Conclusion

A number of different studies have been made of the Hubble flow in nearby regions of space. These investigations yield conflicting results, which indicate that the velocity with which the Local Group is falling into the Virgo cluster may lie anywhere in the range 0 to 500 km sec^{-1}.

Reconciling these discrepant results on the local anisotropy of the Hubble flow is one of the most urgent problems facing contemporary extragalactic astronomy. Until the local flow pattern is better understood, determinations of the Hubble parameter H will have to be derived from exceedingly difficult studies of distant galaxies with redshifts $\gg 1000$ km sec^{-1}. Presently available data indicated that $50 < H < 100$ km sec^{-1} Mpc^{-1}. The corresponding expansion time scale for the universe lies in the range 10 to 20 Gyr. This range brackets ages derived from nuclear chronology and age estimates for the oldest known stars. More accurate determinations of the global value of H and local deviations from it promise to provide valuable new information on the density of the universe, the deceleration of its expansion, and possibly on the size of the cosmological constant.

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Molecular and Cellular Mechanisms of Leukocyte Chemotaxis

Ralph Snyderman and Edward J. Goetzl

Nearly 100 years ago, Eli Metchnikoff noted that coelomic cells accumulated rapidly around a rose thorn that he had placed through the skin of a transparent starfish larva (*1*). Metchnikoff's hypothesis that the cells which had responded to the foreign body functioned to defend the host provided the cornerstone for our current understanding of the role of phagocytic leukocytes in cellular immune responses and inflammatory reactions. The contention that chemical mediators evoked the directed migration of leukocytes led Pfeffer in 1884 to utilize the term chemotaxis and stimulated Leber several years later to speculate that phagocytic leukocytes could sense and follow concentration gradients of specific stimuli (*2*). In prokaryotic cells chemotaxis is a means of finding nutrients, whereas in more complex animals, such as man, it is a process by which cells of the immune system become localized at sites of inflammation. The biochemical mechanisms of bacterial chemotaxis are relatively well defined (*3*). The application of the techniques of modern chemistry and cellular biology to the study of leukocyte function has recently permitted rapid advances in this field as well. Several classes of chemotactic factors have been defined, and cytostructural as well as a number of biochemical prerequisites of leukocyte chemotactic responses have been elucidated.

The ability to quantify the migration of leukocytes in vitro by reproducible techniques has fostered significant advances in the understanding of chemotaxis. In 1962, Boyden (*4*) observed that polymorphonuclear leukocytes (PMN's) placed

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in the upper compartment of a plastic chamber migrated through a microporous filter if a proper stimulant was present in the lower compartment. In subsequent years a number of important improvements were made in this technique and accurate methods were developed for differentiating chemotaxis from random migration or stimulated random migration (chemokinesis). Chemotaxis is directed cellular migration along a concentration gradient of a chemoattractant. Random migration is that cellular motility which occurs in the absence of any known chemical stimulant. Chemokinesis is chemically stimulated, nondirectional cellular motility occurring in the absence of a chemotactic gradient. Most chemotactic factors also stimulate chemokinesis. Methodology to quantify chemotaxis has been reviewed elsewhere (*5*).

Two models that can account for a cell's ability to perceive and to migrate along a chemical gradient have been proposed (*3*, *6*). One involves temporal sensing, the other spatial sensing. Temporal sensing implies that the responding cell perceives a concentration of a chemoattractant at one time, moves in a straight line for a fixed period of time, stops, and perceives the new concentration before moving again. Bacterial chemotaxis is characterized by intervals of linear swimming interposed by periods of tumbling in place. Control of the frequency of tumbling time and swimming time allows bacteria to migrate along chemotactic gradients. When bacteria migrate into a chemotactic gradient, tumbling is suppressed and is replaced by swimming in a relatively straight line. The temporal mechanism best describes the motility of bacteria and theoretically requires only one chemoreceptor and memory.

The chemotactic response of leukocytes differs strikingly from that of bacteria. Leukocytes do not swim but crawl along surfaces. Moreover, when leukocytes are exposed to a chemotactic gradient they alter their shape, become morphologically oriented toward the gradient, and migrate in a curvilinear fashion without discrete pauses (*7*). Corrections in direction finding occur continuously, implying that leukocytes are directly able to detect a gradient across their length. This behavior best fits a spatial response and theoretically requires multiple cellular receptors integrated in such a way that the leukocytes can recognize differential occupancy of receptors for chemotactic factors along their surface.

Summary. The application of modern scientific methods to the study of leukocyte function has begun to reveal the molecular and cytostructural bases of the chemotactic responses of these cells. Leukocyte chemotaxis is initiated by the binding of chemoattractants to distinct plasma membrane receptors; this binding alters transmembrane potential and activates ionic fluxes. The subsequent sequence of metabolic processes leads to a rearrangement of cytoskeletal elements that is manifested by orientation and migration of the cells toward the source of the chemotactic gradient.

