Cytosolic Free Calcium and the Cytoskeleton in the Control of Leukocyte Chemotaxis

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Pettit, Elizabeth J., and Frederic S. Fay. Cytosolic Free Calcium and the Cytoskeleton in the Control of Leukocyte Chemotaxis. Physiol. Rev. 78: 949–967, 1998.—In response to a chemotactic gradient, leukocytes extravasate and chemotax toward the site of pathogen invasion. Although fundamental in the control of many leukocyte functions, the role of cytosolic free Ca\(^{2+}\) in chemotaxis is unclear and has been the subject of debate. Before becoming motile, the cell assumes a polarized morphology, as a result of modulation of the cytoskeleton by G protein and kinase activation. This morphology may be reinforced during chemotaxis by the intracellular redistribution of Ca\(^{2+}\) stores, cytoskeletal constituents, and chemoattractant receptors. Restricted subcellular distributions of signaling molecules, such as Ca\(^{2+}\), Ca\(^{2+}\)/calmodulin, diacylglycerol, and protein kinase C, may also play a role in some types of leukocyte. Chemotaxis is an essential function of most cells at some stage during their development, and a deeper understanding of the molecular signaling and structural components involved will enable rational design of therapeutic strategies in a wide variety of diseases.

I. INTRODUCTION

Almost every cell type known undergoes directed movement for a specific purpose during its lifetime, ranging from the primitive light or nutrient seeking of prokaryotes and single-celled eukaryotes to the highly complex and intricately orchestrated movements taking place during embryogenesis. Multicellular eukaryotes retain specific cell types that are motile in their fully differentiated state and can move around the body of the organism without the constraints imposed on their static neighbors. This was first noted in the late 19th century, when Metchnikoff observed the movement of white blood cells to sites of infection (97, 260). The importance of cell movement, as with many other cellular processes, is well illustrated by observations of organisms in which it malfunctions.
ditions preventing normal motility, such as leukocyte adhesion deficiency, are sometimes fatal (13), and inappropriate motility enables the seeding of cancerous metastases (216, 232, 269). Thus cell movement, and chemotaxis in particular, is an important area for study, shedding light on the functions of different cell types and providing information that will prove useful in the battle against several diseases (216, 224).

There are different terms that describe cellular movement. Chemotaxis is the directional migration of a cell up (or down) a concentration gradient of a chemotactic factor, which must be distinguished from chemokinesis, the random movement of a cell in response to a uniform concentration of a chemoattractant, and haptotaxis, which also describes the directional motility of a cell, but in response to the polarity of the substrate. Cells chemotax to reach sites where chemoattractants are released, either by the organism in response to a stimulus or by the stimulus itself. For example, eosinophils respond to parasitic invasion under the influence of both molecules released by the body in response to the invading organism (59), and factors released by the invading parasite itself (206). Of course, parasites have also evolved to release substances that prevent eosinophil chemotaxis (36). This review describes the range of leukocyte chemoattractants (sect. II), the gross morphology of the chemotaxing cell (sect. III), and the signal cascades (sect. IV) that result in the establishment and maintenance of both a polarized morphology and forward motion (sect. V).

The leukocytes are the most widely studied chemotactic cells in multicellular organisms. They range throughout the tissues of the body, covering distances many times longer than their own dimensions. Other, less dramatically chemotactic cells include the endothelial cells, smooth muscle cells, and fibroblasts that play a vital role in wound healing and angiogenesis (233). Among the unicellular organisms, the slime mold Dictyostelium discoideum is a model for chemotaxis (163, 180), although other unicellular organisms have also been studied (76). There are several contradictory reports in recent literature concerning the role of Ca²⁺ in chemotaxis, and new molecular families are being described in other cell types that may be relevant to leukocytes. In this review, we have attempted to summarize recent findings as they relate to the role of Ca²⁺ in myeloid leukocyte chemotaxis.

II. CHEMOATTRACTANTS

Chemotaxis is initiated when the leukocyte responds to the occupation of a receptor with its ligand, the chemoattractant. Chemotactants fall into different groups, according to their activity, structure, receptor type, and origins. The major chemoattractants for leukocytes are substances released by other cells, such as endothelial cells and other leukocytes, in response to a stimulus such as the presence of an allergen or pathogen. Pathogens such as bacteria, fungi, and other parasites also release chemotacticants, and a variety of other cells release other substances that inhibit or potentiate the inflammatory response. Some of the leukocyte chemoattractants are listed in Table 1.

A. Chemokines

The chemokines are small (8–13 kDa) peptides that share a conserved tertiary structure with one to three disulfide bonds. According to the positioning of the first two cysteine residues, they are classified as either C-X-C, or α-chemokines (cysteines separated by one amino acid), C-C, or β-chemokines, or single cysteine motif chemokines such as lymphotactin, a lymphocyte chemoattractant. Chemokines have been the subject of a number of reviews: for eosinophil chemokines, see Vanacker et al. (250); for their role in inflammation, see Proost et al. (191); and for their actions and classification, see Negus (162) and Baggiolini et al. (17).

B. Anaphylatoxins

Activation of the complement cascade causes the production and local accumulation of a number of small peptides (anaphylatoxins), and several of them are responsible for leukocyte recruitment (Table 1). Leukocytes also express the CD11b/CD18 integrin (CR3; Mac-1) receptor for C3bi (91), a complement component used to opsonize bacteria. CR3 also functions as a receptor for intercellular adhesion molecule-1 (ICAM-1), during leukocyte adhesion to endothelial cells (16), and as a chemoattractant receptor for fibrin degradation products (82).

C. Substances Released by Invading Organisms

Potent chemoattractants and other cellular activators are released by invading bacteria and parasites (109, 206). The bacterial cell wall constituent lipopolysaccharide (LPS; endotoxin) activates neutrophils by an unknown pathway, resulting in phosphorylation and activation of kinases (160, 164). Bacteria, mitochondria, and chloroplasts, unlike the nuclear genome, synthesize formylated peptides, and formyl - methionyl - leucyl - phenylalanine (FMLP), a model for these peptides, is among the best studied neutrophil chemoattractants.

Recent developments in human immunodeficiency virus (HIV) research have focused on the role of the chemo-
TABLE 1. Cross-signaling by some leukocyte cytokines and other chemoattractants

