSTRACT   Locomoting polymorphonuclear leukocytes (PMNs) exhibit a morphological polarity. We demonstrate that they also exhibit a behavioral polarity in their responsiveness to chemotactic factor stimulation. This is demonstrated by (*a*) the pattern of their locomotion in a homogeneous concentration of chemotactic factors, (*b*) their responses to increases in the homogeneous concentration of chemotactic factors, and (*c*) their responses to changes in the direction of a chemotactic gradient. The behavioral polarity is not a function of the rate of locomotion or the particular stimulant used to orient the cells, but may reflect an asymmetric distribution of chemotactic receptors or the motile machinery. The polar behavior affects the chemotactic ability of PMNs. The data are discussed in relation to possible mechanisms of sensing a chemotactic gradient.

Locomoting polymorphonuclear leukocytes (PMNs) have a morphological polarity, with a thin, granule-free pseudopod at the front and a uropod or tail at the rear. Such polarity could merely represent the current direction of locomotion by the cell and not affect the ability of the cell to respond to new stimuli. This would mean that the PMN would be equally capable of extending a new pseudopod in any direction at any time. Alternatively, the morphological polarity could correlate with a structural polarity that biases subsequent responses of the cell. Thus, the cell would also exhibit a behavioral polarity. The extreme of this situation would be analogous to a car with no reverse gear: it can turn but only moves forward and keeps a fixed polarity.

The extent to which the PMN behavior is amorphous or linked to the morphological polarity is important for our understanding of several processes. A polarity that biases behavior would affect the way cells align themselves along a new chemotactic gradient and would affect the interpretation of experiments examining the mechanism by which cells detect a gradient. A behavioral polarity could also stabilize the cell orientation in a constant gradient and therefore serve to effectively amplify the directional signal. Finally, as we begin to study the mechanisms of cell locomotion on a molecular level, an understanding of the cell's behavior will be useful. For example, if a cell is functionally polarized, it may not be

necessary to search for a difference in some mediator of the motility (e.g., pH or calcium ion concentration) between the front and the back of a cell, because the same signal placed in different environments could result in diverse responses, such as extension at the front of a cell and contraction at the rear and along the sides.

Previous studies have shown that PMNs locomoting in a homogeneous solution tend to persist in a given direction, making turns of small angles, usually <90° (2, 14, 25). Other cells, including fibroblasts and slime molds, also show persistence in their direction of locomotion (1, 10, 16).

We now report on an examination of the effect of polarity on PMN responses to chemotactic factors. Using a number of different tests, we find that the front of a locomoting cell is more responsive to stimulation by chemotactic factors than the tail. This asymmetry in responsiveness correlates with the morphological polarity and not with the dynamic features of the locomotion, e.g., the rate of locomotion. Neither the morphological nor the behavioral polarity is dependent upon the presence of microtubules.

MATERIALS AND METHODS

Chemicals

Chemotactic peptides *N*-formylmethionylmethionylmethionine(fMetMetMet) and *N*-formylnorleucylleucylphenylalanine (fNorleLeuPhe) were the gifts of Dr.

Elliott Schiffmann at the National Institute of Dental Health and Dr. Richard Freer at University of Virginia Medical College, Richmond, Va. C5$_a$, a split product of complement factor 5, was a gift of Henry Showell of Farmington, Conn. Colchicine was obtained from Sigma Chemical Co., St. Louis, Mo. Cells were examined in Hanks' Balanced Salt Solution (HBSS) containing 1% Knox gelatin (HBSS-gel).

Cells

Human peripheral blood PMNs were obtained from clot preps. A drop of blood from a finger prick was allowed to clot on a cover slip incubated in a moist chamber at 37°C for 45 min. The red blood cells and plasma were then washed off with physiological saline (0.9% NaCl), leaving a monolayer of white cells.

Filming

Filming was done at 15 or 20 frames/min using a × 16 objective and a × 6 ocular on a Zeiss microscope adapted with a Bolex camera and a Sage time-lapse apparatus (Orion Research, Inc., Cambridge, Mass. 02139). The temperature was controlled by a Sage air curtain. All solutions were equilibrated to 36°C before use.

In studies on rates of locomotion and turning behavior, 3 drops of the peptide in HBSS-gel were applied to cells on a cover slip, which was then inverted onto a microscope slide and sealed with a vaseline-paraffin mixture. Filming began after 5 min, when the transient effects of the peptide had diminished and the cells had begun locomotion. The area of the slide to be filmed was selected for an appropriate cell density. Frequent cell-cell collisions at high cell densities decrease the sequences that are useful for analysis. In studies on the initiation and reversibility of orientation, a visual chemotaxis chamber was used (24). Maintenance of focus during filming was improved with hemocytometer cover slips. The first frame of initiation sequences began with the introduction of peptide, and the first frame of reversal sequences began with the introduction of peptide into the well in the new direction of the gradient. Reversal was accomplished by removing the peptide solution from the well with a 1-ml syringe and a 21-gauge needle. Neutral solution was then placed in the empty well with a Pasteur pipette. The same procedure was then used to empty the previously neutral well and introduce the peptide to reverse the gradient.