Involvement of Cytoskeletal Elements

The initial morphological response of PMN's and mononuclear leukocytes exposed to chemotactic factors is a polarized elongation of the cell with the development of a broad lamellipodium anteriorly and a thin uropod with terminal arborization posteriorly (Fig. 1) (*8*). The morphological polarization of chemotactically stimulated leukocytes is associated with specific topographical reorganization of plasma membrane determinants under the control of cytoskeletal structures. Electron microscopic studies of such polarized leukocytes have revealed a complex arrangement of actin microfilaments just beneath the plasma membrane; these are present in increased numbers in the lamellipodium and uropod, whereas intermediate filaments approximately 10 nanometers in diameter are enriched selectively in the uropod (*9*). The nucleus is located behind the center of the leukocyte and thus is separated from the leading edge of the lamellipodium by the bulk of the granule-containing cytoplasm. Centrioles within nuclear folds serve as the origin of microtubules that extend toward, but not into, the filament-enriched poles. Whereas coated pits and vesicles are distributed diffusely in unstimulated leukocytes, they are found exclusively in the uropods of polarized leukocytes responding to a chemotactic stimulus (Fig. 2). Ruffles and pits in the plasma membranes of the stimulated leukocytes contribute to an increased cell surface to volume ratio (*10*). The degranulation observed at the leading edge of a lamellipodium, a phenomenon involving specific

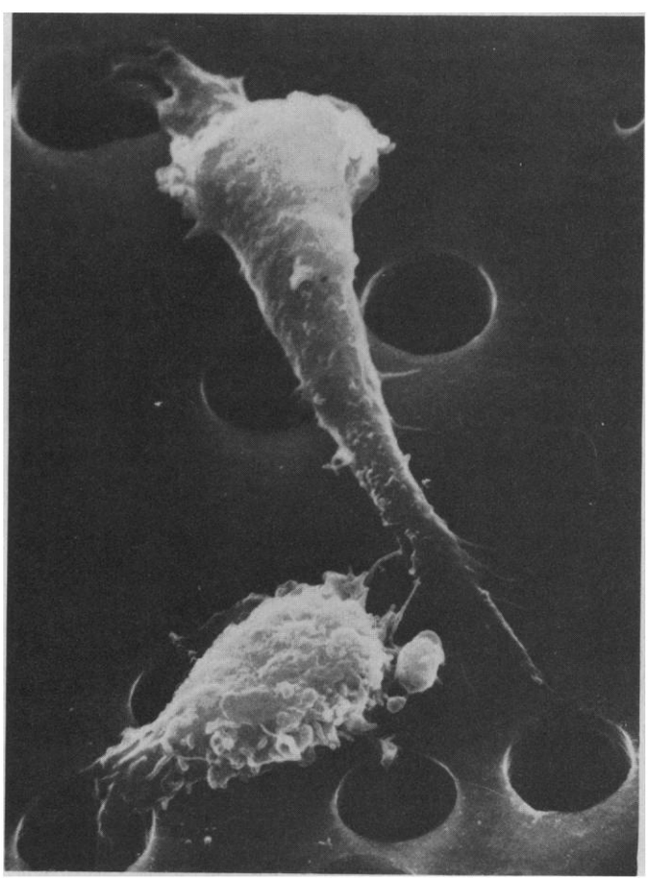

Fig. 1. Scanning electron micrograph of two human blood monocytes migrating through 5.0-micrometer pores of a polycarbonate filter in response to a chemotactic lymphokine (×4000). The cell at the top has completely emerged through a pore and has advanced diagonally across the filter's surface. On the lower left, another cell has begun to emerge through the filter.

granules, is associated with the movement of granule membranes to the surface of the leukocyte followed by fusion of granule and plasma membranes (*11*). Some plasma membrane receptors that are present in a uniform distribution on unstimulated leukocytes are localized asymmetrically on cells polarized by a concentration gradient of a chemotactic factor. Receptors for the Fc portion of immunoglobulin G (IgG), assessed by the attachment of IgG-opsonized erythrocytes, and concanavalin A receptors, assessed by the attachment of fluorescein-conjugated concanavalin A, are initially restricted to the lamellipodium of oriented PMN's but move to the uropod after incubation of the cells at 37°C (*12*).

The exposure of suspensions of human PMN's and monocytes to chemoattractants in the absence of a sustained gradient causes them to rapidly assume the polarized morphology characteristic of migratory cells (*13, 14*). Polarization in suspension occurs within a minute of exposure of the cells to chemoattractants at 37°C and is inhibitable by cytochalasin B, an agent that blocks actin filament rearrangement. The dependence of chemotactic orientation on cytoskeletal structures has been analyzed by using filters with pores too narrow to allow complete entry of PMN's into the filters, a phenomenon termed "frustrated chemotaxis" (*9*). Concentrations of cytochalasin B that abolish the microfilament bundles in the advancing pseudopods of PMN's result in pseudopod retraction without loss of orientation or prevention of reorientation if the chemotactic gradient is reversed. In contrast, levels of colchicine that inhibit the assembly of microtubules lead to a random distribution of nuclei and centrioles, but do not alter the formation of pseudopods. Defects in the chemotaxis of leukocytes with cytoskeletal abnormalities have been associated with specific disease states, such as Chédiak-Higashi syndrome (*15*) and actin dysfunction syndrome (*16*). Such defects support the concept that microfilaments are critical for directed locomotion and the attendant changes in cellular shape, whereas microtubule rearrangement is required to initiate and stabilize cellular orientation toward a chemotactic gradient.

