Chemotaxis: Role in Immune Response

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Secondary article

Chemotaxis is defined as the unidirectional movement of a cell in response to a chemical gradient in the direction from a low to a high concentration of chemoattractant.

Introduction

Chemotaxis refers to the unidirectional movement of a cell in response to a chemical gradient. Leucocytes move from a low to a high concentration of chemoattractant. In the absence of a gradient, chemotactic factors increase the random motion of leucocytes. This has been termed chemokinesis. The directed migration, or chemotaxis, of immune cells is an essential feature of the immune system. It is necessary for the development and homeostatic trafficking of immune cells and for orchestrating the juxtaposition of immune cells and antigen necessary to generate long-lasting antigen specific immunity. Chemotaxis also plays an essential role in the recruitment of leucocytes into sites of inflammation and infection. This article will briefly discuss the discovery of chemotaxis, describe the chemotactic factors and their receptors, the changes induced in cells in response to a chemotactic signal, the biological functions of chemotactic factors in the immune response, and, finally, the measurement of chemotaxis *in vivo*.

Historical Perspective

Over a century ago it was recognized by Cohnheim that phagocytes accumulated at sites of intrusion by foreign substances. These observations led Metchinkoff to propose that chemical signals emanating from foci of invading materials attracted leucocytes, which were an important component of host defences. The first person to describe chemotaxis was Leber, who studied inflammatory reactions induced in the cornea of rabbits injected with various microorganisms. In 1888, Leber described the directional movement of leucocytes outside of vessels, which he attributed to the differences in concentration of substances that the cells contact. He also stated that extravasation of leucocytes is probably due to similar substances. Demonstration of *in vivo* chemotaxis in tissue towards a chemotactic stimulus awaited the studies of Clarke *et al.* in 1936. However, chemotaxis as the principal mechanism in *in vivo* leucocyte accumulation was not fully appreciated until after the *in vivo* experiments of Boyden in 1962.

Chemotactic Factors

Chemotactic factors are substances that cause the directional migration of cells. In the immune system, chemotactic agents are a diverse group of chemicals that include lipids, formylated peptides, proteolytic fragments of complement proteins, and specialized chemotactic cytokines, called chemokines. Chemotactic factors can be divided into the 'classical chemoattractants' and the recently identified 'chemokine' superfamily (**Table 1**). The classical chemoattractants include platelet-activating factor (PAF), *N*-formyl peptides such as formyl-methionyl-leucyl-phenylalanine (fMLP), the proteolytic fragment of complement C5a and leucotriene B_4 (LTB_4). These factors are involved in mobilizing the innate immune system to the site of infection and inflammation, and also activate the microbicidal functions of phagocytic cells, such as degranulation and oxidant production. The chemokines are the largest family of cytokines and, in general, have more specificity in terms of leucocyte selectivity than the classical chemoattractants. In addition to their activity on innate immune cells, the chemokines are also critical for the trafficking of lymphocytes and appear to play a central role in linking the innate and acquired immune responses. All chemoattractants, however, utilize a similar signal transduction system, namely guanine nucleotide-binding protein (G protein)-coupled seven-transmembrane-spanning receptors, which will be discussed below.

Classical chemoattractants

Platelet-activating factor (PAF)

PAF is a potent inflammatory mediator with a wide range of biological activities, including chemotaxis. It is synthesized by acylation of lysoglycerol ether phosphorylcholine, which is derived from a membrane phospholipid by phospholipase A_2 release of a fatty acid, such as arachidonic acid. PAF was originally identified in super-

Table 1 Chemotactic factors and their receptors

Chemoattractant	Receptor
Classical	
PAF	PAFR
N-formylated peptides	fMLPR
C5a	C5aR
LTB_4	BLTR
LXA_4	LXA_4R
Chemokines	
CXC	
IL-8	CXCR1
GCP-2	CXCR1, CXCR2
GRO_α	CXCR2
GRO_β	CXCR2
GRO_γ	CXCR2
ENA-78	CXCR2
NAP-2	CXCR2
IP-10	CXCR3
MIG	CXCR3
I-TAC	CXCR3
SDF-1	CXCR4
BCA-1	CXCR5
PF4	?
CC	
MIP-1α	CCR1, CCR5
MIP-1β	CCR5
RANTES	CCR1, CCR3, CCR5
HCC-1	CCR1
MCP-1	CCR2
MCP-2	CCR1, CCR2, CCR3, CCR5
MCP-3	CCR2, CCR3
MCP-4	CCR1, CCR2, CCR3
MCP-5 (mouse only)	CCR2
Leucotactin-1 (HCC-2, MIP-5)	CCR1, CCR3
Eotaxin	CCR3
Eotaxin-2 (MPIF-2)	CCR3
Eotaxin-3	CCR3
MDC	CCR4
TARC	CCR4, CCR8
SLC (Exodus-2, 6CKine, TCA-4)	CRC, CCR7
MIP-3α (LARC, Exodus-1)	CCR6
ELC (MIP-3β)	CCR7
l-309	CCR8
TECK	CCR9
DC-CK1 (PARC, AMAC-1, MIP-4)	?
MPIF-1 (MIP-3)	?
MIP-5 (HCC-2)	?
HCC-4 (NCC-4)	?
MIP-1γ (mouse only)	?
C-10 (mouse only)	?
C	
Lymphotactin	XCR1
CX_3C	
Fractalkine (neurotactin)	CX_3CR_1