<table>
<thead>
<tr>
<th>C-C chemokines</th>
<th>Chemotaxis</th>
<th>Kinesis</th>
<th>Priming</th>
<th>Secretion</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eotaxin 1, 2</td>
<td>E (204, 259) B, L</td>
<td>E</td>
<td>N, E adhesion</td>
<td>Histamine, LTC₄ from B</td>
<td>E RB (58)</td>
</tr>
<tr>
<td>MCP-1</td>
<td>M, Ma, L (267), DC (266), B</td>
<td>M adhesion</td>
<td>Histamine from B, IL-4 from L</td>
<td>L differentiation</td>
<td></td>
</tr>
<tr>
<td>MCP-3</td>
<td>E, DC (266)</td>
<td>E</td>
<td>E adhesion, kinesis to RANTES</td>
<td>E RB, degranulation</td>
<td></td>
</tr>
<tr>
<td>MIP-1α</td>
<td>E, M, DC</td>
<td>M</td>
<td>E, M adhesion</td>
<td>IFN-γ from L</td>
<td>L differentiation; CCR5 is HIV co-R</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>M, Ma, L, DC</td>
<td>E, B, M, MC, L, DC</td>
<td>E</td>
<td>M adhesion</td>
<td>CCR5 is HIV co-R</td>
</tr>
<tr>
<td>RANTES</td>
<td>E, B, M, MC, L, DC</td>
<td>E, M adhesion</td>
<td>E</td>
<td>M adhesion</td>
<td>E RB, degranulation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C-X-C chemokines</th>
<th>Chemotaxis</th>
<th>Kinesis</th>
<th>Priming</th>
<th>Secretion</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRO-α, -β, -γ</td>
<td>N, L, M/Ma (weak) E, NK</td>
<td>E (60)</td>
<td>E adhesion, cytotoxicity</td>
<td>E adhesion, cytotoxicity</td>
<td>Angiogenesis, growth</td>
</tr>
<tr>
<td>IL-2</td>
<td>E, NK</td>
<td>E</td>
<td>IL-2 adhesion, chemotaxis to MCP-1 and MCP</td>
<td>E</td>
<td>IL-2 adhesion, chemotaxis to MCP-1 and MCP</td>
</tr>
<tr>
<td>IL-5</td>
<td>E</td>
<td>E</td>
<td>IL-5 adhesion</td>
<td>IL-5 adhesion</td>
<td>E</td>
</tr>
<tr>
<td>IL-8</td>
<td>N, L, M/Ma (weak) L, N</td>
<td>L</td>
<td>L adhesion, chemotaxis to MCP-1 and MCP</td>
<td>Elastase from N</td>
<td>N</td>
</tr>
<tr>
<td>IP-10</td>
<td>NK, L, M/Ma (weak) L</td>
<td>L</td>
<td>NK chemotaxis</td>
<td>NK chemotaxis</td>
<td>NK chemotaxis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Complement components</th>
<th>Chemotaxis</th>
<th>Kinesis</th>
<th>Priming</th>
<th>Secretion</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1q</td>
<td>E (125)</td>
<td>E (125)</td>
<td>E</td>
<td>cytotoxicity</td>
<td>IgG-dependent E killing of S. mansonii (88)</td>
</tr>
<tr>
<td>C3a</td>
<td>E, MC (167)</td>
<td>E</td>
<td>E adhesion, chemokinesis to RANTES</td>
<td>MCP-1 adhesion, IL-4, IL-13, CCL2, from B, many cytokines from M</td>
<td>E, N RB; E aggregation</td>
</tr>
<tr>
<td>C5a</td>
<td>E, N (30), MC (107)</td>
<td>E</td>
<td>E adhesion, chemokinesis to RANTES</td>
<td>E</td>
<td>E adhesion, chemokinesis to RANTES</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Others</th>
<th>Chemotaxis</th>
<th>Kinesis</th>
<th>Priming</th>
<th>Secretion</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS</td>
<td>N, M, Ma</td>
<td>PAF, LTB₄, C5a response, E adhesion</td>
<td>AA from N (67), several cytokines from Ma and hepatocytes</td>
<td>E</td>
<td>Bacterial cell wall constituent, phagocytosis</td>
</tr>
<tr>
<td>LTB₄</td>
<td>E, N</td>
<td>N</td>
<td>M BB, E taxis to FMLP, AA, LTB₄ secretion from N</td>
<td>M</td>
<td>BB, E taxis to FMLP, AA, LTB₄ secretion from N</td>
</tr>
<tr>
<td>ETE</td>
<td>E (172), N (171)</td>
<td></td>
<td></td>
<td>AA from N</td>
<td>N vesicle translocation, E degranulation</td>
</tr>
<tr>
<td>FMLP</td>
<td>N, M, Ma, E</td>
<td>N (50)</td>
<td>N, M adhesion, N response to PAF, E adhesion</td>
<td>N granule contents, LTB₄ from E</td>
<td>N, M RB</td>
</tr>
<tr>
<td>PAF</td>
<td>E (60), N</td>
<td>E</td>
<td>N adhesion, E aggregation, N response to FMLP</td>
<td>N</td>
<td>adhesion, E aggregation, N response to FMLP</td>
</tr>
<tr>
<td>Substance P</td>
<td>L</td>
<td></td>
<td></td>
<td>L adhesion, E chemotaxis, N RB</td>
<td>Pain neurotransmitter</td>
</tr>
</tbody>
</table>

Roles of a range of chemoattractants in leukocyte chemotaxis, chemokinesis, priming, secretion, and other responses are shown. AA, arachidonic acid; B, basophils; DC, dendritic cells; ETE, eicosatetraenoates; E, eosinophils; GRO, growth-related peptide, IFN, interferon; IL, interleukin; IP, inducible protein; L, lymphocytes; Ma, macrophages; M, monocytes; MC, mast cells; MIP, macrophage inhibitory peptide; N, neutrophils; NK, natural killer cells; RANTES, released on activation normal T cells expressed and secreted; RB, respiratory burst; PAF, platelet-activating factor; LT, leukotrienes; MCP, monocyte chemotactic protein; FMLP, formyl-methionyl-leucyl-phenylalanine; LPS, lipopolysaccharide; HIV, human immunodeficiency virus.

for HIV entry into leukocytes (reviewed by Broder and Dimitrov, Ref. 27). The mechanism by which HIV achieves this is currently being studied (263). In addition, the HIV releases HIV-Tat, which is both a chemoattractant for and a primer of chemotaxis in monocytes (130).

D. Others

Platelet-activating factor (PAF) is both expressed on, and secreted by, activated endothelial cells. The PAF receptor on leukocytes is therefore occupied firstly during adhesion by the membrane-bound form, potentiating the CD11b/CD18 integrin-ICAM-1 interaction (38), and then by soluble PAF. Soluble PAF is produced by other cells (209), including leukocytes (18).

Substance P, a peptide released by pain-sensing neurons, is both a chemoattractant for T cells (252) and a primer of chemotaxis in eosinophils (55, 56). Other neuro-peptides inhibit chemotaxis. Primers are substances or interactions that do not cause intracellular signals that
lead to a particular response but do cause changes in a
pathway that increase the cell’s response to subsequent
stimulation of that pathway. Such changes include tyro-
sine phosphorylation, or receptor activation. Other prim-
ers of chemotaxis include adhesive interactions and sub-
stances released by invading organisms (140, 206).

Leukocytes themselves release chemoattractants
when stimulated (Table 1); for example, neutrophils re-
lease granule contents (241) and interleukins (201) that
are potent chemoattractants for monocytes and T cells,
and eosinophils release monocyte chemotactic protein-1
(105). Leukocytes and other cell types also synthesize and
release leukotrienes (LT), such as LTA₄ (207), LTB₄ (18),
LTC₄ (41, 212), and LTD₄, and arachidonic acid (203),
which are chemoattractants.

E. Cross-Signaling

It must be noted that the majority of chemoattrac-
tants also function to augment some other aspect of leu-
kocyte behavior associated with chemotaxis (Table 1).
Chemoattractants can alter the avidity and cause the
upregulation of adhesion molecules and chemoattractant
receptors (32, 35, 119, 256); mediate secretion (48), aggreg-
ation, and other responses associated with pathogen kill-
ing; and inhibit apoptosis (191). Many of the chemokines
are also leukocyte primers (122) and chemokinetants (Ta-
ble 1; Refs. 90, 122).