Chemoattractants for Leukocytes

Since leukocytes are critical for host defense and also have the capacity to damage host tissues in some immunological reactions, the initial investigations of chemotactic factors were directed to principles generated by immune effector pathways. Boyden showed that the addition of antigen-antibody complexes to fresh serum generated PMN chemotactic activity (*4*). Since heating serum at 56°C for 30 minutes prevented the generation of chemotactic activity by the immune complexes, he surmised correctly that the complement system might be involved. The major chemoattractant produced upon the activation of serum complement was found to be a cleavage product of the fifth component of complement termed C5a (*17*). This molecule is a polypeptide containing 74 amino acids with the carboxy-terminal amino acid being arginine (*18*). In human serum the terminal arginine is rapidly cleaved (des) by a carboxypeptidase-B–like enzyme, thereby producing C5a-des-Arg which has roughly 10 percent of the chemotactic activity of native C5a. Evidence has been presented that, to be chemotactic, C5a-des-Arg requires an anionic protein "helper factor" that is present in normal serum (*19*). C5a is chemotactic for neutrophils, monocytes, macrophages, eosinophils, and basophils.

Other chemotactic factors generated by immunological reactions include diverse lymphokines. These protein molecules are produced by lymphocytes that have been stimulated by antigens or mitogens. One lymphokine, with a molecular weight of approximately 12,500, is a potent chemoattractant for monocytes as well as for macrophages (*20*). Chemotactic factors for PMN's and fibroblasts are also produced by stimulated lymphocytes (*21*). Chemoattractants are released by PMN's and macrophages ingesting antigen or other particulate materials. A factor produced when PMN's engulf monosodium urate crystals has a molecular weight of about 8400 (*22*). This substance, termed crystal-induced chemotactic factor (CCF), may be responsible for the acute inflammatory response seen in individuals with gouty arthritis.

Mast cells and basophils also produce chemoattractants that are released upon interaction of these cells with specific antigens or with the complement cleavage products C3a or C5a. Two tetrapeptides, valylglycylserylglutamine (Val-Gly-Ser-Glu) and alanylglycylserylglutamine (Ala-Gly-Ser-Glu), which are principal constituents of the eosinophil chemotactic factor of anaphylaxis (ECF-A), are chemotactic for eosinophils and to a lesser degree for neutrophils (*23*). Larger peptides of 1000 to 3000 daltons which are chemotactic for eosinophils, as well as a high molecular weight substance (>150,000) termed neutrophil chemotactic factor of anaphylaxis (NCF-A) are released by immunologically challenged mast cells in vitro and appear in the venous effluent from lesions of patients with various types of physical urticaria (*24*). These compounds are mediators of inflammatory cell accumulation in allergic reactions.

An additional class of chemoattractants consists of the major initial metabolites of the lipoxygenation of arachidonic acid. In most types of leukocytes this is 5-hydroperoxyeicosatetraenoic acid, which is converted both to 5-hydroxyeicosatetraenoic acid (5-HETE) and to a family of complex HETE's, termed leukotrienes, that contain three conjugated double bonds, a fourth double bond, and additional polar substituents (*25, 26*). The 5-HETE's with 6-sulfido-glutathione, 6-sulfido-cysteinyl-glycine, and 6-sulfido-cysteine substituents (leukotrienes C, D, and E, respectively) are the functionally critical components of slow reacting substance of anaphylaxis (SRS-A), while a specific 5,12-di-HETE termed leukotriene B is the most potent chemotactic factor generated from arachidonic acid (*26, 27*). Many of the products of arachidonic acid oxygenation modulate leukocyte adherence, migration, and lysosomal degranulation, but only the HETE's stimulate leukocyte chemotaxis. A rank order of chemotactic potency is manifested, such that leukotriene B > > 5-HETE > > 8-HETE = 9-HETE > 11-HETE = 12-HETE > > 15-HETE, whereas neither leukotriene C nor tri-HETE's from platelets are chemotactic. The chemotactic potency of leukotriene B in vitro and in vivo is similar to that of C5a. Furthermore, acetyl-leukotriene B and 5-HETE-methyl ester, which exhibit less chemotactic activity than the parent lipids, inhibit the PMN responses to leukotriene B and 5-HETE, respectively, but not to C5a or to the synthetic *N*-formyl-methionyl chemotactic peptides (*28*).

Evidence for Chemoattractant Receptors on Leukocytes

Supernatants from cultures of rapidly growing bacteria contain potent chemoattractants for leukocytes (*29*). Schiffmann and co-workers (*30*) observed that much of the activity present in culture supernatants of *Escherichia coli* had blocked NH_2-terminal amino acids. Since bacteria but not eukaryotic cells initiate protein synthesis with an NH_2-terminal-formylated methionyl residue, it was reasoned that such peptides might be recognized as chemoattractants by

leukocytes (*30*). This supposition led to the discovery that certain synthetic peptides containing *N*-formylated methionine were potent chemoattractants for PMN's, monocytes, and macrophages. Thus, researchers in the field of chemotaxis had available purified, structurally defined chemotactic peptides.