Data Analysis

The films were projected by a L-W film analyzer (L-W International, Woodland Hills, Calif.), and the outlines of the cells were traced. After the cells in the first frame were numbered, each cell path was traced by drawing successive cell outlines at intervals (of 30 or 40 s, depending on the particular study). Cell orientations in a gradient were measured as the angle between the cell polarity vector (a line from the center of cell mass to the midpoint of the front of the lamellipodium) and the vector perpendicular to the peptide well, e.g., a cell going directly up the gradient would have an orientation of 0°.

When analyzing the mean-free path, a "turn" was defined as a change in direction, i.e., center of mass movement over 2 min, >20° from the previous 2-min time period. For the summation of turns per time, vectors indicating the direction of movement were drawn for each cell every 30 s, and the sum of the angles turned, left and right, was determined over a 2-min interval. Three separate slide preparations for 10^{-7} M, two for 10^{-6} M, 10^{-8} M, and 10^{-5} M, and one for 10^{-9} M fNorleLeuPhe were analyzed.

To test the significance of the result, the "F-test" was used. Because the variances between slides at the same concentration of peptide were not significantly different, we use n, the number of independent observations, as the cell number and not the number of slides.

The studies with the micropipette were done in collaboration with Dr. Daniel Kiehart (currently at the Department of Anatomy, Johns Hopkins University). A micropipette with a tip diameter between 0.5 and 1 μm was used. A drop of mercury was drawn into the pipette, followed by a drop of mineral oil. Then ~1 nl of 10^{-4} M fMetMetMet was drawn into the pipette, followed by an equal volume of Wesson oil. After the pipette was moved by means of a micromanipulator to the vicinity of the cell to be tested, the Wesson oil was expelled. The pipette was then rapidly adjusted so that the tip was about 1 cell diameter away from the cell, and the location of the pipette relative to the direction of locomotion by the cell was recorded. The cell response was observed in the microscope and the times up to the cell's first turn and correct orientation (±10°) toward the pipette were recorded. For control tests, HBSS was drawn into the pipette, followed by the Wesson oil. Again, the oil was expelled and the pipette situated approximately one cell diameter from a cell. For studies on the effect of colchicine, cells were preincubated for at least 50 min in 10^{-4} M colchicine before being tested.

Electron Microscopy

For scanning electron microscopy, cells on glass cover slips were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate, pH 7.2. Cells were postfixed in 1% osmium tetraoxide in 0.1 M cacodylate. The cells were dehydrated through gradual changes of methanol. Cells in 100% methanol were put directly into liquid CO_2 in a Sorvall critical point drying apparatus (DuPont Instruments-Sorvall, DuPont Co., Newtown, Conn.). The methanol was carefully flushed out before the cells were critical-point dried. After drying, they were shadowed with about 50 Å gold/palladium 60/40. The specimens were examined in an AMR 1000A microscope at 20 kV.

RESULTS

To determine whether the polar morphology characteristic of locomoting PMNs contributes to the cell behavior, we examined the relationship of the morphological polarity to the behavior under three different conditions: (a) cells locomoting in a homogeneous solution, or (b) exposed to an increase in the concentration of a chemotactic factor, and (c) cells subject to a change in the direction of a chemotactic gradient.

Cell Behavior in Homogeneous Solutions

The patterns of turns exhibited by cells locomoting in a homogeneous solution demonstrate a polarity of the cell behavior. Analysis of the change in direction of movement over 160-sintervals shows a distribution with a mode of 0° and few changes in direction over this time interval >90° (Fig. 1). These results are consistent with those of Allan and Wilkinson (2). Furthermore, analysis of cells exhibiting a turn indicates that even turns of large angles are usually achieved through a series of small turns and not by the cell suddenly sending out a new pseudopod at an angle obtuse to the previous direction of locomotion. Rather, as illustrated in Fig. 2, new pseudopods continually extend near the cell front, and a turn is usually achieved by extension of the lateral portion of an existing pseudopod and withdrawal of cytoplasm from the other side (Figs. 2 A and B). Only occasionally does a cell turn by initiating a new pseudopod from a region of the cell surface not previously extending pseudopods (Fig. 2 C). The percentage of the cell length that projects new pseudopods varies with the degree of cell elongation. Projections tend to be limited to
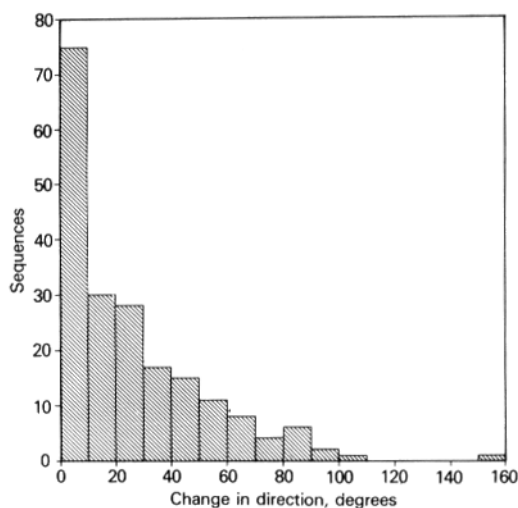

FIGURE 1 Distribution of changes in direction over 160-s intervals. Human PMNs were filmed locomoting in 10^{-9} M fNorleLeuPhe. Cell outlines were traced every 40 s, and the sum of change in direction of movement after every four outlines was evaluated. Data represent analysis of nine cells selected randomly.

the anterior 20% of an elongated cell, but can arise from the anterior 80% of a compact cell.