AMAC, alternative macrophage activation-associated CC chemokine; BCA, B-cell attracting chemokine; BLTR, leucotriene B_4 receptor; C, cysteine; CC, cysteine–cysteine; CXC, cysteine–X amino acid–cysteine; DC-CK1, dendritic-cell-derived CC chemokine; ELC, Epstein–Barr virus (EBV)-induced gene 1 ligand chemokine; ENA, epithelial cell-derived neutrophil-activating peptide; fMLP, formyl-methionyl-leucyl-phenylalanine; GCP, granulocyte chemotactic protein; GRO, growth-regulated oncogene; IL, interleukin; IP-10, interferon-inducible protein of 10 kDa; I-TAC, interferon T-cell alpha chemoattractant; LARC, liver and activation-regulated chemokine; LT, leucotriene; LX, lipoxin; MCP, monocyte chemotactic protein; MDC, macrophage-derived chemokine; MIG, monokine induced by interferon γ; MIP, macrophage inflammatory peptide; MPIF, myeloid progenitor inhibitory factor; NAP, neutrophil-activating peptide; PAF, platelet-activating factor; PARC, pulmonary and activation-regulated chemokine; PF4, platelet factor 4; R, receptor; RANTES, regulated on activation normal T cell expressed and secreted; SDF, stromal cell-derived factor; SLC, secondary lymphoid-tissue chemokine; TARC, thymus and activation-regulated chemokine; TCA-4, thymus-derived chemotactic agent; TECK, thymus-expressed chemokine.

natants from sensitized basophils challenged with antigen as an inducer of platelet aggregation. PAF has since been shown to be generated by several human cell types, including macrophages, neutrophils, eosinophils and endothelial cells following a variety of physiological stimuli. PAF is a potent chemoattractant for monocytes, neutrophils and eosinophils. It has additional biological activities including platelet activation and bronchoconstriction, and is also an anaphylatoxin.

N-formyl peptides

A major advance in the study of chemotaxis was the discovery that synthetic *N*-formyl oligopeptides are chemoattractants for phagocytes. Schiffmann et al. (1975) had discovered that chemoattractant peptides in bacterial supernatants have blocked N-termini, and he noted that bacterial proteins start with *N*-formyl-methionyl. Structure–activity analysis suggested that *N*-formylation was required for peptide potency. Several natural *N*-formyl peptide chemoattractants, including the prototype synthetic *N*-formyl peptide (fMLP), have since been purified from bacterial supernatants. In addition, mitochondrial proteins are also *N*-formylated and are chemoattractants for neutrophils. Thus mitochondria from damaged tissues could also release *N*-formyl peptides that

might attract phagocytes. fMLP is a potent chemoattractant for neutrophils, monocytes and eosinophils.

Complement peptide C5a

Proteolysis of the complement proteins C3, C4 and C5 gives rise to N-terminal cationic fragments, C3a, C4a and C5a. These biologically active peptides are collectively called anaphylatoxins because they induce the release of mediators from mast cells, which cause a rapid increase in vascular permeability which is characteristic of anaphylaxis. The most potent of these, C5a, elicits the broadest responses, including chemotaxis. C5a is an 11-kDa cationic glycopeptide released from the first 74 amino acids of the α chain of C5 by the action of either classical or alternative pathway C5 convertase. At subnanomolar to nanomolar levels, C5a elicits chemotaxis of cells of the myeloid lineage, including neutrophils, eosinophils, basophils, monocytes and macrophages. Higher nanomolar concentrations also elicit degranulation and activation of reduced nicotinamide–adenine dinucleotide phosphate (NADPH) oxidase. C5a is also thought to have vasoactive properties, mediating vasodilatation, increased vascular permeability, smooth muscle contraction, and release of histamine from mast cells. Thus, C5a is a potent proinflammatory mediator released into the fluid phase following complement activation. Several studies in complement-depleted animals or in mice genetically deficient for C5 have demonstrated reduced injury in anaphylaxis, in the Arthus reaction, and other complement-dependent inflammatory models.

Leucotriene B_4

Leucotrienes constitute a class of potent biological mediators of inflammation that have been implicated in a wide variety of human inflammatory diseases. Their biosynthesis derives from 5-lipooxygenase-catalysed oxygenation of arachidonic acid in cells of the myeloid lineage into an unstable epoxide intermediate, LTA_4. This derivative can be converted enzymatically by hydration into LTB_4 and, by the addition of glutathione, into LTC_4. LTC_4 is metabolized to LTD_4 and LTE_4 by the successive elimination of a γ-glutamyl residue and glycine. Slow-reacting substance of anaphylaxis consists of a mixture of LTC_4, LTD_4 and LTE_4. Although 5-lipooxygenase and thus LTA_4 generation is found mainly in cells of the myeloid lineage (e.g. neutrophils, eosinophils, mast cells, basophils, monocytes and macrophages), the enzymes determining the next step in the arachidonic cascade, either to LTB_4 or to the cysteinyl leucotrienes (LTC_4, LTD_4 and LTE_4), are more widely distributed. Thus the export of LTA_4 from myeloid cells enables a much broader range of cells to act as leucotriene secretors. Among the myeloid cells, considerable variation exists in both the type and amount of leucotrienes secreted. Most of these cells produce appreciable quantities of either LTB_4 or LTC_4 but not both, with the exception of monocytes and macrophages which produce both in response to immunological stimuli such as Fc receptor activation. LTB_4 is the principal 5-lipooxygenase product released from activated neutrophils and immunoglobulin (Ig) E-sensitized basophils stimulated with antigen.