Studies of the structure and function of these agents demonstrated a strict relation between the amino acid sequence of the *N*-formylated peptides and their biological activity (*31*). Formylation of the NH_2-terminus was necessary for biological activity. The most potent NH_2-terminal amino acid was methionine, but an aliphatic amino acid of similar hydrophobicity such as norleucine (Nle) was active albeit to a lesser degree. The second position required a neutral amino acid such as leucine for optimal activity. In tripeptides, a phenylalanine in the third position produced maximum activity. The most potent tripeptide thus far synthesized is *N*-fMet-Leu-Phe.

On the basis of these structure-function relations it was speculated that rabbit PMN's had receptors for *N*-formylated methionyl peptides (*31*). The synthesis of radioactively labeled *N*-formylated methionyl peptides and *N*-formylated norleucyl peptides of high specific activity, that had the same biological potency as the unlabeled peptides, allowed studies of the binding of the peptides to plasma membrane receptors on rabbit and human PMN's, human monocytes, and guinea pig macrophages (*32–34*). With the use of fMet-Leu-[^{3}H]Phe, it was shown by direct binding techniques that human PMN's have specific, high-affinity receptors for the *N*-formylated oligopeptides (*33*). The equilibrium dissociation constant (K_D) for the ligand is approximately 20 n*M*, with binding at 37°C being rapid ($t_{1/2}$ = 3 minutes) and readily reversible. Human PMN's have approximately 50,000 receptors per cell (*35*). The ability of a series of unlabeled *N*-formylated peptides to compete for binding with the radiolabeled ligand exactly parallels the ability of these peptides to initiate chemotaxis. Neither the chemotactic peptide C5a nor leukotriene B binds to the receptor for *N*-formylated peptides.

The inhibition of chemotaxis to *N*-formylated methionyl peptides by covalent chemical modification of human neutrophil receptors and the results of microscopic analyses with fluorescent derivatives of *N*-formylated norleucyl peptide (*36*) have confirmed the plasma membrane localization of the receptors. The characteristics of the specific chemotactic factor receptors have been elucidated further by affinity labeling techniques. The labeled peptide fNle-Leu-Phe-Nle-[^{125}I]Tyr-Lys was cross-linked to the receptor with dimethyl suberimidate, fNle-Leu-Phe-Nle-[^{125}I]Tyr-Lys-Nϵ-bromoacetyl reacted spontaneously, and fNle-Leu-Phe-Nle-[^{125}I]Tyr-Lys-Nϵ-azido-2-nitrophenyl was cross-linked to the receptor by photoactivation. Labeling of the receptors on human neutrophils with each of the reactive derivatives led to the identification of a membrane protein of molecular weight 55,000 to 70,000, the selective labeling of which was suppressed by the native peptide at concentrations that competitively protected the receptor (*37*). The use of affinity chromatography with formylated methionyl peptide coupled to Sepharose has permitted the isolation of soluble membrane protein constituents of specific human neutrophil receptors, which retain selective binding activity for the formylated methionyl peptides (*38*). Plasma membrane constituents of disrupted neutrophils that were bound by the fMet-Leu-Phe-Sepharose column in a buffer containing non-ionic detergent were eluted specifically with the chemotactic peptide fMet-Leu-Phe. Resolution of the constituents of the eluate by gel filtration in sodium dodecyl sulfate (SDS) buffer and by SDS-polyacrylamide gel electrophoresis revealed three membrane proteins of approximate molecular weights 94,000, 68,000, and 40,000, of which the 68,000 m.w. constituent accounted for 74 to 93 percent of the total protein. The 68,000 and 40,000 m.w. proteins, but not the 94,000 m.w. protein, bound fMet-Leu-[^{3}H]Phe in equilibrium dialysis chambers with a mean valence of 0.68 and 0.63. Each receptor protein exhibited either a high-affinity or a low-affinity site, and the association constant (K_A) for the high-affinity site of the 68,000 m.w. protein was equal to that of receptors on intact neutrophils. The specificity of the interactions of the purified membrane proteins with fMet-Leu-Phe was shown by their failure to bind radioactively labeled lipid chemotactic factors.

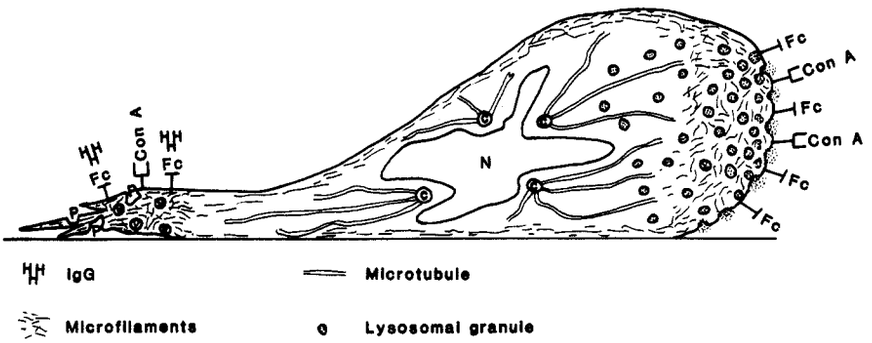

Fig. 2. Polarization and cytoskeletal organization of a leukocyte engaged in chemotaxis. *N*, nucleus; *c*, centriole; *P*, coated pit; *v*, coated vesicle; *Fc*, receptor for Fc portion of IgG; *Con A*, receptor for concanavalin A. Although the depiction of the granules at the leading edge of the leukocyte is intended to emphasize the fusion of granules with the plasma membrane, the lamellipodium characteristically has a submembrane zone of clear cytoplasm.