Only by observing cells in the process of turning is the extent of the cell polarity fully appreciated. Analysis of the cell movement from tracings of cell paths, where the paths are considered to consist of straight regions intercepted by turns, has shown the mean angle of turn to be between 30° and 50° (25). However, the magnitude of the turn analyzed in this way is more a reflection of the time that the cell continues to favor one side of an existing pseudopod than of the location on the cell surface that initiates the pseudopod, as has been suggested previously (1).

Polarity and the Rate of Locomotion

To determine whether the polar behavior is a result of a dynamic property of a moving cell, we examined the behavior as a function of the rate of locomotion. Cells were stimulated
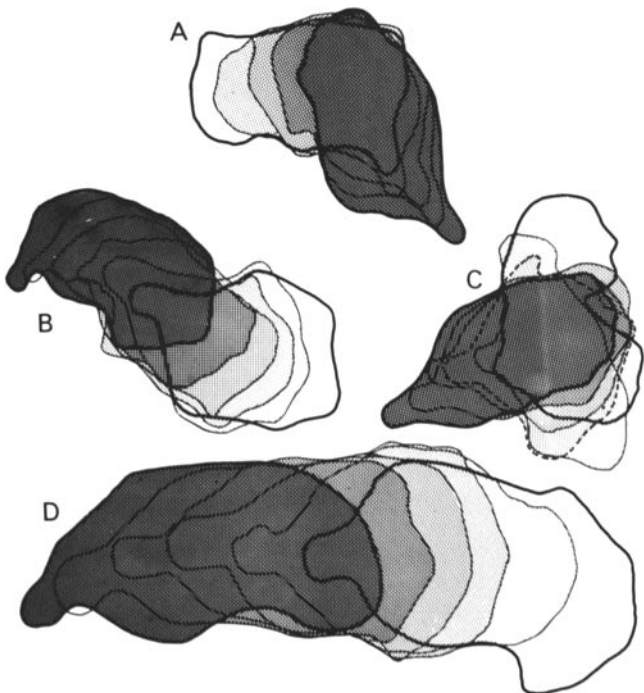
FIGURE 2 Tracings of human PMNs moving in homogeneous concentrations of 1 × 10^{-7} M fNorleLuePhe. The sequential tracings, 40 s apart, decrease in density. One sees that most new cell projections arise from existing pseudopods, even when a cell turns (A, B, and D). C illustrates the exception, an instance when in the fourth outline a new pseudopod arises from a region of the cell that had not been extending a pseudopod. In the fifth and sixth outline, this pseudopod grows and determines the new direction taken by the cell.

to move at different rates by incubating them in various concentrations of chemotactic peptide. As seen in Fig. 3 a, the rate of locomotion of human PMNs evaluated by tracing cell paths from time-lapse films increases nearly 100% when the concentration of fNorleLeuPhe is increased from 10^{-9} to 10^{-7} M. The faster-moving cells exhibit longer mean-free paths (defined as the distance between changes in direction of 20° or more) as seen in Fig. 3 b. The frequency of turns (i.e., turns per time interval), evaluated either as the sum of degrees change in direction over a 160-s interval or the mean angle of turn per 40 s, shows no significant correlation with the rate of locomotion (Table I). In fact, there is a tendency for the sum of turns to increase with increasing concentration of peptide, but the difference is not statistically significant. Thus, although the rapidly moving cell moves farther between turns than a slowly moving cell, the number of turns made over a given period of time is not altered.

The persistence of direction of locomotion was also examined in cells after they were transiently stopped. Immediately after increasing the concentration of a chemotactic peptide, the cells stop translocation (27). At this time, ruffles are present over most of the surface, except for the uropod (Fig. 4). We examined the relationship between the direction of locomotion that the cell resumes and the direction before the simultation. As seen in Fig. 5, cells exhibiting locomotion (and having a uropod structure) at the time they are stopped tend to resume locomotion in the same direction. Changes in the direction of
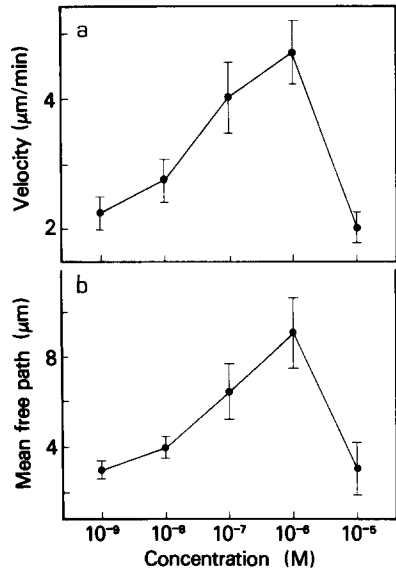
FIGURE 3 (a) velocity (rate of locomotion in µm/min) or (b) mean-free path (distance moved between turns of ≥20°) as a function of fNorleLeuPhe concentration. Data are plotted as the mean ± SD.