LTB_4 is primarily active on leucocytes, causing chemotaxis, adhesion and activation, while the cysteinyl leucotrienes are primarily active on smooth muscle, causing bronchoconstriction, capillary leakage and mucus production. LTB_4 secretion by myeloid cells, as well as by nonmyeloid cells caused by transcellular metabolism, induces a range of cellular and molecular responses that coordinate and amplify the inflammatory response. LTB_4 is the most potent neutrophil chemotactic agent produced by the arachidonic cascade. Intratracheal instillation of LTB_4 induces the selective recruitment of functionally active neutrophils into bronchoalveolar fluid in humans. Subcutaneous injection of LTB_4 into humans causes neutrophils to accumulate rapidly in the affected tissue. LTB_4 induces the adhesion of neutrophils to endothelial cells *in vitro* and *in vivo*, and causes the release of glucuronidase and lysozyme from neutrophils. Although LTB_4 was discovered based on its potent chemotactic activity on neutrophils, it has also chemotactic activity on activated eosinophils, monocytes and lymphocytes.

Lipoxins

Lipoxins are a newer class of bioactive lipooxygenase-derived eicosanoids. The lipoxins are functionally distinct from leucotrienes and other eicosanoids, and are generated primarily in human tissues during cell–cell interactions as exemplified by leucocyte–platelet interactions. Lipoxin (LX) A_4 has been shown to inhibit the chemotaxis of neutrophils toward LTB_4 and fMLP. In addition, LXA_4 and LXB_4 have also been shown to inhibit fMLP-induced migration of neutrophils across intestinal epithelium as well as to reduce neutrophil adherence to human endothelial cells. In contrast to their effects on neutrophils, lipoxins have been shown to activate monocytes and induce their migration to sites of inflammation. LXA_4 promotes monocyte activation, adherence and chemotaxis, while suppressing neutrophil function.

Chemokines

The chemokines are 8–10-kDa secreted proteins with 20–70% homology in structure, and share the common functional activity of being chemotactic for leucocytes. Over 40 chemokines have been identified and subdivided into families based on the relative position of their cysteine residues. There are at least four families of chemokines, but only the α and β chemokines, which contain four cysteines, appear to contain many members (**Table 1**). The α, or cysteine–X amino acid–cysteine (CXC), chemokines have

their first two cysteine residues separated by one amino acid, whereas the first two cysteine residues of the β, or cysteine–cysteine (CC), chemokines are adjacent to each other. Two chemokines that do not fit into this classification, lymphotactin, with only two cysteines and hence the classification of (C), and fractelkine, in which the first two cysteine residues are separated by three amino acids (CXXXC), may represent additional families. Fractelkine is also unique in that it is a membrane-bound protein with a chemokine-like domain that sits on top of a mucin stalk.

The α chemokines can be further subdivided into those that contain the sequence glutamic acid-lysine-arginine (ELR) near the N-terminus (preceding the CXC sequence) and those that do not. ELR-containing chemokines are chemotactic for neutrophils and include interleukin (IL)-8, growth-regulated oncogene (GRO) α, β, γ, epithelial cell-derived neutrophil-activating peptide (ENA) 78 and neutrophil-activating peptide (NAP) 2. In contrast, non-ELR containing α chemokines are active on lymphocytes and include interferon-inducible protein of 10 kDa (IP-10), monokine induced by interferon γ (MIG), interferon T-cell alpha chemoattractant (I-TAC), stromal cell-derived factor (SDF) 1 and B-cell chemoattractant (BCA) 1.

The β chemokines, in general, are inactive on neutrophils, and instead attract monocytes, eosinophils, basophils and lymphocytes with variable selectivity. Like the CXC family, the N-terminal amino acids preceding the CC residues of the β chemokines are critical for their biological activity and leucocyte selectivity. For example, the addition or deletion of a single amino acid residue at the N-terminus of monocyte chemoattractant protein (MCP) 1 reduces its biological activity on monocytes by 100–1000-fold, and the deletion converts it from an activator of basophils to an eosinophil chemoattractant. Several chemokines undergo N-terminal proteolytic processing after secretion that alters their activity. For example, the inactive platelet granule chemokine, platelet basic protein, is N-terminally processed by monocyte proteases to generate NAP-2, which is active on neutrophils. This may be a general mechanism whereby local factors can regulate and amplify chemokine activity.

Chemotactic Receptors

Although chemoattractants constitute a diverse array of molecules, including proteins, peptides and lipids, they all appear to signal leucocytes through a related family of seven-transmembrane-spanning G protein-coupled receptors (GPCRs) (**Figure 1**). GPCRs are the largest family of cell surface receptors and represent a versatile signal transduction paradigm that is also used in vision, olfaction, hormone action and neurotransmission. Chemoattractant receptors on leucocytes are homologous to the cyclic adenosine monophosphate (cAMP) chemotactic receptor of the unicellular amoeba *Dictyostelium discoideum*, suggesting that they have an evolutionarily conserved role in controlling chemotaxis.

Biochemical analysis of leucocyte chemoattractant receptors began with labelling of pure chemoattractant molecules that retained their biological activity and bound specifically to membrane-associated protein. Unsuccessful efforts to purify and sequence receptor proteins were replaced by successful expression cloning and homology hybridization cloning strategies. Genes encoding all the classical chemoattractant receptors and 16 chemokine receptors have been identified, and include the receptors for C5a, PAF, fMLP, LTB_4, LXA_4 and the chemokines. In addition, through the more recent sequencing of complementary deoxyribonucleic acid (cDNA) libraries and the human genome, many additional orphan receptors with a high degree of homology to the chemoattractant receptors have been identified that probably represent additional members of the chemoattractant receptor family.