Human C5a can be radiolabeled and thus is useful for the study of chemotactic factor receptors (*39*). Human PMN's have specific receptors for C5a with binding of [^{125}I]C5a being rapid and saturable. Specificity of the receptor for C5a is indicated by the finding that C5a lacking the carboxy terminal Arg (C5a-des-Arg) has approximately one tenth of the biological potency of C5a, and binds to the receptor with an affinity corresponding to its biological activity. C3a, another biologically active peptide derived from complement, but devoid of chemotactic activity, does not bind to the C5a receptor. The median effective dose (ED_{50}) for the binding of [^{125}I]C5a to the human PMN receptor is approximately 3.3 n*M*, and the number of binding sites is about 190,000 per cell. The *N*-formylated peptides do not compete with [^{125}I]C5a for the C5a receptor.

The crystal-derived chemotactic factor (CCF) has been radioiodinated and used to demonstrate the existence of yet another chemotactic factor receptor on human PMN's (*40*). The binding of ^{125}I-labeled CCF is rapid, saturable, and specific in that binding of this radioactive ligand is not inhibited by fMet-Leu-Phe, or by C5a.

Regulation of Chemotactic Factor Receptors on Leukocytes

Polymorphonuclear leukocytes are able to detect and to migrate directionally in response to extremely small chemotactic gradients. It is estimated that these cells can differentiate gradients of as

little as 0.1 percent across their surface (*7*). In view of this sensitivity, the discriminating mechanisms for chemotaxis must be subject to fine regulation. One means of modulating the sensitivity of cells to chemoattractants would be to alter the number, affinity, or distribution of chemotactic factor receptors along the surface of leukocytes during the chemotactic response. That the number of chemotactic factor receptors on cells exposed to chemoattractants changes is indicated by the finding that the expression of *N*-formylated peptide receptors on human PMN's can be altered by agents that induce degranulation (*41*). The exposure of human PMN's to agents that induce limited secretion of lysosomal enzymes enhances the number of chemotactic factor receptors on the cells apparently, because of the fusion of receptor-bearing granule membranes with the plasma membrane of the PMN during exocytosis. Up to a third of the formylmethionyl peptide receptors in PMN's may be present on granule membranes.

Further support for the contention that chemotactic factor receptors on responding leukocytes may be subject to "up-regulation" has been provided. Specific binding of *N*-formylated chemotactic peptides to receptors on rabbit and human PMN's is enhanced up to threefold by the aliphatic alcohols *n*-propanol and *n*-butanol (*42, 43*). The effect of 2.5 percent butanol on the affinity of the human fMet-Leu-Phe receptor is more dramatic since in the presence of this agent the K_D is decreased almost tenfold from approximately 22 n*M* to 2.5 n*M*. *Cis*-vaccenic acid which, like the aliphatic alcohols, is a hydrophobic modulator of membranes, also enhances the number and the affinity of chemotactic factor receptors on human PMN's (*43*). Thus, chemotactic factor receptors on PMN's can exist in more than one affinity state, and the affinity of the receptor can be altered by agents that alter membrane properties. Since binding of chemoattractants to their receptors may produce changes in the compositions of membrane phospholipids in the proximity of the receptor (*44, 45*), the affinity of occupied and perhaps of neighboring receptors might be altered. In support of this contention is the finding that incubation of guinea pig macrophages with inhibitors of transmethylation reactions, including methylation of membrane phospholipids, blocks chemotaxis and lowers the affinity of the formylated methionyl peptide chemotactic factor receptor on these cells (*46*).

Exposure of chemotactically responsive cells to large doses of chemoattractants renders them unresponsive to chemoattraction by the same agents. This phenomenon has been termed "deactivation" or "desensitization" and suggests an involvement of down-regulation of the receptors for chemotactic factors. Incubation of rabbit PMN's with unlabeled fNle-Leu-Phe at 37°C causes a decrease in the number of receptors available for binding at 4°C (*47*). The disappearance of these receptors from rabbit PMN's occurs in minutes and is dose- and temperature-dependent. Recovery from down-regulation occurs rapidly at 37°C, being nearly complete by 20 minutes. Along with down-regulation of receptors, receptor-mediated pinocytosis of the ligand occurs. Similar down-regulation and chemotactic factor uptake by cells is seen in human PMN's and monocytes exposed to fNle-Leu-Phe-Nle-Lys-Tyr (*34, 36*). By using a fluorescent (rhodamine conjugated) derivative of the *N*-formylated sextapeptide, specific binding, aggregation, and rapid internalization of the peptide was observed directly. Up- and down-regulation of chemotactic factor receptors on leukocytes may provide a means for controlling cellular sensitivity for chemotactic responses.