TABLE I

Angle of Turn per 40 and 160 s

	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M
Mean angle of turn per 40 s					
x̄	23.37	23.67	25.93	29.02	27.17
N	188	145	216	203	125
SEM	1.96	2.29	2.15	2.33	2.56
Sum of degrees turned per 160 s					
x̄	90.0	75.3	98.7	107.4	114.3
N	44	33	52	49	28
SEM	7.6	7.3	8.2	8.2	12.8

Mean angle of change in direction over a 40-s or a 160-s interval, as determined from tracings of cells moving in various concentrations of fNorLeuLeuPhe.
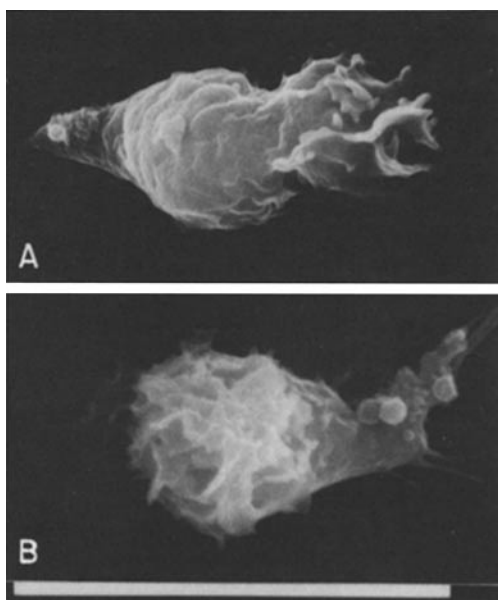
FIGURE 4 SEM pictures illustrating the distribution of cell ruffles. Control cells in 1×10^{-8} fNorleLeuPhe for 30 min form ruffles primarily from the front (A). When these cells are treated with 10^{-7} M fNorleLeuPhe for 30 s, they form ruffles over their surface but not over the tail (B). Bar, 10 μm.
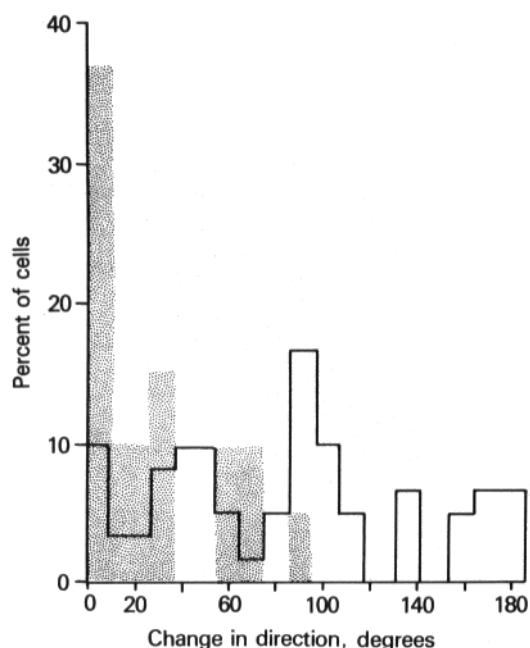

FIGURE 5 The angle between original and resumed direction of locomotion was evaluated in cells that either had (white bars) or had not withdrawn (stippled bars) uropods. Human PMNs were filmed during locomotion in 10^{-9} M fNorleLeuPhe. Addition of 1 $\times 10^{-7}$ M fNorleLeuPhe caused the cells to stop translocation. When the cells recommenced locomotion, the new direction was compared to the original direction and expressed as degrees change in direction (stippled bars). Another preparation of cells was filmed during locomotion in 10^{-9} M peptide but, before the addition of peptide, the cells were washed and incubated for 5 min in HBSS. During this time, the cells round and resorb their tails. The cells were then treated with 10^{-7} M fNorleLeuPhe, and, again, the new direction of locomotion was compared with that before treatment (white bars).

locomotion >90° are virtually excluded, just as they are in a continually locomoting cell. Thus, persistence in the direction of locomotion is not dependent on continued movement by the cell. Nor is the persistence affected by the ruffles or lamellipodia over most of the cell surface just before the resumption of locomotion.

A different result is obtained if cells are allowed to withdraw their uropod and round up completely before stimulation. Cells were filmed locomoting in the presence of chemotactic peptide. The peptide was then removed and replaced with a HBSS-gel. Again, the cells stopped locomotion. If the peptide is returned immediately, the cells continue locomotion in the direction they had been moving. However, if the cells are allowed to remain in the HBSS for ~10 min, most of the cells withdraw their uropods and have a round morphology. When these rounded cells are perfused with peptide, they form ruffles over their entire surface and spread symmetrically onto the substrate. After a short period they resume locomotion. As seen in Fig. 4, the new direction of locomotion is not correlated with the direction of locomotion before rounding. Thus, the component of polarity important for the behavior is at least randomized, if not lost, in a round cell.