Based on the known structures of bacteriorhodopsin and rhodopsin, a model has been proposed for GPCRs (**Figure 1**). The major domains consist of: (1) an extracellular N-terminus; (2) an intracellular C-terminus; (3) seven α helical transmembrane domains oriented perpendicularly to the plasma membrane and kinked in transmembrane domains II, IV, VI and VII by intrahelical prolines; (4) three intracellular and three extracellular connecting loops composed of hydrophilic amino acids (i1–3 and e1–3); and (5) a disulfide bond linking cysteine residues in the e1 and e2 loops. As a subfamily of the GPCRs, the chemoattractant receptors share some common features: (i) they are among the smallest GPCRs, being only about 350 amino acids in length due to a relatively short i3 loop and a C-terminal segment; (ii) they share more than 20% sequence identity; and (iii) they have an acidic N-terminus. As for many other GPCRs, sites for potential N-linked glycosylation of the chemoattractant receptors are usually found in the N-terminal segment and, in some cases, the e2 loop. The C-terminus is rich in serine and threonine residues which become phosphorylated following receptor activation that leads to receptor internalization and desensitization. In this way a chemotactic agent may blunt its own signal as well as that of other chemoattractants through homologous and heterologous desensitization.

Platelet-activating factor receptor

PAFR cDNA has been cloned in humans and rodents and has been identified as a unique molecular species (approximately 80% identity across species), although the possible existence of PAFR subtypes has been suggested by biochemical or pharmacological methods. PAF induced mobilization of calcium in human kidney epithelial cells stably transfected with the human PAFR with a half-

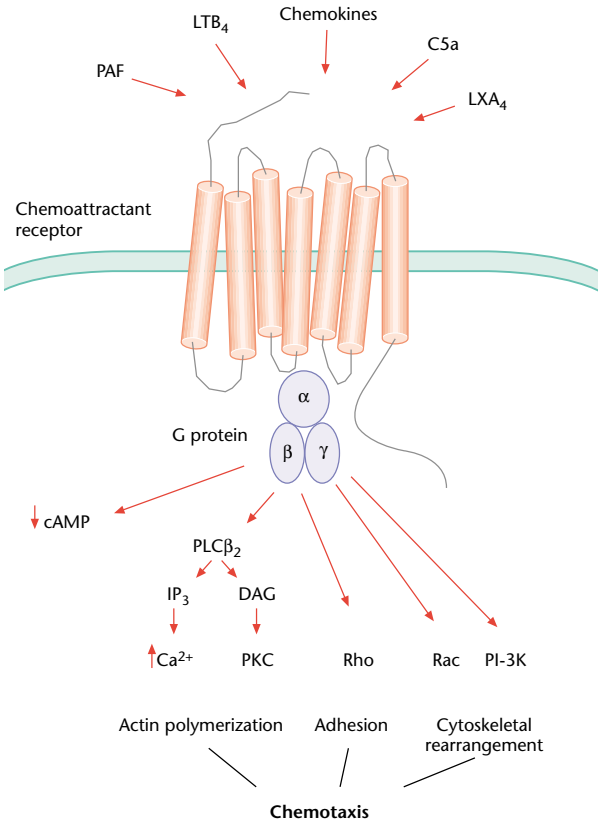

Figure 1 Chemoattractant receptors. Chemoattractants activate cells through seven transmembrane-spanning G protein-coupled receptors that share common structural and functional motifs. A multitude of signalling pathways, some of which are indicated, are activated to control the complex process of directed cellular locomotion. cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; IP_3, inositol trisphosphate; LT, leucotriene; LX, lipoxin; PAF, platelet-activating factor; PI-3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PLC, phospholipase C.

maximal response concentration (ED_{50}) of 0.5–1.0 nmol L^{-1}. This response could be blocked by PAF antagonists L-659 898 and CV-3988 but not by pertussis toxin, exactly paralleling the properties of the native neutrophil calcium response to PAF. PAFRs have been identified in lung, brain, platelets, leucocytes and activated endothelial cells. Pharmacological studies have suggested a role for PAF in pregnancy, neuronal cell migration, anaphylaxis and endotoxic shock. However, PAFR-deficient mice developed normally, were fertile, and remained sensitive to bacterial endotoxin, but had a marked reduction in systemic anaphylactic symptoms following ovalbumin immunization and intravenous challenge (Ishii *et al.*, 1998). In addition, PAFR-deficient mice were spared the allergen-induced airway obstruction associated with this active anaphylaxis model, demonstrating that PAFR plays a dominant role in eliciting anaphylaxis.

Formyl-methionyl-leucyl-phenylalanine receptor

The cDNA for the human fMLPR was cloned based on its ability to confer high-affinity *N*-formyl peptide binding in COS cells. The selectivity of this receptor for binding to different *N*-formyl peptides is similar to that of neutrophils. Moreover, activation of transfected fMLPR by fMLP induces calcium mobilization, cytoskeletal reorganization and degranulation in heterologous cell types. Thus fMLPR is considered the receptor that mediates migratory and cytotoxic responses of phagocytic cells to *N*-foryml peptides. fMLPR expression is seen principally in cells of the myeloid lineage such as neutrophils, eosinophils, monocytes and macrophages.

C5a receptor

The C5aR was initially identified as a high-affinity binding site on neutrophils with an equilibrium dissociation constant (K_d) of about 1 nmol L^{-1}. The number of receptors on neutrophils is extremely high, up to 200 000 sites per cell. The cloned C5aR also binds C5a with a K_d of 1 nmol L^{-1} when transfected into heterologous cells. C5aR is approximately 35% homologous to the fMLPR and, like fMLP, is expressed principally in cells of the myeloid lineage such as neutrophils, eosinophils, monocytes and macrophages. C5aR has also been detected in endothelial cells, vascular smooth muscle cells, bronchial and alveolar epithelial cells, and hepatocytes. Mutational analysis of C5a has led to a two-site model for C5aR activation which involves the large N-terminal core of C5a binding to the N-terminal region of C5aR, thereby promoting the interaction of the C-terminus of C5a with the interhelical region of the receptor. This model has served as a general paradigm for other chemoattractants. C5aR-deficient mice exhibit decreased migration of neutrophils and decreased tumour necrosis factor (TNF) α and IL-6 in the peritoneal reverse passive Arthus reaction. In addition, C5aR-deficient mice have an impaired ability to clear intrapulmonarily instilled *Pseudomonas aeruginosa*, despite a marked neutrophil influx, and succumb to pneumonia, suggesting a nonredundant role in host defence (Hopken *et al.*, 1996).