Cellular Models for Studying Chemotactic Factor Receptors

Eukaryotic cells with specific defects of chemotactic responses have been described recently and should allow better definition of the biochemical events that occur after the binding of chemoattractants to their receptors. Equine PMN's contain on their surface specific high-affinity receptors for the *N*-formylated peptides (*48*). The number of receptors on equine PMN's, however, is only 630 ± 184, a value substantially less than that found on human PMN's, rabbit PMN's, or guinea pig macrophages. Despite having specific receptors for the *N*-formylated peptides, equine PMN's do not orient nor do they respond chemotactically to the *N*-formylated peptides, but they do orient normally and respond chemotactically to equine C5a. Although the *N*-formylated peptides do not initiate chemotaxis in equine PMN's they do induce the release of lysosomal enzymes and stimulate the production of superoxide anions. Why the occupancy of the receptors on equine PMN's is ineffective in initiating chemotaxis despite stimulating secretion and superoxide production is unknown. One possibility is that the coupling processes that link the receptor to effector mechanisms for different biological activities are discrete and the transducer for chemotaxis is nonfunctional. Alternatively, there may be insufficient numbers of *N*-formylated peptide receptors to initiate chemotaxis since a high density of receptors may be required for orientation and directed migration.

Two continuous lines of human leukocytes are available in chemotactically responsive and unresponsive states. A human monocyte-like cell line, U937, can be stimulated to differentiate into macrophages in vitro in the presence of supernatants from lectin-stimulated lymphocytes (*49*). Unactivated U937 cells do not respond chemotactically to the *N*-formylated peptides, to C5a, or to lymphocyte-derived chemotactic factor. After exposure of the U937 cells to lymphocyte supernatants, however, they develop chemotactic responsiveness to all three types of chemoattractants. In the nonchemotactically responsive state, the U937 cells contain no detectable receptors for fMet-Leu-[^{3}H]Phe. After incubation with lymphokines, however, there is a parallel increase in the ability of the cells to migrate chemotactically and to specifically bind fMet-Leu-[^{3}H]Phe (*50*).

Another human myeloid precursor, the HL-60 cell line, develops into a granulocytic cell upon treatment with dimethyl sulfoxide. A small proportion (about 2 percent) of unstimulated cells possess receptors for the *N*-formylated peptides, but when the cells are stimulated with dimethyl sulfoxide approximately 30 percent of the cells develop chemotactic factor receptors (*51*). The chemotactic responsiveness of the stimulated and unstimulated HL-60 cells parallels the expression of the chemotactic factor receptor. Continuous cell lines that can be induced to differentiate and express receptors for chemotactic factors will be useful not only for characterizing these receptors, but also for delineating the regulatory mechanisms in cellular differentiation and the biological responses mediated by chemotactic factor receptors.

Biochemical Coupling of Chemotactic Stimulation to Migration

A wide range of plasma membrane and intracellular events are initiated in leukocytes by chemotactic factors. The results of analyses of the time course of such events and of their dependence on the concentration of the chemotactic factor relative to similar parameters of leukocyte chemotaxis have suggested some integral relationships. However, in most

instances, the lack of selective inhibitors has prevented the unequivocal establishment of the sequence of biochemical prerequisites of chemotactic activation.

Plasma membrane potential and ionic conductances. Direct measurements of electrical potentials of leukocyte plasma membranes have been limited to monocytes and macrophages, because of technical difficulties with studies of the smaller PMN's (*52*). Mononuclear leukocytes exhibit resting potentials of approximately −15 millivolts with occasional spontaneous hyperpolarizations. The introduction of a chemotactic concentration of C5a or of an *N*-formylated methionyl peptide produces a brief depolarization followed by a hyperpolarization that achieves a maximum level of approximately −50 mV at 2 to 5 minutes. The phase of hyperpolarization appears to reflect a calcium-mediated potassium conduction. Exposure of PMN's to a chemotactic factor leads to changes in surface charge, as assessed by altered movement in an electrical field, and evokes hyperpolarization of the plasma membrane, as quantified by modified rates of uptake of lipophilic cations or ionic dyes. The leukocyte membrane hyperpolarization induced by a chemotactic factor may begin within 10 seconds and thus is one of the earliest detectable consequences of specific receptor occupancy. Although the replacement of extracellular sodium with potassium ion or choline suppresses membrane hyperpolarization in parallel with chemotactic factor-mediated biological effects, the nature of the coupling of the electrical events to the biological effects has not been elucidated.

The rapid increase in the intracellular concentration of calcium elicited by chemotactic factor stimulation of PMN's is a function both of displacement of calcium from membranous stores and a selectively increased plasma membrane permeability that results in a substantial net influx of calcium (*53*). While the influx of calcium appears to be linked to several processes that may be critical to chemotactic responses, such as activation of phospholipases and increases in adenosine 3′,5′-monophosphate (cyclic AMP), a more direct role in motility also has been envisioned. The shortening of actin filaments in macrophages, which is required for the dissolution of the cross-linking of actin by actin-binding protein, is accomplished by the calcium-dependent linkage of a 90,000-dalton heat-labile protein, termed gelsolin, to the actin (*54*). Thus, portions of the actin lattice and other leukocyte structures move within the cell from regions of high calcium concentration to those of low calcium concentration during chemotaxis.

Phospholipid methylation, phospholipase activation, and arachidonic acid metabolism. Transmethylation reactions mediated by *S*-adenosylmethionine (AdoMet) are required for the chemotaxis of leukocytes. The critical involvement of these reactions in leukocyte chemotaxis was demonstrated by the nearly complete suppression of the chemotactic response by treatment of the cells with agents that cause an intracellular increase in the concentration of *S*-adenosylhomocysteine, a competitive inhibitor of AdoMet-mediated methylation reactions (*55, 56*). Treatment of rabbit PMN's with chemoattractants causes a rapid stimulation of protein carboxy-*O*-methylation (*57*). This effect has not been observed, however, in guinea pig macrophages or human PMN's (*56*). Chemoattractants do, nevertheless, alter methylated phospholipid synthesis and turnover in both mononuclear phagocytes and PMN's (*44, 45*).