Polar Responses to Chemotactic Gradients

The response of cells to chemotactic gradients also reflects their polarity. Cells in a chemotactic gradient remain responsive to changes in the gradient and reverse their orientation when the gradient is reversed (25, 26). However, rather than forming a new pseudopod from their tail, most cells reverse their direction by maintaining their polarity and walking around in a circle as shown in Fig. 6. (The gradient was reversed at the time of the first drawing, but it took ~3 min for the peptide to diffuse over the bridge and affect the cells.) Of >25 cells filmed reversing their direction in three separate trials, no cell formed a pseudopod from its tail. Furthermore, analysis of tracing of cell paths during the reversal indicated that cells rarely projected a new pseudopod from the posterior half of their body. 80% of the cells clearly "walked around" in a circle by making a series of small turns initiated near their
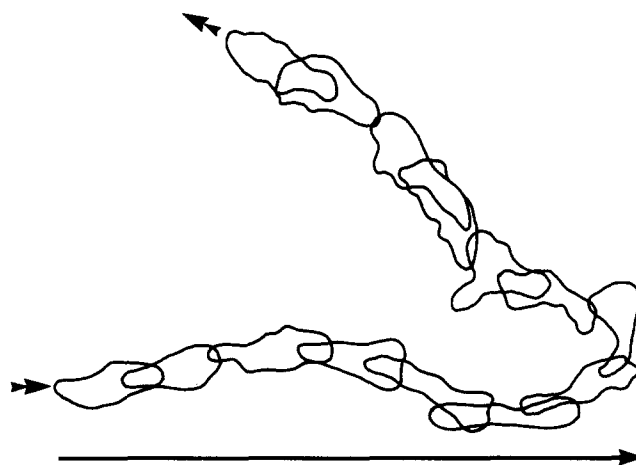

FIGURE 6 Tracing of human PMN outlines at 30-s intervals from a time-lapse film. The cell was originally oriented toward a chemotactic factor in the right well (gradient of $0-10^{-5}$ M fMetMetMet across the bridge). At the time of the first profile, the gradient was reversed and peptide was added to the left well. The long arrow indicates the direction of the previous chemical gradient. The short arrows indicate the direction of cell movement at the beginning and end of this sequence.

front. Most cells also rounded to some extent as they reversed their direction, and 20% rounded so much that it was impossible to determine the location on the cell which gave rise to the new pseudopod. Approximately equal numbers of cells turned to the left as to the right, indicating that the behavior was not due to the gradient developing from one direction.

The polar responses of PMNs to chemotactic factors could be caused by an asymmetric distribution of a component required for pseudopod formation, such as chemotactic receptors, transducers, or elements of the motile machinery. $C5_a$ and peptide interact with the cell via separate receptors (4, 23).
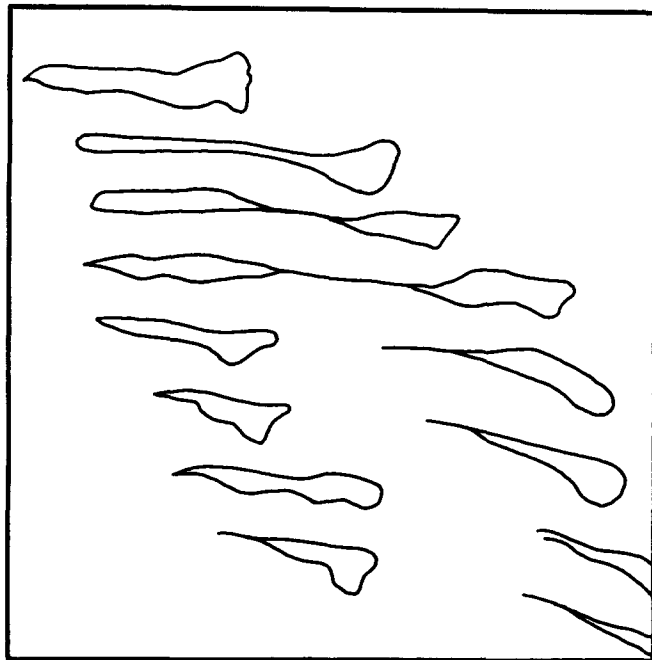

FIGURE 7 Sequential tracings (top to bottom) of a human PMN moving toward a chemotactic factor in the right well. In the fifth drawing, the cell splits into two parts. The front of the rear half, which was not ruffling before the split, now broadens and forms a typical pseudopod. Both halves of the cell progress to the right. The sequence took ~4 min.

Cells oriented in a gradient of peptide walk around in a circle to reverse their direction when the peptide is removed and replaced by a gradient of $C5_a$ in the opposite direction.

Further observations suggest that if the polarity is due to elements which exhibit an asymmetric distribution, this asymmetry must either be gradual or of a reversible nature. A cell, moving up the gradient and extending pseudopods only from its front, became very elongated and eventually broke into two fragments of approximately equal size (Fig 7). Within seconds after the split, the front of the rear portion of the cell, which had not been extending pseudopods before the break, began to extend pseudopods. Clearly, neither all the receptors nor all the protrusive machinery were exclusively localized at the front.

The response of cells to chemotactic gradients was confirmed by exposing cells to chemotactic peptide emanating from a micropipette placed less than one cell diameter away from the side or rear of a locomoting cell. The stimulus here would be expected to be both an increase in the peptide concentration and a spatial gradient of peptide concentration. As shown in Table II, cells respond directionally with respect to the peptide placed at their side. This was true whether or not 10^{-4} M colchicine was present. Seven of nine cells responded to a pipette placed at their side by forming a pseudopod toward the pipette within 30 s. Two cells initially moved away from the pipette but then returned. Seven of nine cells oriented their locomotion directly toward the pipette ($\pm$ 10°) within 2 min. A pipette filled with HBSS served as a control. When it was placed near the side of a cell, only one of four cells altered its direction within 1 min, and none of the cells oriented toward the control pipette within the 4 min of observation.