III. MORPHOLOGICAL CHANGES IN CHEMOTAXIS

To introduce the molecular changes taking place in
the leukocyte in response to chemoattractants, it is neces-
sary first to describe the gross changes in leukocyte mor-
phology that are immediately obvious by light micro-
scopic observation. Similar observations have been pub-
lished with reference to neutrophils (144), metastatic
tumor cells (46), D. discoideium (47), and Entamoeba his-
tolytica (76).

The cell in its “resting” state is spherical, and the
plasma membrane appears to be “static.” Within seconds
of the arrival of the chemotactic stimulus, membrane ruf-
fling occurs over the whole surface of the cell. Several
membrane ruffles may become larger membranous filo-
podial spikes, or sheetlike lamellipodia, and ruffling
ceases as they extend from the cell body. This change in
morphology has an oscillating periodicity (51). The pseu-
dopod is formed when granules and other constituents
from the cell body begin to flow into the lamellipod, which
expands and flattens over the substrate. More granules
continue to flow forward, stopping just short of the very
tip of the advancing pseudopod (222). After some time,
the cell is stretched across the substrate, and the cyto-
plasmic components are separated, with granules occupy-
ing the leading 60% of the cell, and the nuclear lobes
at the back, left behind by the moving granules. After this
polarized morphology is assumed, the nuclear lobes move
with the cell toward the stimulus, either driven forward
by constriction of the uropod, the cytoplasmic “tail” at
the back of the cell, or pulled forward by the advancing
lamellipod.

Another cytoplasmic component, visible in the larger
types of leukocyte (29), is the centrosomal region. In the
nonpolarized cell, this group of organelles is located close
to the nucleus, often nestled between the lobes (29, 181).
In studies of the giant newt eosinophil, as the cell becomes
polarized, the region moves forward in the direction of
the leading pseudopod; its movement is in fact an accurate
predictor of where the first pseudopod will form (29).

Reorientation of the chemoattractant gradient causes
the leukocyte to cease movement, and the centrosomal
region returns, sometimes rapidly, back toward the nu-
cleus. Ruffling begins again, even on the parts of the cell
furthest from the stimulus, but not on the leading pseu-
dopod. A new pseudopod forms, and the previous one is
retracted. The new stimulus may cause the cell to “round
up” before repolarization, or if the change in the angle of
the gradient is small, the repolarization will sometimes be
accomplished by a change in the direction of the original
pseudopod. The time taken for the cell to perform this
reorientation depends on the angle between the old che-
moattractant source and the new one. A change of 180°
gives rise to prolonged (2–6 min) reorganization of the
cytoplasmic components, including the centrosomal re-
region, which moves around, or over, the nuclear lobes,
often accompanied by the granules, which eventually
move to the part of the cell nearest the new chemoatrac-
tant source.

These are fascinating processes to watch and raise
many questions for the observer. At what point does the
leukocyte become polarized? Is this polarity solely a result
of the action of the chemoattractant, or does the cell have
some inherent polarity, even in its resting state? How does
a chemotaxing cell continue to sense a concentration gra-
dient which, along its length, is small (As low as 1%; Ref.
273)? Is it the polarity of the cell that enables this, by
some change in the signaling capacity of the front and
back areas of the cytosol?

IV. CALCIUM SIGNALING

The questions raised by observation of the chemotac-
tic leukocyte can only be approached with some under-
standing of the signal transduction pathways involved in
the generation and maintenance of the polarized and mo-
tile state.
A. Receptor-to-Cytosol Signaling

The majority of receptors for chemotactic agents in leukocytes are the seven transmembrane, or serpentine, type (7TMR; Ref. 159). The extracellular NH₂-terminus contains the ligand binding site, and cytoplasmic loops of varying sizes between the transmembrane domains are thought to interact with heterotrimeric G proteins (25). A variable-length COOH-terminus may contain tyrosine phosphorylation sites.

The receptors have varying degrees of redundancy (for example, CCR1, -4, and -5 are all receptors for both RANTES and MIP-1α), but because the receptor types are expressed to differing extents on different cells, release of a particular chemokine can give rise to a specific infiltrate of one cell type. The leukocyte may have several different receptors, with different affinities for the same chemoattractant (264). The interactions between receptor and chemokine have been reviewed (142, 162).

The most fully characterized 7TMR is the FMLP receptor (25), but an increasing number of receptors, such as the human eotaxin (190) and CCR5 receptors (198), are being cloned, and their structures and functions are becoming clearer. Indeed, the identification of receptors exceeds the number of identified ligands, leaving some 7TMR classified as “orphans” (142), although they, too, are being assigned ligands, as new chemoattractants are described (268).

Both heterotrimeric and small G proteins (85, 86) are involved in leukocyte movement, and the latter are also active during chemokinesis (131, 195) and embryogenesis (141). The role of the heterotrimeric G proteins in general is well characterized, the released α- and βγ-subunits causing activation of phospholipase subtypes, phospholipase C (PLC)-β and phospholipase D (PLD; Refs. 63–65, 106). The action of PLC-β on the lipid phosphatidylinositol 4,5-bisphosphate (PIP₂, itself an important molecule in signaling, see below) causes cleavage of the cytoplasmic “head” region, releasing inositol 1,4,5-trisphosphate (IP₃) into the cytosol, and leaving the lipid diacylglycerol (DAG) in the plasma membrane. Phospholipase D also produces DAG, by an indirect mechanism (see sect. IV D). In addition, phosphatidylinositol 3-kinase (PI3K)-γ is activated by G proteins and tyrosine kinases and phosphorylates PIP₂ to phosphatidylinositol 3,4,5-trisphosphate (PIP₃) and other phosphatidylinositols at the 3'-position. Thus two types of second messenger are formed in the cytosolic (IP₃; Ref. 2) and lipid (DAG; PIP₃) fractions of the leukocyte.

B. Cytosolic Second Messengers: Inositol 1,4,5-Trisphosphate and Calcium

A small and highly diffusible molecule, IP₃, rapidly occupies its receptors on the surface of membranous Ca²⁺ stores (within 75 ms in neutrophils, Ref. 183). The IP₃ receptor is a channel consisting of four subunits, and IP₃ binding causes channel opening, releasing Ca²⁺ into the cytosol. The IP₃ receptor may be regulated by phosphorylation by the tyrosine kinase, Lyn, induced by the increase in cytosolic free Ca²⁺ concentration (108). The released Ca²⁺ has a multitude of intracellular effects (2).

There is more than one type of Ca²⁺ store in leukocytes. The site at the central, centrosomal region, present in both neutrophils (181) and eosinophils (29), has been demonstrated to be IP₃ sensitive (66), but the sensitivity of the other, peripherally located stores is not so well established. The presence of IP₃-insensitive Ca²⁺ stores is being demonstrated in an increasingly large number of cell types (189). Evidence from human neutrophils suggests that chemoattractants cause Ca²⁺ release from the central store (185), but interactions with the adhesive substrate release Ca²⁺ from peripheral stores (184), by an uncharacterized, but possibly cytoskeletonally linked mechanism. Thus there is an interplay between these two Ca²⁺ release pathways in the leukocyte chemotaxing over a physiological substrate, engaging both the 7TMR and adhesion receptors.