In mononuclear phagocytes, chemoattractants cause inhibition of the methylation of phosphatidylethanolamine to form phosphatidylcholine. The inhibition appears to be due to curtailment of methylated phospholipid synthesis and not to increased breakdown of the phosphatidylcholine. Studies in rabbit PMN's have also shown that chemoattractants reduce the content of methylated phospholipids, but in these cells the decrease in these substances appears to be due to increased destruction of phosphatidylcholine by activation of phospholipase A_2 and results in the release of arachidonic acid (*45*). The release of arachidonic acid by leukocytes exposed to chemotactic factors and the initiation of chemotactic migration are suppressed in parallel by inhibitors of transmethylation reactions (*58*). Regardless of the mechanism by which chemoattractants depress the content of methylated phospholipids in different types of leukocytes, there is agreement that decreases in phosphatidylcholine produced by the methylation pathway are important for the chemotactic response of leukocytes.

The relative contributions of phospholipase A_2 and C in the release of arachidonic acid from leukocytes are not entirely clear and probably vary with the cell type. The effects of preferential inhibitors of phospholipase activity have been used to support a major role for phospholipase A_2 in the release of arachidonate from stimulated mast cells, leukemic basophils, PMN's and lympho-

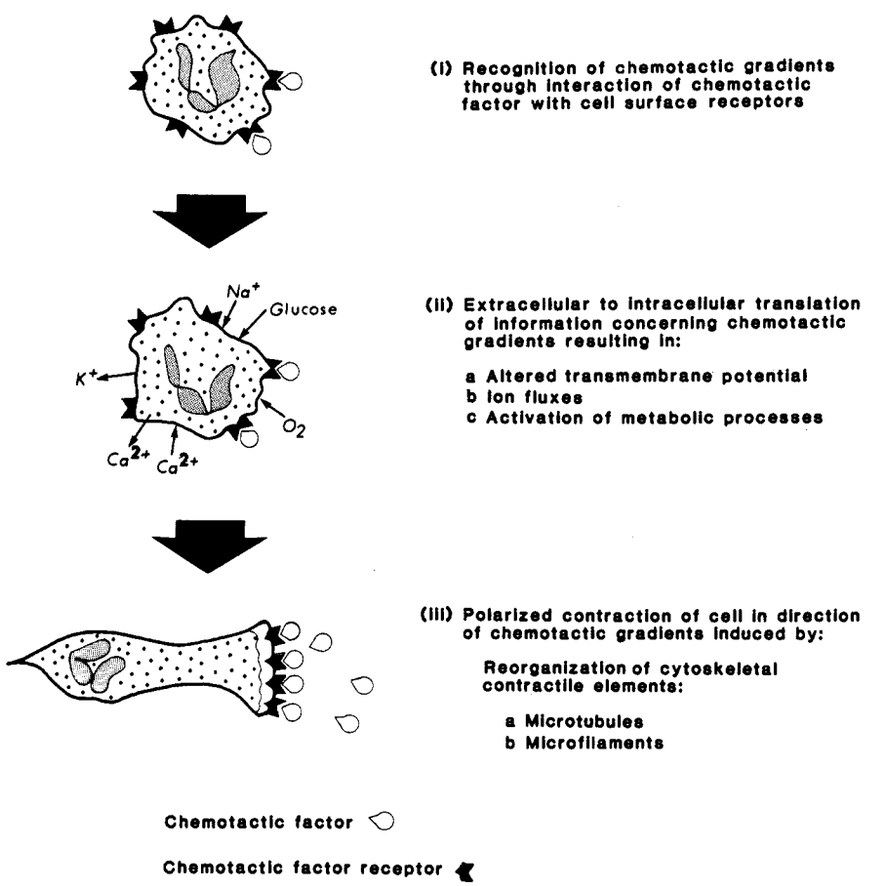

Fig. 3. Metabolic events in leukocyte chemotaxis.

cytes, but the specificity of these inhibitors is open to question. Measurements of specific intermediates indicate that phospholipase C is also involved in the release of arachidonic acid from phosphatidylinositol in mast cells and platelets (*59*), but similar studies have not been performed in PMN's or monocytes. Free arachidonic acid in leukocytes is converted by cyclooxygenase to thromboxanes and prostaglandins (PG's), predominantly PGE_2, and by lipoxygenation to a wide variety of HETE's (*60*). The rates of generation of the metabolites of both pathways are stimulated by chemotactic factors, and concentrations of the metabolites achieve peak intracellular levels within a few minutes.