Leucotriene B_4 receptor

Radioligand binding studies have identified an LTB_4 receptor on leucocytes that has been given the designation BLTR. LTB_4 can also bind and activate the intranuclear transcription factor peroxisome proliferator-activated

receptor (PPAR) α, resulting in the activation of genes that terminate inflammatory processes. BLTR has been characterized as a binding site for LTB_4 on human neutrophils and on guinea-pig eosinophils with a K_d of about 1 nmol L^{-1}. Recently, human and mouse BLTRs have been molecularly identified as GPCRs with approximately 30% identity with other chemoattractant receptors. Membrane fractions of COS-7 cells transfected with human and mouse BLTRs were shown to bind LTB_4 with a K_d of about 1 nmol L^{-1}. In Chinese hamster ovary (CHO) cells stably expressing this receptor, LTB_4 induced a calcium flux, D-myoinositol-1,4,5-trisphosphate (IP_3) accumulation, inhibition of adenylate cyclase and chemotaxis.

Specific antagonists of LTB_4 have revealed a role for LTB_4 in regulating the recruitment of eosinophils into tissues *in vivo*. In a guinea-pig model of antigen-induced pulmonary inflammation, pretreatment of sensitized guinea-pigs with a selective LTB_4 antagonist before antigen provocation decreased the influx of eosinophils into the lung and bronchoalveolar (BAL) fluid but had no effect on the influx of neutrophils. Likewise, in a murine model of experimental allergic encephalomyelitis, pretreatment of mice with a selective LTB_4 receptor antagonist markedly blocked the recruitment of eosinophils into the spinal cord and completely inhibited the development of paralysis. The influx of lymphocytes was unaffected by pretreatment with the LTB_4 receptor antagonist, thus revealing an unrecognized role for eosinophils in the pathogenesis of this disease. Furthermore, the specific inhibition of 5-lipooxygenase with zileuton reduced eosinophil influx into the lung tissue and BAL fluid in a murine model of allergic pulmonary inflammation and also prevented airway mucus release in these mice. Studies in humans have also implicated leucotrienes in the pathogenesis of allergic airway inflammation. Increased levels of LTB_4 and LTC_4 were recovered in the BAL fluid following endobronchial antigen challenge compared with prechallenge levels. Nocturnal levels of LTB_4 and cysteinyl leucotrienes were raised in the BAL fluid of patients with nocturnal asthma compared with normal controls. In these asthmatic subjects, zileuton decreased BAL fluid LTB_4 levels and blood eosinophil levels, and improved the forced expiratory volume in 1 s (FEV_1). In fact, zileuton is now widely used clinically in the treatment of human asthma.

Lipoxin A_4 receptor

LXA_4 binds to high-affinity receptors (K_d approximately 1 nmol L^{-1}) on human neutrophils. The LXA_4R cDNA was identified based on its ability to bind and signal with LXA_4 following transfection in CHO cells. LXA_4R shares a surprising 78% identity with the fMLPR and was initially identified based on its sequence homology with the fMLPR. LXA_4 has recently been identified in enterocytes where it inhibits TNFα-induced IL-8 release, suggesting a broader role for LXA_4R in suppressing the inflammatory response.

Chemokine receptors

A major breakthrough in the chemokine field came with the cloning of the chemokine receptors and the realization that their receptors were members of the GPCR chemoattractant subfamily of seven-transmembrane spanners. Chemokine receptors are named based on the subfamily of chemokines they principally interact with. Although most chemokine receptors bind more than one chemokine, CCR receptors bind CC chemokines and CXCR receptors bind CXC chemokines. Five human CXC chemokine receptors (CXCR1–5), nine human CC chemokine receptors (CCR1–9), one human C chemokine receptor (XC1) and one human CX_3C chemokine receptor (CX_3CR1) have thus far been identified.

Chemokine receptors are expressed on different types of leucocytes. Some receptors are restricted in their expression (e.g. CCR3 predominantly to eosinophils and basophils; CXCR1 and CXCR2 predominantly to neutrophils; CXCR3 to activated T cells; and CXCR5 to B cells), while others are more widely expressed on leucocytes (e.g. CXCR4 on monocytes, T cells and B cells, and CCR2 on monocytes, T cells and basophils). In addition, chemokine receptors are expressed constitutively on some cells, whereas they are inducible on others. For example, CCR1 and CCR2 are expressed constitutively on monocytes, but are expressed on lymphocytes only after stimulation by IL-2. Conversely, some constitutive chemokine receptors can be downregulated by inflammatory stimuli. For example, lipopolysaccharide (LPS) downmodulates CCR2 on monocytes and CCR6 on dendritic cells, making these cells unresponsive to MCP-1 and MIP-3α, respectively. Still other chemokine receptors are functional only on activated cells. For example, CXCR3 appears to be functional only on IL-2-activated T lymphocytes. In this way, transient upregulation of chemokine receptors on leucocytes allows for the amplification of the immune response. In addition, some chemokine receptors (e.g. CXCR4) are also expressed on nonhaematopoietic cells, suggesting additional roles for the chemokine system in addition to leucocyte chemotaxis.