When a pipette containing chemotactic peptide was placed directly behind a locomoting cell, none of the eight cells tested responded by forming a pseudopod from the tail. Rather, the cells either turned to their side (5/8) or rounded (3/8).

Colchicine treatment at 10^{-4} M has previously been shown to depolymerize microtubules of PMNs (14). The colchicine-treated cells were more rounded than control cells, but nevertheless did have a uropod. Because the response of these cells was similar to that of control cells, we conclude that microtu-

TABLE II

Response of Cells to 10^{-4} M fMetMetMet Released from a Micropipette One Cell Diameter from a Locomoting Cell

Cell	Colchicine	Direction to pipette (relative to cell direction)	Direction of first turn	Time of first turn	Time to correct orientation
				min:s	
1	−	120° (side)	120°	0:36	0:36
2	−	120° ″	30°	:30	1:00
3	−	135° ″	135°	—	1:40
4	−	165° ″	75°	:30	5
5	−	165° ″	−45°*	:20	4
6	−	180° (tail)	90°	:34	—
7	−	180° ″	60°	:21 (rounds)	1:30
8	−	180° ″	95°	:50	1:40
9	−	180° ″	45°	:50	4:00
10	+	60° (side)	60°	:30	:30
11	+	60° ″	60°	:20	:20
12	+	60° ″	−60°*	:30	1:30
13	+	120° ″	120°	:30	:30
14	+	180° (tail)	Rounds	2:00	3:00
15	+	180° ″	150°	:25	3:00
16	+	180° ″	90°	:50	2:00
17	+	180° ″	60°	:20 (rounds)	3:30

* Cells turn in direction away from the pipette.

bules are not essential for either the structural or behavioral polarity.

Development of Polarity

We examined the initiation of locomotion by round cells by subjecting them to a chemotactic gradient. Cells were rounded by placing them in a nonstimulatory medium of HBSS-gel for 10 min and then placing the cells in a visual chemotaxis chamber with one well filled with HBSS-gel and filming while 10^{-5} M fMetMetMet was added to the other well. The results of such an experiment are shown in Figs. 8 and 9. Over 80% of the cells in the field filmed responded to the addition of peptide by spreading onto the cover slip; many respond within 1 min. 85% of these cells formed an initial lamellipodium of at least one third of a cell diameter toward the 180° sector and 63% into the 90° sector of the peptide well. Thus, the direction of the first pseudopod of this magnitude is biased toward the higher concentration. An even greater bias is seen in the direction of initial translocation, i.e., the direction of center of mass movement of at least half a cell diameter. With this measure, 94% of the cells move into the 180° sector and 75% into the 90° sector toward the peptide. Thus, some refinement in the gradient or in the cells' response to the gradient occurs between the formation of the initial pseudopod and the translocation of at least half a cell diameter.

The accuracy of this initial orientation is not sufficiently great to eliminate the possibility that the round cells have a behavioral polarity. For example, if cells were polarized but could form pseudopods up to 45° from the direction of their polarity, 75% of the cells could immediately send lamellipodia into the 180° sector toward the peptide. If they could form pseudopods up to 90° from the direction of their polarity, they could all move into the 180° sector. The initial orientation observed here was 85%.

Once a pseudopod of about 3–5 μm is formed, the behavioral polarity appears to be determined and the cell has a high probability of proceeding in the general direction of the pseudopod. Cells rarely extend a pseudopod of this size and then withdraw it to extend a pseudopod in a different direction. We were interested to see whether we could define an even earlier
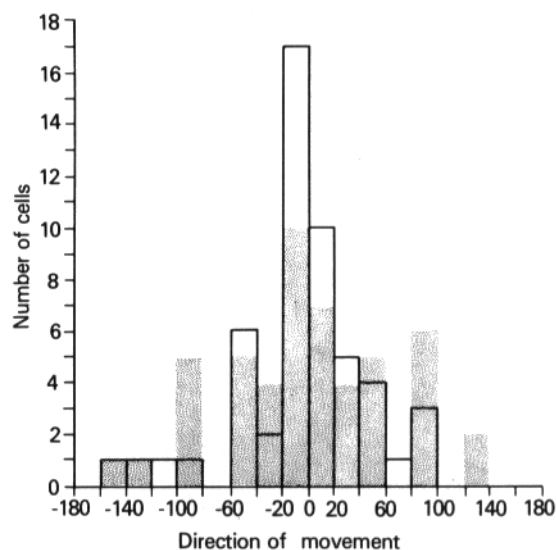
FIGURE 9 Bar graph of data presented in Fig. 6. Stippled bars represent the direction of the initial pseudopod; the white bars represent the initial translocation of center of mass in degrees deviation from the direction of the gradient.
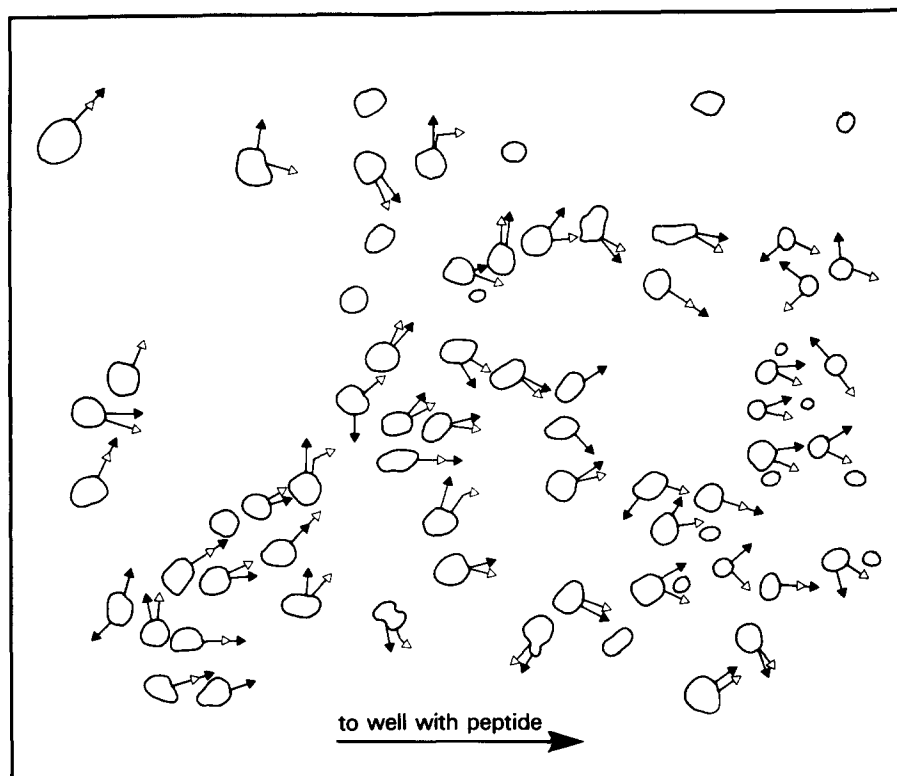
FIGURE 8 Drawings of cell outlines before addition of 10^{-5} M fMetMetMet to the right well of the chemotaxis chamber. The direction of the initial pseudopod (of a length ≥1/3 cell diameter) is marked with a filled arrow; the direction of the initial cell translocation (movement of center of mass of half a cell diameter) is indicated by the white arrow.