Release of Ca²⁺ from the central store is “coupled” to Ca²⁺ influx, a relationship termed “capacitative Ca²⁺ influx” (19, 98, 179, 194). The nature of the coupling is thought to be a diffusible factor, Ca²⁺ influx factor (197), but the actions (and even existence) of this factor have been subject to much debate (20, 43). Peripheral Ca²⁺ store release is not coupled to influx, and because Ca²⁺ diffuses only 2–3 µm before it is buffered, or taken up into stores (2), is a mechanism for achieving a degree of accuracy in the location of the Ca²⁺ signal.

Calcium store release (in the absence of influx) generates only localized increases in cytosolic free Ca²⁺ concentration, because of the high buffering capacity of the cytoplasm. This is at least partly because of the high concentrations of Ca²⁺-binding proteins in the leukocyte. Some of these proteins, such as the low-affinity calreticulin and calsequestrin, are found in Ca²⁺ stores, which also contribute to Ca²⁺ removal from the cytosol by activation of inwardly driving Ca²⁺-ATPase pumps. Some Ca²⁺ is expelled extracellularly by pumps across the cell membrane. However, a proportion of the released Ca²⁺ is bound by proteins whose functional activity is altered by the binding.

C. Calcium-Binding Proteins

There are three types of Ca²⁺-binding proteins. Storage proteins sequester Ca²⁺ in stores, in preparation for store release. They bind as many as 25 Ca²⁺ each. The second type of Ca²⁺-binding protein also contains binding
sites for phospholipids and are typified by protein kinase C (PKC-α and -β), which are activated by Ca\(^{2+}\) and DAG. The third, and largest group, are the “EF hand” Ca\(^{2+}\)-binding motif proteins. Calmodulin may be the major Ca\(^{2+}\)-binding protein in this group, which also includes calpain, possibly involved in integrin-receptor recycling. The full range of Ca\(^{2+}\)-binding proteins and their functions have been reviewed (166).

Calmodulin binds to four Ca\(^{2+}\), two at each “end” of the molecule. In its Ca\(^{2+}\)-bound form (Ca\(^{2+}/CaM\)), it associates with, and alters the function of, CaM-dependent protein kinases (CaM kinases) and unconventional myosin heavy chains. The functional association between actin and myosin II is also modulated by Ca\(^{2+}\)/CaM binding to myosin light-chain kinase (MLCK), which then becomes activated and phosphorylates the light chain part of the myosin head domain. This alters the affinity of myosin for actin, the effects of which are explained in section V, B and C.

D. Lipid Messengers

Both chemoattractants and adhesive interactions during chemotaxis generate intracellular messengers within the lipid fraction of the cell. Phospholipase D (73) activation generates phosphatidic acid. Phosphatidic acid is phosphohydrolyzed to DAG, causing a second, more prolonged burst of DAG production, after the initial one caused by the action of PLC-β. Diacylglycerol and Ca\(^{2+}\) together activate PKC-α and -β. Protein kinase C-β is activated, in neutrophils, by DAG alone (115). The PKC activates a range of other proteins by phosphorylation. In addition, phosphatidic acid can also cause activation of the respiratory burst (245), tyrosine phosphorylation (173), Ca\(^{2+}\) store release, and actin polymerization (215) and, because it is restricted to the lipid fraction of the cell, may be responsible for Ca\(^{2+}\) release from stores located close to the plasma membrane.

Cytosolic phospholipase A\(_2\) (PLA\(_2\)) is translocated to the plasma membrane after Ca\(^{2+}\) concentration increases, and it then cleaves lipids, including PIP\(_2\), leaving arachidonic acid in the lipid bilayer. Arachidonic acid is secreted as a chemoattractant, and its production is essential for macrophage spreading (242). Arachidonic acid is also a precursor in the formation of LTA, LTB, LTC, LTD, and LTE\(_4\), PAF, and prostaglandins, which are also primers, and chemoattractants (203). Other chemoattractants formed from lipid precursors include lipoxin A\(_4\), diepoxides of linoleic acid, and eicosanoids.

Activation of PKC, by either small G proteins or tyrosine kinases (Lyn in neutrophils; Ref. 193), causes the formation of PIP\(_3\). The lipid intermediate PIP\(_3\) has been implicated in the activation of the akt protein kinase (21, 120) and some isoforms of PKC (44).

Another group of lipid messengers, formed as a result of the action of a variety of stimuli (226), including those acting through 7TMR (161), is sphingosines and ceramide. Ceramide, sphingosine, and sphingosine-1-phosphate all serve as second messengers, causing PIP\(_2\) hydrolysis, Ca\(^{2+}\) store release, activation of mitogen-activated protein kinase (226), and cAMP-dependent kinases (26, 84). Sphingosine-1-phosphate production causes localized actin disassembly and inhibition of focal adhesion formation (26).

E. Other Intracellular Effectors

There are other proteins involved in the regulation of chemotaxis whose role is not clearly defined, because, as yet, little is known about their activation, mechanisms of action, or intracellular effects. One of these is the glycosyl-phosphatidylinositol-linked mono(ADP-ribo)syl transferase, which is required for the assembly of F-actin. In mouse muscle cells, mono(ADP-ribo)syl transferase ADP-ribosylates the extracellular domain of the integrin α\(_cβ_1\), regulating its adhesiveness for fibronectin and collagen (6). It is possible that a similar function may exist in leukocytes. Alternatively, mono(ADP-ribo)syl transferase may cause desensitization of G proteins, lowering the cytosolic Ca\(^{2+}\) concentration and promoting actin assembly by that route (7).

The cyclic nucleotides are important signaling molecules, and leukocytes have receptors for them, which are involved in phagocytosis. In addition, cAMP is an intracellular second messenger, which increases in concentration in leukocytes after stimulation by chemoattractants. However, the functional significance of this increase has not been elucidated.

Another signaling molecule released by chemoattractants is nitric oxide, a regulator of vascular function, with a range of effects on endothelial cells and leukocytes.

V. CALCIUM AND THE CYTOSKELETON IN CHEMOTAXIS

With the discussion of some of the Ca\(^{2+}\) signaling events in leukocytes, it is important to remember that not all of them occur at all times throughout the cell during chemotaxis. Whereas one process occurs at the leading edge, a very different one may be regulating activity 15–20 μm away, at the back of the cell. In addition, the timing of the different signaling pathways regulating chemotaxis must be under tight control mechanisms. Our knowledge of the precise temporal and spatial control over the mechanisms of chemotaxis is poor compared with our knowledge of the mechanisms themselves and the structural components of the cytoskeleton on which they act. Understanding how pathways are regulated in time and space...
within the cell is now the major challenge for science in this field (87, 133).

A. Prechemotaxis Events and Adhesive Interactions

As described previously, inflammatory conditions cause the release of chemokines and other factors which, by upregulating and activating adhesion molecules on both endothelial cells and leukocytes, promote adhesion, shape change, and extravasation (101, 227) before chemotaxis through the tissues. To understand the signaling processes occurring in the chemotaxing cell, it is necessary to consider those before chemotaxis.