Chemokine receptor expression may also reflect the state of differentiation of a cell. An interesting example of this is the differential expression of chemokine receptors on T helper (T_H) 1 and T_H2 CD4 + lymphocyte subsets. These cells have been defined functionally based on their expression of cytokines. T_H1 cells express interferon (IFN) γ and promote cell-mediated immunity, whereas T_H2 cells express IL-4 and IL-5 and promote IgE production and allergic inflammation. It has recently been demonstrated that T_H1 cells preferentially express CCR5

and CXCR3, whereas T_H2 cells express CCR3, CCR4 and CCR8.

Biological Functions

Chemoattractants are thought to provide the directional cues for the movement of leucocytes in development, homeostasis and inflammation, and appear to play an important role in bringing together T cells, B cells and dendritic cells to generate an immune response.

Development

The targeted deletion of the SDF-1 gene revealed an unexpected role for chemokines in development (Nagasawa *et al*., 1996). Mice deficient in SDF-1 or its receptor CXCR4 have defects in B-cell lymphopoiesis and the recruitment of haematopoietic progenitors from the fetal liver into the bone marrow. In addition, these mice have defects in cardiac, cerebellar and vascular morphogenesis. This dramatic phenotype underscores the possibility that, as well as directing the migration of leucocytes to sites of inflammation and infection, chemokines may play an important role in orchestrating the movement of cells during development.

Homeostasis

Chemokines are also believed to control the baseline trafficking of leucocytes through tissues. Lymphocytes recirculate continuously through the blood, tissues and lymphatics in an organized manner, bringing naive lymphocytes into the lymph nodes where they encounter antigen, and memory lymphocytes into inflamed tissue to ensure immunity. T cells routinely patrol the body in search of foreign antigens and recirculate through the blood, tissue and lymphatics, making over 20 round trips each day. Several recently identified chemokines seem to participate in guiding T cells in their journey. One such chemokine independently identified by several groups, and given the various and sundry names of secondary lymphoid-tissue chemokine (SLC), exodus-2, 6Ckine and TCA-4, appears to play an important role in directing T cells into peripheral lymph nodes. A role for SLC in the trafficking of T cells into lymph nodes has been suggested from studies that have examined the mutant mouse strain DDD, which has a paucity of lymph node T cells owing to a defect in the lymph node stroma. It has recently been shown that lymph nodes from the DDD mouse do not express the SLC chemokine. Furthermore, genetic analysis has revealed that the mutant allele maps to mouse chromosome 4, the same chromosome to which SLC has been mapped.

Much the same way in which T cells recirculate, B cells also traffic through the body, and this process is also controlled by chemokines. The B cell-specific CXC chemokine, independently identified as B cell-attracting chemokine (BCA) 1 and B lymphocyte chemoattractant (BLC), is an important participant in this process. BCA is a potent chemotactic factor for B cells and is expressed in the follicles of Peyer patches, the spleen and lymph nodes. BCA is a ligand for CXCR5, which is highly expressed on peripheral blood B cells. A role for BCA and CXCR5 in B-cell trafficking was revealed by the generation of a mouse strain deficient in CXCR5 (Forster *et al*., 1996). These mice have an impairment in the trafficking of peripheral blood B cells into lymph nodes.

Other leucocytes, such as macrophages, eosinophils and mast cells, also traffic into tissue. While these cells are produced in the bone marrow, they reside primarily in other tissues. The role of chemokines in regulating this process has begun to be elucidated from studies in mice deficient in chemokines. For example, eotaxin plays a critical role in normal eosinophil recruitment into tissues (Matthews *et al*., 1998).

Role in orchestrating the immune response

Chemokines also play an important role coordinating the movement of T cells, B cells and dendritic cells necessary to generate an immune response (**Figure 2**). Dendritic cells are thought to play a pivotal role in generating an immune response by capturing and presenting antigen to lymphocytes in a process that leads to the activation of T and B cells. Dendritic cells in the tissue are thought to pick up antigen and bring the antigen back to the lymph nodes where the antigen-loaded dendritic cells then interact with lymphocytes to generate an immune response. Several recent studies have suggested that chemokines may guide the dendritic cells in their journey (Dieu *et al*., 1998).

Immature dendritic cells reside in the tissue where they are very efficient at engulfing antigen but are not efficient at activating lymphocytes. Immature dendritic cells respond to a number of chemokines, including the recently identified CC chemokine called macrophage inflammatory protein (MIP)-3α, liver and activation-regulated chemokine (LARC) or exodus. MIP-3α is a ligand for CCR6, which is highly expressed on immature dendritic cells. MIP-3α is expressed in tonsils by inflamed epithelium, a site known to be infiltrated by immature dendritic cells. Moreover, MIP-3α is induced by inflammatory stimuli such as LPS or TNFα. It has been hypothesized that a stimulus such as LPS induces the local tissue production of MIP-3α, which attracts immature dendritic cells into the tissue. Once in the vicinity of the inflammatory stimulus, immature dendritic cells pick up antigen and then differentiate into cells more capable of activating lymphocytes. During this maturation process, dendritic cells