characteristic that signified polarity had been determined. For these studies, we filmed at higher power (× 40 objective) and analyzed the initial movements of the cells as the gradient was developing. Under these conditions, we could see that, although cells may remain for some time without translocating, they are not immobile. When stimulated by the increasing concentration of chemotactic factors as the gradient develops, the cells often spread or extend small projections, usually <20% of the cell diameter in length. Then, in a fairly abrupt transition, a cell will extend a larger pseudopod, develop a polarized form, and translocate across the substrate. As this pseudopod begins to extend, there is a concomitant movement of the body of the cell. This movement appears to be a contraction of the cell body, resulting in a narrowing or a movement forward of what will become the rear of the cell. Thus, the cell can commence locomotion, keeping the overall cell profile round. In 25 cells examined, once a margin of the cell body had moved centripetally >20% of the cell diameter, this region of the surface did not reverse and extend a pseudopod (0/25 observed). The centripetal movement is unlikely to merely result from a requirement to maintain constant volume while the pseudopod extends because, during spreading, a cell can extend to three times its round diameter. Rather, it appeared to be an active contraction of the body of the cell, suggesting that a contracted area had reduced its probability of forming a projection.

DISCUSSION

In this study we have demonstrated that locomoting PMNs exhibit a morphological polarity that is not the consequence of exposure to a chemotactic gradient but of locomotion. We have shown that the morphological polarity correlates with a behavioral polarity on the part of the locomoting cell in response to chemotactic factors. The behavioral polarity was observed in several ways. First, in randomly locomoting cells, most new pseudopods protrude near the existing front. Because the pseudopod determines the direction of locomotion, this results in the cells persisting in a given direction and making turns of small angles (2, 15). Second, increasing the concentration of a chemotactic peptide induces pseudopods to form more readily from the front than from the rear of a cell. Even when a gradient is formed from the rear, cells usually reorient by responding at their front and walking around in a circle. Others have made similar observations (10, 13). However, Ramsey (18) studied PMN respones to a moving chemotactic stimulant and described cells that reverse direction by forming new pseudopods from their tails. In our experience, this is a rare event and did not occur in any of the reversal experiments carried out here.

It would be useful to identify the cell structures or activities that determine the behavioral polarity. Microtubules do not appear to be necessary. Yet, Malech et al. (13) noted that as a cell changes its direction, the centriole can be seen to move toward the cell front. Certain aspects of the behavior described can be correlated with the cell morphology, particularly with the presence of a uropod. The unresponsiveness of the tail region could be caused by absence or inactivation of (a) chemotactic receptors, (b) membranous components involved in transduction of the chemotactic signal, or (c) particular contractile and cytoskeletal elements in the uropod. Because a cell oriented by one chemotactic factor still exhibits polar behavior when stimulated by a different factor, it is unlikely that selective capping of bound receptors accounts for the polar behavior. Nevertheless, the tail is the site to which concanavalin

A (Con A) and other lectins cap (20). Recent papers suggest that the Fc receptor and Con A binding may be preferentially localized at the front of a locomoting PMN (21, 22). Braun et al. (6) have demonstrated that certain receptors move to the uropod of a lymphocyte even in the absence of a ligand. Thus, it is possible that some receptors or enzymatic activities are selectively localized in or excluded from the uropod of a locomoting PMN. We are currently investigating the distribution of the chemotactic peptide receptor. Electron microscopic studies as well as immunofluorescence studies demonstrate differences in distribution and organization of contractile proteins between the front and the tail of moving leukocytes (S. H. Zigmond. Unpublished observations; 17, 19). However, whether these differences account for the observed polarity is unknown.