Engagement of adhesion molecules such as integrins (39) activates PLC-γ2, causes localized release of Ca^{2+} from peripheral storage organelles, followed by periodic influxes of Ca^{2+} (152, 184), upregulation, or shifts in the affinity of other receptors, and tyrosine phosphorylation (77). These effects give rise to changes in the cytoskeleton (225), associated with alterations in cell shape (132, 221) and secretion (112, 223). These events may “potentiate” cellular polarization (1) and prime the response to chemoattractants (10, 170). Prevention of β_{2}-integrin ligation prevents pseudopod protrusion and IP_{3} production (112).

Each integrin has an α-subunit cytoplasmic tail domain that interacts with cytoskeletal components, forming a bridge to the actin cytoskeleton (34, 178). Talin, vinculin, paxillin, and α-actinin are the main constituents of these bridges, but there are others, and these proteins can be arranged in different configurations (110). α-Ac- tinin stabilizes the actin network by forming interactions between neighboring F-actin microfilaments and also completes the bridge between the extracellular matrix and F-actin. The assembly and disassembly of this bridge complex depends on the phosphorylation status of its components and may also be mediated by the activity of proteases associated with the cytosolic (calpain II; Refs. 93, 230) or extracellular (urokinase) regions of the membrane binding complex. Clustering of integrins therefore promotes changes in the actin cytoskeleton, both by association with other cytoskeletal components and by the initiation of Ca^{2+} signaling (136).

Initial interaction with the endothelial cell layer is followed by extravasation, which requires further cytoskeletal reorganization (208). Leukocytes then locomote across the basement membrane, which includes in its structure proteins that can modulate chemotaxis and phagocytosis (81, 248, 265), and enter the extracellular matrix.

Recent studies suggest that cell migration in a threedimensional mesh, such as extracellular matrix, is more complicated than migration up the chemotactic gradient across a two-dimensional substrate (126, 147). The type of extracellular matrix, the number of ligands it contains for specific adhesion receptors, and the affinity with which these interactions occur all affect the ability of cells to migrate (92, 175).

There is not sufficient space here to describe in detail the effect of adhesive interactions on chemotaxis, but throughout the process, they play an important role (34, 62, 92, 137, 147), and one which must be taken into account to fully understand chemotaxis.

B. Initiation of Chemotaxis

The result of the arrival of a chemoattractant is the activation of intracellular signaling pathways, leading to the first stages of cell shape change.

1. Calcium signaling

In some leukocytes, such as the giant newt eosinophil, the resulting cytosolic and lipid mediators of chemotaxis show gross differences in their intracellular distributions. Inositol 1,4,5-trisphosphate diffuses rapidly throughout the cytoplasm, whereas DAG stays at the location of 7TMR occupation, which is highest in the area of the cell closest to the stimulus (66). Inositol 1,4,5-trisphosphate causes Ca^{2+} release from the central storage organelle, and cytosolic free Ca^{2+} concentration increases to 400–500 nM (74). The distribution of IP_{3}-sensitive and -insensitive Ca^{2+} stores and the dissociation constants of the IP_{3} receptors on those store membranes at this point in the initiation of chemotaxis are unknown. The presence of Ca^{2+} and DAG at the part of the cell nearest the chemoattractant source locally activates PKC.

Other types of leukocytes, including human neutrophils, are able to initiate polarization and chemotaxis without a measurable change in cytosolic free Ca^{2+} concentration (8, 57). These observations may suggest that a Ca^{2+} signal is not essential for the generation of polarity. In the giant newt eosinophil, establishment of a polarized morphology may depend on elongation of the cell along an innate axis, determined by the arrangement of microtubules (182), rather than the chemotactic gradient.

Immediately after the initial Ca^{2+} release from the central store, in both the giant newt eosinophil (28, 29, 74) and Dictyostelium (271), the Ca^{2+} concentration falls most rapidly in the part of the cell nearest the chemoattractant source so that a back-to-front Ca^{2+} concentration gradient forms. This small gradient, from 250 nM in the uropod to ~100 nM in the lamellipod, could be responsible for the differing functions of Ca^{2+}-dependent processes in the newly polarized cell. In migrating fibroblasts, a similar gradient in the distribution of Ca^{2+}/CaM has also been reported (83). The Ca^{2+} gradient could be because...
of the relative distributions of IP$_3$ and DAG in the newly stimulated cell (66). Thus, giant newt eosinophils and Dictyostelium amoebae become polarized, not only in the distribution of major components of the cytoplasm such as nucleus and granules, but in the gradients of cytosolic free Ca$^{2+}$ concentration and DAG distribution (74).

A Ca$^{2+}$ gradient of this type has been difficult to demonstrate or is apparently absent in smaller cell types, such as human neutrophils (129, 151), and these cells are able to undergo actin polymerization (8) and effective chemotaxis (276) in the absence of, or incidental to (10, 54), Ca$^{2+}$ signaling, which seems to be necessary for eosinophil chemotaxis (57). Neutrophils do, however, undergo transient Ca$^{2+}$ oscillations (151) during migration, which may be Ca$^{2+}$ dependent, according to the substrate on which the cell is chemotaxing (148, 150) and what the stimulus is (53). Large (micromolar) global increases in the cytosolic free Ca$^{2+}$ concentration in neutrophils are usually associated with termination of chemotaxis (129).

Whether an increase in Ca$^{2+}$ concentration is required, is incidental, or is not necessary for reorganization of the cytoskeleton is therefore unclear.

2. Shape change

The signaling pathways activated by chemoattractants mediate their effects on the cell by modulation of the cytoskeleton, first to produce shape change and a polarized morphology, and then to exert force and generate forward motion.

A) ACTIN AND MYOSIN. The activation of PI3K, described in section IV, A and D, is required for leukocyte chemotaxis, such as that elicited by the stimulation of neutrophils by interleukin-8 (121), and for secretion (237). The PI3K subtypes $\alpha$ and $\beta$ are activated by the adhesion receptors (193) and $\gamma$ by G protein-coupled receptors, such as the chemoattractant receptors. Translocation of PI3K to the cytoskeleton (71) is associated with the establishment of membrane ruffling and reorganization of the actin cytoskeleton, possibly mediated by its effect on PKC isofoms (44), including PKC-$\delta$, which also associates with the intermediate filament network in HL-60 cells (174). There is insufficient space to discuss intermediate filaments here, but they are important in maintaining cell structure (70).

Precise mechanisms are not fully understood, but the Rho subfamily of small GTP binding proteins become activated in a PI3K-dependent manner. They are activated by guanine nucleotide exchange factors (GEF) and inactivated by GTPase activating proteins (85, 86). One Rho GEF has been localized to the polarized cell buds of Saccharomyces cerevisiae (149). Activation of each member of the Rho family has a different effect on the actin cytoskeleton (86, 240). In macrophages (and Swiss 3T3 cells; Ref. 275), Cdc42 activation stimulates filopodia formation, Rac activation forms lamellipodia by increasing the availability of actin barbed ends, Rho activation (which may indirectly involve PKC), stress fibers (5, 169), and focal adhesions (34). One myosin, in Drosophila, can interact directly with Rho, deactivating it (199). Some of the effects of Rho are mediated by the activation of phosphatidylinositol 5-kinase (PI5K) and MLCK, which undergoes an increase in activity due to the inhibitory effect of Rho kinase on myosin light-chain phosphatase (118). Activation of PI5K causes the formation of PIP$_2$, which binds gelsolin, and thus allows actin polymerization to occur (see sect. VE). Rac has similar effects (12). Phosphatidylinositol 4,5-bisphosphate also binds to vinculin, causing a conformational change that exposes binding sites for actin and talin (75), so that adhesive interactions with the cytoskeleton can form. Myosin light-chain kinase activates myosin II, which provides the force necessary for lamellipod extension and uropod retraction (see sect. VC).