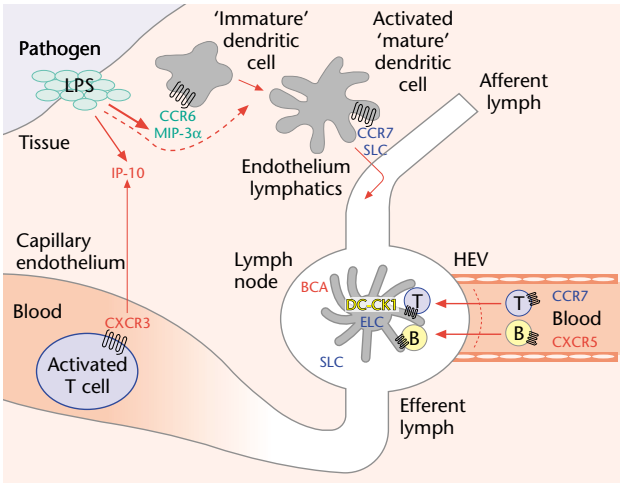

Figure 2 Chemokines orchestrate the movement of immune cells necessary to generate antigen-specific immunity. Dendritic cells in the tissue are thought to pick up antigen and bring it back to the lymph nodes where they interact with lymphocytes to generate an immune response. Chemokines appear to control this process by guiding activated antigen-loaded dendritic cells into lymph nodes as well as T and B cells. T cells activated in regional lymph nodes find their way back to sites of inflammation and infection by sensing chemokine gradients established at these local sites. BCA, B cell-attracting chemokine; CCR, CC chemokine receptor; CXCR, CXC chemokine receptor; DC-CK1, dendritic-cell-derived CC chemokine; ELC, Epstein–Barr virus-induced gene 1 ligand chemokine; IP-10, interferon-inducible protein of 10 kDa; HEV, high endothelial venule; LPS, lipopolysaccharide; MIP, macrophage inflammatory peptide; SLC, secondary lymphoid-tissue chemokine.

downmodulate the expression of CCR6 and hence responsiveness to MIP-3α. At the same time, they upregulate their expression of CCR7 and hence responsiveness to SLC and ELC. This switch in chemokine receptor expression and chemokine responsiveness results in the mature dendritic cells leaving the tissue and being drawn into the lymphatics and, ultimately, into the T cell-rich regions of lymph nodes.

While the molecular details still remain to be elucidated, it is likely that the expression of chemokines by lymph node stroma and dendritic cells coordinates the juxtaposition of antigen-loaded dendritic cells with recirulating T and B cells. T cells activated in regional lymph nodes, following encounter with antigen-loaded dendritic cells, find their way back to sites of inflammation and infection by sensing chemokine gradients established at these local sites. It is likely that IP-10, a chemokine induced by LPS and IFNγ, and a ligand for CXCR3, which is highly expressed on activated T cells, plays a role in this process (**Figure 2**).

Inflammation and infection

The attraction of leucocytes to sites of inflammation and infection is an essential component of the host response to disease. This process is controlled by chemoattractants. The secretion of chemoattractants by cells in inflamed tissue increases dramatically during an inflammatory response, resulting in the selective recruitment of leucocytes into that tissue. It is likely that chemoattractants play a role in most disease processes that result in the accumulation and activation of leucocytes in tissues. Chemoattractants have been detected during inflammation in most organs, including the skin, brain, joints, meninges, lungs, blood vessels, kidneys and gastrointestinal tract. In these organs chemoattractants, and in particular chemokines, can be detected in many types of cell, suggesting that most if not all cells have the capacity to secrete them given the appropriate stimulus. The major stimuli for chemokine production are early proinflammatory cytokines such as IL-1 and TNFα, bacterial products such as LPS, and viral infections. In addition, IFNγ and IL-4, products of T_H1 and T_H2 lymphocytes respectively, can induce the production of chemokines and also synergize with IL-1 and TNFα to stimulate chemokine secretion. The capacity to control precisely the movement of inflammatory cells suggests that the various chemokines and their receptors might provide novel targets for therapeutic interventions to modify the courses of these diseases.

The type of inflammatory infiltrate that characterizes a specific disease is controlled, in part, by the subset of chemokines expressed in the disease. For example, many acute disease processes, such as bacterial pneumonia and the adult respiratory distress syndrome, are characterized by a massive influx of neutrophils into the tissue. The concentration of chemokines that are potent neutrophil chemoattractants, such as IL-8, are increased in the bronchoalveolar fluid of patients with these diseases. Other acute diseases, particularly nonbacterial infectious diseases such as viral meningitis, are characterized by the recruitment of monocytes and lymphocytes into the tissue. The cerebrospinal fluid concentrations of chemokines active on these cells, such as IP-10 and MCP-1, are increased in these patients, and the concentrations are correlated with the extent of mononuclear cell infiltration of the meninges.

Many chronic disease processes are characterized by tissue infiltration of lymphocytes and macrophages. The delayed-type hypersensitivity granulomatous lesions of tuberculoid leprosy and sarcoidosis are characterized by the accumulation of activated lymphocytes, and high concentrations of IP-10 have been detected in these lesions. In addition, levels of IP-10 in the bronchoalveolar fluid of patients with active sarcoidosis correlates with the number of T lymphocytes in the fluid. In atherosclerosis, macrophages and lymphocytes are the major inflammatory cells found in the diseased blood vessels. These cells are thought to be central to the pathogenesis of this disease, both as progenitors of lipid-laden foam cells and a source of growth factors that mediate intimal hyperplasia. MCP-1 has been detected in diseased carotid endarterectomy

specimens but not in normal carotid arteries. A functional role for MCP-1 in the recruitment of monocytes into atherosclerotic lesions was revealed when mice deficient in MCP-1 or its receptor, CCR2, were bred with mouse strains prone to develop atherosclerosis, such as the apolipoprotein E-deficient and low density lipoprotein receptor deficient mouse strains. These 'bigenic' mice had a decrease in the number of lesional macrophages and in atherosclerotic lesion formation (Boring *et al*., 1998).