Whatever determines the location of pseudopod formation, its effect is reversible. Thus, after a cell split in half, the front of the posterior half of the cell started to ruffle, although this section was previously quiescent. The reversibility of the polar behavior in split cells indicates that the inhibition of ruffling is not produced by the exclusive sequestering of certain components in either the cell front or rear. One possibility that fits with our observations on the development of polarity is that once a region of the cell cortex has undergone a contraction, it has reduced its potential to form a pseudopod. During contraction, the filamentous organization in the region behind a pseudopod could become crosslinked or altered in some manner that inhibits protrusion of a pseudopod. In locomoting cells, the constriction ring and rounded body of the cell, both of which appear contracted, do not normally extend pseudopods.

The polar behavior of PMNs has consequences for their chemotactic ability. The polarity decreases the efficiency of orienting along a new gradient but stabilizes the orientation once achieved. Thus, once the cell's polarity and the gradient are aligned, both will contribute to the maintenance of this orientation.

In addition, the existence of the cell polarity affects the interpretation of experiments investigating the mechanism that cells use to detect a gradient. We reported previously and confirm here, by use of a different chemoattractant, that round PMNs are able to initiate locomotion up a concentration gradient of a chemotactic factor without first translocating in the gradient (25). Furthermore, locomoting PMNs are able to turn toward a new source of chemotactic factor (5). Clearly, the PMN exhibits vectorial responses that bacteria do not. This led us to suggest that PMNs sense the direction of a gradient by detecting a difference in receptor occupancy on different parts of the cell surface at a given time. That is, a cell would form a pseudopod from the region of its surface with the highest concentration of occupied receptors. This has been termed a "spatial" mechanism, because the cell compares different locations at one time. Although this may be valid, several new pieces of information suggest an alternative hypothesis.

It has recently been demonstrated that increasing the concentration of a chemotactic factor transiently induces pseudopod formation, whereas decreasing the concentration of a factor causes pseudopod withdrawal (8, 27). Although these responses alone are not directional, if a pseudopod that extends up a concentration gradient and thus encounters an increase in concentration, were induced to extend farther, while a pseudopod that extends down the gradient were withdrawn, chem-

otaxis would occur. The mechanism would be temporal because the signal would be a change in the mean number of occupied receptors on a particular region of the cell surface over time. This is a local response and requires no comparisons across the cell's dimensions. A temporal signal has been suggested by Gerisch (11) to be functional in slime mold chemotaxis and by Alt for PMNs (3).

Several aspects of PMN behavior seem consistent with a temporal mechanism. The responses to temporal changes as described above are exactly those that would lead to chemotaxis. In addition, the manner in which PMNs turn, i.e., usually by further extending a region of an already extending pseudopod, is consistent with a temporal mechanism. The extending pseudopod would be probing the environment and, where favorable, would be induced to extend farther. It is of interest that a number of chemotactic factors for PMNs including $C5_a$ (8), a cell-derived factor (S. H. Zigmond. Unpublished observations), and the N-formyl methionyl peptides (27) all induce a transient surface ruffling. Furthermore, chemotactic factors for other cell types, including cAMP for slime molds[1] and NGF (7) on nerve cells, induce the trasient ruffling. Thus, the ability to induce a ruffle or pseudopod may be a common feature of chemotactic factors.

It is difficult to determine whether PMNs are using a temporal or spatial mechanism of sensing the direction of a gradient. A spatial signal could be detected by a stationary cell in a stable gradient, whereas a temporal signal requires either that the concentration be changing or that some region of the cell be moving. It is difficult to obtain a stationary cell in a stable chemotactic gradient because chemotactic factors cause cells to move. Furthermore, it is only by moving, at least forming a pseudopod or polar morphology, that we can recognize that a cell has sensed the direction of a gradient. As soon as a cell moves, it could be using a temporal mechanism.

The fact that a cell does not respond to a reversed gradient by extending a pseudopod from its tail does not rule out a spatial mechanism. The polarity would modulate a signal received by the spatial mechanism and the cell response at any point on its surface would be a combination of the strength of the spatial signal and the relative responsiveness of that portion of the cell. On the other hand, the fact that PMNs can move smoothly up a concentration gradient with their shape remaining nearly constant does not rule out a temporal mechanism. If the induction of pseudopods depended solely upon the increase in concentration over time, the tail of a cell moving up a gradient should be induced to form as many pseudopods as the front. Clearly, this is not the case, and pseudopods are not even observed forming laterally from the tail. The polarity would modulate the responses to the same temporal signal and thus make these observations consistent with a temporal model.

At the moment none of the experiments definitively differentiates between a temporal and a spatial mechanism of sensing the gradient. Regardless of the mechanism of sensing the

gradient, it is clear that much of the coordinated movement of a cell up a gradient is not due solely to responses to the gradient. Rather, it is achieved through the behavioral polarity which exists in the absence of a gradient and continues to exert its effect in a gradient.

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