A role for lipoxygenase products of arachidonic acid as functional intracellular constituents has been suggested by the ability of several classes of inhibitors of lipoxygenation to suppress PMN leukocyte migration, hexose transport, aggregation, and lysosomal degranulation. Such a role is also suggested by the ability of exogenous 5-HETE to restore, in some instances, the functions of HETE-depleted leukocytes (*61*). Although the mechanisms of lipoxygenase dependence have not been defined, two possibilities have been proposed. Human PMN's reincorporate into cellular phospholipids some of the endogenous 5-HETE, which would alter the properties of the plasma membrane, possibly at specific sites. In addition, the reactive intermediates, such as 5-hydroperoxyeicosatetraenoic acid, can covalently derivatize intracellular proteins and other polar constituents that are vital to cell function, some of which have been recovered by chromatography of extracts of stimulated PMN's (*62*).

Cyclic nucleotides. The exposure of PMN and mononuclear leukocytes to chemotactic factors evokes increases in the intracellular concentrations of both cyclic AMP and guanosine 3′, 5′-monophosphate (cyclic GMP) (*63*). Both C5a and formylated methionyl peptides induce up to threefold increases in the concentration of cyclic AMP in PMN's at 5 to 60 seconds, with the peak elevation evident at or just after the time of maximum hyperpolarization of the plasma membrane and subsiding within minutes. The increase in cyclic GMP in PMN's stimulated by a chemotactic factor attains a similarly early peak, but persists far longer. The doses of chemoattractants necessary to produce these increases in cyclic nucleotide levels, however, are about 10- to 100-fold greater than those necessary to produce chemotaxis. The increase in cyclic AMP elicited by a variety of chemotactic factors activates a calcium-dependent protein kinase, which phosphorylates a specific 85,000- to 90,000-dalton protein in PMN's. The lack of correlation between the concentration-dependence of chemoattractants for producing chemotaxis and cyclic AMP changes indicate the difficulties involved in assigning a role for alterations of this cyclic nucleotide in the chemotactic response.

Effects of chemotactic factors on lysosomal degranulation and other leukocyte functions. Chemotactic factors elicit an array of other leukocyte responses, including increased adherence, aggregation, enhanced oxidative metabolism, increased expression of complement receptors, and lysosomal degranulation (*64*). These effects generally require 5 to 30 minutes to attain maximum levels and appear not to be involved in the initiation of chemotaxis, but may have consequences that modulate the chemotactic response. For example, increased oxidative metabolism may generate chemotactic and chemokinetic factors from lipids. Lysosomal constituents are capable of enhancing or inhibiting chemotaxis by modifying the adherence of leukocytes to the substratum. The late effects of chemotactic factors on leukocytes serve mainly to provide more efficient phagocytosis and microbicidal activity after the leukocytes have been mobilized for host defense.

Conclusion

Leukocyte chemotaxis is initiated by the binding of chemoattractants to specific plasma membrane receptors. Receptor occupancy induces rapid alteration in cellular transmembrane potential, initiates changes in cyclic nucleotide levels and ion fluxes, enhances glucose and oxygen utilization, alters membrane phospholipid composition, and modifies transmethylation reactions mediated by *S*-adenosylmethionine. Arachidonic acid released after the activation of phospholipases is metabolized into a number of biologically active products including leukotrienes and prostaglandins. Within minutes of encountering chemoattractants, phagocytes alter their morphology and become oriented toward the chemotactic gradient. Reorganization of cytoskeletal elements, such as microtubular structures and actin filaments as well as membrane receptors, is manifest in oriented cells and appears to be required for directed migration (Fig. 3).

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Regulation of Technological Activities: A New Approach

Simon Ramo

Success in satisfying the requirements and aspirations of the American citizenry depends greatly on the wise employment of advancing science and technology. The potential gains from proper use of these tools include reduced costs of production, the discovery of new resources and invention of substitutes for those in shrinking supply, and the design of new products whose manufacture would create needed jobs. Unfortunately, there is a deterrent to our full realization of the fruits of technology. It is that technological activities produce negatives along with positives. Build any machine or set up any process and, along with the benefits, detrimental consequences also may result. Appreciation of possible disbenefits is now so widespread that government regulation of technological activities is a permanent policy, even if in practice it is an ambiguous one, difficult to implement.

Critics of present technological regulation abound. They complain that the regulation often does not provide needed, minimum protection; over-regulation is frequent; Congress has created bad regulatory legislation; the courts are called upon to do what they cannot and should not be asked to do; agencies sometimes have conflicts of interest; regulators often make inadequate investigations and stall to play it safe; value judgments are confused with economic or scientific factors; an unintegrated hodgepodge of disconnected decisions dominates; balanced decisions, with the risks and benefits of all alternatives compared, are rarely made. Whether these criticisms are justified is itself a value judgment, my own being that all have considerable validity.

In this article I will discuss the nation's present pattern of regulating technology-based activities, arguing that it is overly beset with shortcomings. I will propose a new approach which I believe merits consideration for two reasons: (i) it satisfies some of the criteria fundamental to any more satisfactory system, and (ii) it constitutes beginning theoretical support for the belief that superior systems are inventable.

Difficulty of Technological Regulation

Before considering the shortcomings of present regulatory policy, it is essential to recognize the inherent difficulty of technological regulation. To begin with, defining accurately what hazards are tolerable is essentially impossible. The unwanted ills conceivably present are too numerous and not always quantifiable. Even if for every activity we could measure every possible menace, we would not learn thereby what threshold level of impairment is acceptable. What we define as tolerable must depend on how much we are willing to risk losing. What degree of lowering of our life expectan-

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