In addition, Rho activates integrins in the $\beta_1$ and $\beta_2$ families (34).

Because MLCK itself is directly activated by Ca$^{2+}$/CaM binding, it can thus be regulated by two pathways: a Ca$^{2+}$-dependent one, via CaM, and a Ca$^{2+}$-independent one, via Rho. Inhibition of the Ca$^{2+}$/CaM activity of newt eosinophils causes them to cease chemotaxing; thus, in these cells, where an elevated cytosolic free Ca$^{2+}$ concentration is important during chemotaxis, the Rho pathway may not be so prominent (254). The relative contribution of these two regulatory pathways in different leukocytes may explain the differing role of Ca$^{2+}$ in the chemotactic response, on various types of substrate.

Increasing evidence from both Dictyostelium and other cell types (168) suggests that lamellipod formation and “whole cell” shape change are mediated by different signaling pathways. In Dictyostelium, myosins I (257) and II (214) are primarily required for gross changes in cell shape, and their absence or inhibition prevents organized polarization, but not pseudopod extension. In giant newt eosinophils and erythroleukemia cells (168), a similar situation is observed, whereby prevention of actin assembly and control does not prevent pseudopod extrusion but does prevent organized, persistent chemotaxis. These observations support the hypothesis that the formation of pseudopodia, and particularly the direction in which they are extended, is at least partially because of changes in the microtubule network (49, 182, 251). Thus, although the force required for cytoplasmic protrusion is provided by actin and its associated proteins, such as myosins I and II and $\beta$-actinin, the microtubules and microtubule organizing center (MTOC) also play a fundamental role.

B) CENTROSOMAL AREA. The centrosomal area directs cell migration. In some cell types, the lamellipod is formed according to where the centrosomal area is before the
arrival of the stimulus (184), and in others, the centrosomal area moves upon stimulation, before extension of the lamellipod (218).

The role of the centrosomal area may be related to the two structural features located here: the MTOC (11, 29, 168) and a Golgi-like Ca\(^{2+}\) store (23, 217), which has been described as the site for Ca\(^{2+}\) release in response to the chemoattractant FMLP (181, 185). Close association between microtubules and Golgi is suggested by the observation that disruption of microtubules (196), or dynein, a microtubule motor (89), causes reorganization of the Golgi. The MTOC nucleates microtubules (45), which play an important role in lamellipod extension, particularly in directing the lamellipod, and the Golgi traffic a supply of new membrane for the leading edge (218). Exactly how the MTOC moves is subject to debate. Changes in the distribution of Ca\(^{2+}\) during the initiation of chemotaxis would cause local changes in the cytoskeleton, which may exert a torsional effect on the MTOC, causing it to move into the position required for lamellipod extension. The importance of the MTOC has been reported in several cell types, including endothelial cells, where the location of the centrosomal area was similar to that described here (61), an erythroleukemia cell line, in which the location seemed unimportant (168), and Dictyostelium, where the centrosomal area stabilizes direction, rather than providing it (247).

Calcium release from this storage site at the initiation of chemotaxis would have a localized effect on the proteins in the centrosomal region. A number of regulatory proteins involved in chemotaxis are associated with the centrosome, including Ca\(^{2+}/CaM\)-dependent kinase II (188). Centrin is a major component of the centrosome, and although its exact functions are unknown, there are several indications that it may regulate the cytoskeleton. It can associate with both \(\gamma\)-tubulin and with 70-kDa heat-shock protein (HSP70), the actin-related protein-like (69) protein activated by microtubule-associated protein kinase-activated protein kinase-2 (MAPKAPK2) which regulates actin polymerization (278). In addition, centrin is structurally similar to EF hand Ca\(^{2+}\)-binding motif proteins and contains tyrosine phosphorylation sites, so its activity could be regulated by Ca\(^{2+}\) or protein kinases (177). \(\gamma\)-Tubulin is a specialized tubulin monomer that is found only at the centrosome. Its exact function is unknown, but its location would suggest that it nucleates microtubules (157, 187, 277).

Microtubules. The role of microtubules in the establishment of leukocyte pseudopodia remains unclear, but they increase in both number and stability after monocyte or macrophage treatment with LPS (3,4) and seem to be able to regulate the process from the MTOC, where the minus ends are anchored, not just at the tip of the advancing lamellipod (202). Using fluorescent, microinjected tubulin monomers to observe the relationship between microtubules and membrane ruffle formation shows that ruffles are formed at areas of membrane adjacent to the tips of microtubules, regardless of whether that point is where the main pseudopod will form (Fay, unpublished data; Ref. 49).

Microtubules are long polymers, consisting of \(\alpha\) and \(\beta\)-tubulin heterodimers (33) and microtubule-associated proteins (MAP; Refs. 143, 145). Microtubule-associated proteins regulate organelle transport along microtubules (24, 95, 210) and microtubule assembly dynamics (253) and are subject to regulation by MAP kinase (MAPK), which is also associated with microtubules and the centrosome (156). Microtubule-associated protein kinases regulate the microtubular cytoskeleton via the phosphorylation of MAP and thus have a central role in chemotaxis (79, 127), as well as other leukocyte functions requiring cytoskeletal alterations. Microtubules are either maintained in equilibrium (treadmilling) or undergo shortening (catastrophe) and lengthening (rescue) (238). Microtubule-associated protein kinases are activated by leukocyte chemoattractants including LPS (164), FMLP (202), and others (160). Of the three families of MAPK, the members of the ERK family probably play the greatest role in chemotaxis. Microtubule-associated protein kinases are heterodimers in the cytoplasm, which dissociate and become active upon 7TMR occupation, via a cascade of intracellular effectors (138), principally Raf (MAPKK) and MEK1/2 (MAPKK) (15, 21, 235).

Activators of Raf include the small G protein ras, which is activated by the binding of GTP after 7TMR occupation, and causes the recruitment of Raf to the plasma membrane, where it becomes activated and itself activates MEK1/2 by serine phosphorylation (40). Protein kinase C may also activate Raf (80).

Microtubule-associated protein modulation of microtubules may also be directly regulated by MAP binding proteins, such as mapmodulin (249), but these interactions have not been fully described in leukocytes. Different agonists acting on the same cell to produce a similar end response can do so through different MAP kinases (165), and indeed, it is impossible to describe here the full range of MAP kinase activity in leukocytes (139). Their roles and the roles of other kinases in chemotaxis are currently under investigation (90) and have been the subject of recent reviews (21, 235).

Other proteins associated with microtubules, although not usually classified with MAP, may anchor the plus ends of microtubules to the cell cortex, either acting directly on the tip of the microtubule or interacting with the length of the tubule, near its tip. Some of these molecules (the cytoplasmic linker protein, CLIP; Ref. 200) also link vesicles to the plus ends of microtubules. Although the importance of these proteins has not been demon-
strated in chemotaxis, they are necessary for dynein and kinesin function, vesicular transport, and microtubule orientation (123).

At least one of the MAPK is also involved in the regulation of the actin cytoskeleton. P38 MAPK (not a member of the ERK cascade) and its associated MAPKAPK2 dissociate upon neutrophil activation by FMLP, and MAPKAPK2 serine/threonine phosphorylates HSP70, which, by an unknown mechanism, modulates actin dynamics (124).

Thus chemoattractant stimuli give rise to shape change, actin and myosin providing the force, and microtubules, the direction, for lamellipod extension, which results in a polarized morphology.

C. Structural Polarity and Forward Motion

The newly polarized cell depends on the activity of the cytoskeleton to move in the direction of the chemoattractant source. The lamellipod must extend forward, forming new cell-substratum contacts (135, 272), whereas the uropod must detach from the substratum. Control of the cytoskeleton to accomplish this in an organized fashion depends on the correct subcellular localization of either structural components themselves, the molecules controlling those components, or both. The major force-producing proteins utilized in leukocyte motility are actin and the myosins.

The majority of recent research in this area has focused primarily on the amoeboid stage of D. discoideum, the rapid fundamental changes in myosin localization, and the actin cytoskeleton taking place in this cell during chemotaxis (107). Phosphorylation of myosin I occurs locally, by transient activation of guanylyl cyclase (211), and causes its association with actin at the leading edge of the cell, and pseudopod formation (128). Concomitantly, inhibition of phosphorylation of the myosin II heavy chains causes active myosin II localization to the back of the cell, where the uropod will form (158). The activity of myosin II here causes the actin cytoskeleton to contract, and the cytoplasm is pushed forward (271). Myosin II may also play a subsidiary role at the periphery of the cell in preventing the formation of pseudopodia perpendicular to the leading pseudopod (231). This pattern of myosin activity and localization, a useful “model” for chemotaxis, remains to be confirmed in leukocytes.

Myosins I (unconventional) and II (conventional) are both found in leukocytes (114, 234). In the migrating leukocyte, F-actin is localized to the leading lamellipod, where it undergoes polymerization. Myosin I is also thought to locate here, and, with α-actinin, may provide the force required for pseudopod expansion (114). Myosin I is a group of single-headed myosins, with a small tail and two to six light chains, which associate with the neck region, which links the head and tail domains. Myosin I tails do not associate with each other and probably function to link actin to membranes bounding vesicles and organelles, via electrostatic association with anionic phospholipids, or with “docking” proteins in the organelle, or plasma membrane. Myosins I and V, another unconventional myosin, may therefore be involved in transport of organelles within the cell. The rate of myosin I translocation along an actin filament in vitro is most rapid at cytosolic free Ca\(^2+\) concentrations below 100 nM, the resting Ca\(^2+\) concentration. Energy for this translocation is supplied by the Mg\(^2+\)-ATPase function of the myosin, which is activated in the presence of F-actin (244, 261). The requirement of a low Ca\(^2+\) concentration for myosin I function may explain its preferential activation at the front of the cell in those leukocytes that show a Ca\(^2+\) concentration gradient during chemotaxis.

In the uropod, the actin cytoskeleton contracts, allowing progress to be made across the substrate and forcing the cytoplasmic contents forward. Myosin II is thought to provide the force necessary for these functions. The tail regions of myosin II homotypically associate, forming bundles that cross-link adjacent actin filaments. Bound to actin, the myosin II molecule can rapidly translate along its length from the minus end to the plus end, utilizing energy generated by the hydrolysis of ATP to function as a “molecular motor.” Thus a contractile force is generated (114, 244). Myosin II is regulated by phosphorylation of its light chains, which are structurally similar to the EF-hand Ca\(^2+\) binding proteins but have a lower affinity for divalent cations. Inhibition of MLCK (72, 254) prevents chemotaxis and chemokinesis of both neutrophils and eosinophils and secretion by basophils (237), which requires retraction of the actin network from the secretory site. Myosin functions are also dependent on the availability of G-actin (274).

Other actin-associated proteins, which both stabilize F-actin and produce force, include the “cross-linkers,” such as α-actinin, calpain, fimbrin, and filamin. They are necessary for normal polarization and chemotaxis (68). The actions of this group of proteins can cause actin microfilaments to assume different configurations; filamin stabilizes actin in a “gel” form, where the individual filaments are randomly oriented with respect to each other, but α-actinin, calpain, and fimbrin “bundle” the actin filaments into larger groups.

Intermediate filament components, such as vimentin, in neutrophils undergo phosphorylation (103) chiefly in the uropod of polarized cells, because of the redistribution of G-kinase (192), and this mechanism may be important for chemotaxis. However, their roles in leukocytes and chemotaxis have not been fully investigated.

Alterations in the cytoskeleton may be translated to redistribution of membranous Ca\(^2+\) stores. The peripheral
Ca\textsuperscript{2+} storage organelles of some leukocytes are mobile, moving to the site of phagocytosis (229, 243). These movements are probably mediated by the actin cytoskeleton (9). Integrin-containing vesicles are transported by the microtubules (117). Changes in distribution of these organelles, and variations in the IP\textsubscript{3} receptor sensitivity on different types of Ca\textsuperscript{2+} stores, are probably involved in the sustained Ca\textsuperscript{2+} concentration gradients observed in eosinophils (66).

D. Maintenance of Polarity and Motion

Once shape change has been initiated by rapid signaling events, such as G protein activation, IP\textsubscript{3} production, Ca\textsuperscript{2+} signaling, and kinase cascades, long-term maintenance of intracellular signals must be established for chemotaxis to continue.

The role of Ca\textsuperscript{2+} influx and Ca\textsuperscript{2+} store release during sustained chemotaxis has been debated (66). Calcium influx and store refilling seems to be required for prolonged chemotaxis in eosinophils (57, 74). Neutrophils undergo repeated Ca\textsuperscript{2+} influx events as chemotaxis proceeds (52, 94, 152), and their intracellular Ca\textsuperscript{2+} concentration may correlate with speed of migration (146). Locally high cytosolic free Ca\textsuperscript{2+} concentration in the tip of the advancing lamellipod has also been reported (102).

Maintenance of polarity will also involve stabilization of the chemoattractant sensing mechanism, possibly by receptor redistribution, up- or downregulation, or changes in affinity. There is not yet a consensus of opinion concerning the position of chemoattractant receptors relative to the lamellipod and F-actin distribution (155) or the importance of their internalization in chemotaxis.

Adhesive contacts of the type described above can be found throughout the basal membrane of the chemotaxing cell (135). As the cell moves forward, they form at the leading lamellipod, and then remain stationary, and gather in the uropod so that chemotaxis cannot continue if they remain attached to both the matrix and the cell. Different cell types have different mechanisms for dealing with this and allowing the cell to continue to move. Fibroblasts, which migrate small distances, leave most of their contacts behind on the extracellular matrix (176), but leukocytes recycle their contacts and their constituents are endocytosed and returned to the leading pseudopod, a process requiring Ca\textsuperscript{2+}, ATP, and protein tyrosine kinase (134). An increase in Ca\textsuperscript{2+} concentration at the uropod, as well as allowing recycling, also causes contraction of the actin network, via myosin II, or filamin-desmin crosslink shortening (216), which may push cellular components forward. In the absence of Ca\textsuperscript{2+} influx, grossly elongated uropods may form, motility ceases (93), and the cell may eventually rip apart (176). It has been proposed that contact recycling is a process by which myosin V may supply the leading edge of some chemotaxing cell types with a constant supply of new membrane (154, 244), although microtubules, the Golgi apparatus, and the motors, kinesin (205) and dynein (96, 113, 200, 213), have been implicated in this function in myeloid cells (117). A smaller number of contacts dissociate in the membrane, scattering integrins across the cell, or contacts may release from the extracellular matrix and migrate in their entirety along the side of the cell to the leading lamellipod (176).

Thus, through extension of the lamellipod and formation of new adhesive interactions at the front of the cell, with controlled recycling of the receptors and retraction of the uropod at the back of the cell, chemotaxis continues.

E. Alteration of Polarity and Termination of Chemotaxis

Reorientation of the chemotactic gradient, which ultimately results in reorientation of cellular polarity, has several marked effects on the cell. The key event in some leukocyte types seems to be an increase in cytosolic free Ca\textsuperscript{2+} concentration throughout the cytoplasm (29), but this has not been observed in human neutrophils (146). The effects of an increase in Ca\textsuperscript{2+} concentration would be to "scramble" the cytoskeleton, via the activity of proteins such as gelsolin, to prepare for reconstruction in the new polarity.

Gelsolin is the major actin-binding protein in leukocytes. In unstimulated cells, it (and thymosin \(\beta_4\)) sequesters free actin monomers. Gelsolin is usually membrane bound (to PIP\textsubscript{2}, where it competes with PLC; Ref. 236) but is released into the cytoplasm, where it binds actin filaments, upon increase of Ca\textsuperscript{2+} to micromolar concentrations (255). Calcium at these concentrations causes gelsolin to sever actin filaments, cap the barbed growing ends, and to nucleate actin assembly (100). These functions may allow the cell to reorientate and increase its locomotion speed (37). Gelsolin has two actin binding sites, which straddle across two actin monomers in the F-actin. One of the binding sites is Ca\textsuperscript{2+} dependent, and the other is IP\textsubscript{3} dependent; in the presence of both Ca\textsuperscript{2+} and IP\textsubscript{3}, gelsolin and actin dissociate, and the two actin monomers bound by that gelsolin molecule also dissociate (14). In addition to regulation by cytosolic free Ca\textsuperscript{2+}, PIP\textsubscript{2} inhibits the activity of gelsolin, and thus the reorientation of the stimulus regulates its activity by both an increase in Ca\textsuperscript{2+} and a decrease in PIP\textsubscript{2}. Gelsolin has a role in the regulation of PLD, increasing the formation of phosphatidic acid (228) and PB3K, increasing the phosphorylation of PIP\textsubscript{2} (219). CapZ (153), another actin-binding protein,
is regulated by PIP2 alone and functions only as a capping protein. Arrival of the stimulus and concomitant decrease in PIP2 causes actin and capZ to dissociate. Myosin I- and V-mediated functions cease because of the dissociation of the Ca2+/CaM light chains and loss of the actin-myosin interaction. Calcium also causes microtubule disassembly, via Ca2+/CaM-dependent kinase II activation, which results in the phosphorylation of MAP, including MAP2, tau (22, 220, 253), and oncoprotein 18 (78), which dissociate from microtubules, and cause them to shorten (188, 253). Thus there is a “resetting” effect on the cytoskeleton.

Myosin II has an additional function during chemotactic turns induced in Dictyostelium. In this case, active myosin II locates not only at the back of the cell, continuing to push the contents of the cytoplasm forward, but also forms a plug in the old leading pseudopod, preventing any further extension in that direction (270).

At some point, chemotaxis has to resolve into a decision that either the point has been reached when secretion and “attack” has to begin, or the original need for the cell has passed, chemotactic turn is no longer being released, and chemokinesis takes over. The latter scenario can be envisaged when the concentration of chemoattractant has fallen to below the level required for chemotaxis, or a process of desensitization has taken place (23, 99). The leukocyte can then chemokinese until called upon to chemotax again.

As the cell nears the source of the chemoattractant, its concentration increases. This may cause Ca2+ influx, which prevents further motion, as described above, or the cell may require other stimuli for chemotaxis to terminate, such as changes in the basal membrane distribution of adhesive contacts (246). The function of the cytoskeleton now modulates from that of migration to the regulation of phagocytosis and secretion. Although monocytes are the only leukocytes that undergo differentiation in the tissues, neutrophils, eosinophils, and other leukocytes undergo marked changes in morphology and in their Ca2+ signaling capacity (42, 111, 186), which prime them for more efficient adhesion to opsonized particles, rapid specific secretion, and phagocytosis.

Thus, through an orchestrated series of controlled intracellular changes, the resting leukocyte in the bloodstream has become a potent anti-infectious fighter located at the point where it is required for bacterial or parasitic killing.

VI. CONCLUSION

As we have seen, chemotaxis is a fascinating and complex process, with relevance to many fields of biology, biochemistry, and medicine. However, many fundamental issues remain to be tackled in the quest for fuller understanding of chemotaxis.

A. Future Directions

Future research in chemotaxis will tackle some important, unresolved questions (87). The role of Ca2+ will be one of the central ones and, as we have discussed, will have to take the full range of cellular stimuli into account, including the changing types of adhesive interactions that the leukocyte experiences during the process of chemotaxis. The mechanisms for achievement of polarity, and the interplay between the centrosome and microtubules, actin, and the direction of the chemotactic gradient remain to be ascertained.

“Mapping” how the distribution of 7TMR, IP3-sensitive and -insensitive Ca2+ stores, and the activity of cellular effectors such as CaM and MLCK change in the leukocyte upon arrival of the chemoattractant will be necessary. Microinjected fluorescent analogs and ligands, as well as inhibitory “caged” peptides, Ca2+ and IP3 will all prove useful.

Green fluorescent protein techniques are proving invaluable in the study of molecular movements such as these in a wide range of different cell types (258) but are difficult in leukocytes because they do not survive for long enough to culture and undergo very little de novo protein synthesis. However, new models of transformed myeloid cells, such as U-937, which can be induced to undergo chemotaxis (116), are candidates for green fluorescent protein transfection and will add to our knowledge in the future.

B. Summary

Chemotaxis is a fascinating process that raises many interesting questions for the scientist. It occurs to a greater or lesser extent in almost every cell type at some time during its development and in mature organisms is a major component of the inflammatory, allergic, and wound-healing responses. Chemotaxis relies on sensing the extracellular chemotactic concentration gradient and coupling of chemoattractant receptor occupation to intracellular establishment and maintenance of polarity, mediated by changes in the cytoskeleton. Many of the molecular processes involved have been described.

The signaling of chemotaxis can hardly be described as a pathway, a more accurate term would be a “signaling network,” especially when the adhesive interactions of the cell are taken into account, which they must be, for full understanding of the process. When unanswered questions are finally tackled, it will be possible to observe the leukocyte migrating in response to chemoattractant in a physiological situation and to understand the observations. This will represent a huge step forward in the understanding of one of the basic properties of life, purposeful
movement, and enable a logical approach to the many devastating human diseases that result when this process fails.

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