In allergic diseases, such as asthma, rhinitis and atopic dermatitis, the inflammatory reaction is characterized by the selective accumulation and activation of eosinophils and mast cells, and their mediators are strongly associated with the pathogenesis of these diseases. Agents that induce the release of histamine from mast cells and basophils, so-called histamine-releasing factors, are also strongly associated with the pathogenesis of allergic diseases. Chemokines, in particular eotaxin and the monocyte chemoattractant proteins, are potent eosinophil chemoattractants and histamine-releasing factors, making them particularly important in the pathogenesis of allergic inflammation. In fact, the chemokines may be the major histamine-releasing factors in the absence of antigen and IgE antibody. Many chemokines have been detected in the airways of patients with asthma. In addition, several eosinophil-active chemokines are increased in the epithelial tissue of patients with atopic dermatitis, allergic rhinitis and asthma after antigen challenge, making it likely that the chemokines are one of the molecular links between antigen-specific immune activation and tissue recruitment of eosinophils. In animals with allergic pulmonary inflammation, expression of eotaxin, MIP-1α and MCP-1, -3 and -5 precedes the massive airway recruitment of mononuclear cells and eosinophils. From studies using antibodies that inhibit the action of these chemokines and studies of mice with a targeted disruption of the eotaxin gene, it is clear that all these chemokines are participating in the recruitment of eosinophils into the airways.

Chemotactic Gradients

The mechanism by which eukaryotic cells sense a chemical gradient has remained elusive but is thought to involve both temporal and spatial sensing. Eukaryotic cells respond temporally to increases in receptor occupancy. Stimulus increments elicit sharp rises in the production of polymerized actin, inositol trisphosphate and Ca^{2+} levels, as well as the phosphorylation of myosins, the production of intracellular cAMP, and the activation of mitogen-activated protein kinase (MAPK) and signal transducer and activator of transcription (STAT). All these responses are transient and recur with each increase in receptor occupancy. Eukaryotic cells are also capable of spatial sensing. When a stimulus is applied, eukaryotic cells preferentially extend pseudopods up the gradient, suggesting that they have the ability to sense surrounding chemoattractant concentrations before moving. For cells to sense gradients, a signalling system must become asymmetrically activated. In fact, it has recently been shown that G-protein signalling events are activated at the leading edge of chemotactic cells and serve to establish spatial signalling (Parent *et al*., 1998).

Cells can sense and respond to both soluble and fixed gradients of chemoattractants. Chemotaxis refers to the directional migration of cells towards the source of a soluble chemotactic signal. Haptotaxis refers to the directional migration of cells towards the source of a chemotactic signal that is bound to a substrate. Haptotaxis is likely to play an important role *in vivo*. Chemokines are thought to be presented to leucocytes bound to endothelial cell surface and matrix glycosaminoglycans (Middleton *et al*., 1997). In this way, a chemotactic gradient can be maintained that is not subject to the forces of the fluid phase. In contrast, the classical chemoattractants have not been demonstrated to bind to matrix components and this may be an important difference between these factors and chemokines. This could theoretically result in a much more transient gradient for the classical chemoattractants compared to the chemokines.

Cellular Locomotion

Cell locomotion involves extension and contraction of the cell's organelle-less outer rim, or cortex. As cells begin to crawl, part of the cortex flows out to form a plate-like projection known as the leading lamella. The bottom of the lamella attaches to the underlying surface, primarily through the action of membrane adhesion proteins. Binding between these proteins and molecules on the substrate provides a traction force that enables the cell body to pull itself forward. The lamella then detaches from the substrate and flows forward again. The protrusion, attachment, contraction and detachment steps are often so tightly coordinated that the cell appears to glide along. Continued locomotion requires the cycling of cytoskeletal and membranous components between the front and rear. Chemotaxis is achieved by orienting the direction of locomotion along a chemoattractant gradient. This orientation results from the preference of pseudopod extension toward the higher chemoattractant concentration. Stationary unstimulated cells become oriented toward the higher concentration of a gradient when stimulated by a chemotaxin. In doing so they become triangular or droplet shaped, put out pseudopods in the front, known as lamellipodia, and retraction fibres or uropods at the tail end, as observed by phase-contrast microscopy, and both scanning and transmission microscopy (**Figure 3**). None of these processes is understood in

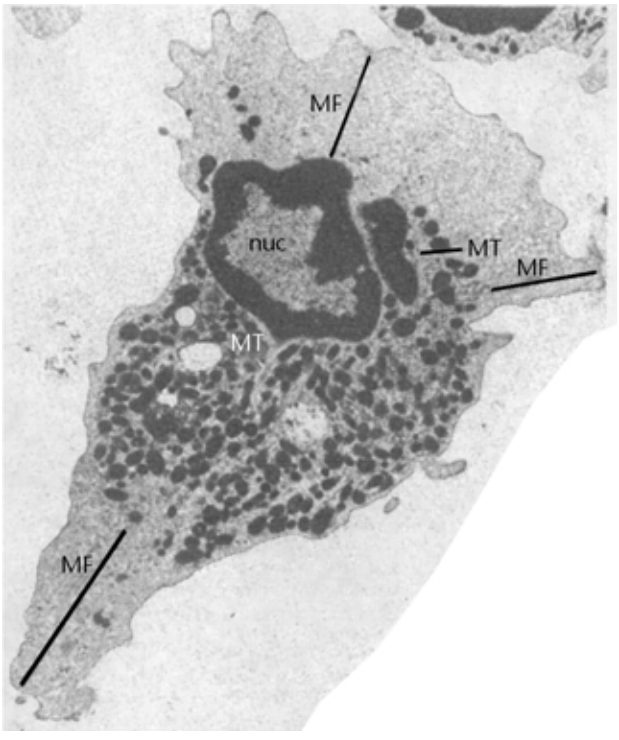

Figure 3 Human neutrophils polarized by formyl-methionyl-leucyl-phenylalanine (fMLP). The lamellipodium (top right) is free of organelles and contains microfilaments (MF, with double-headed arrow) not seen at this magnification. The uropod (bottom left) is also free of organelles and contains microfilaments. Microtubules (MT) are barely recognizable at this magnification. They extend toward, but not into, both pods of the cell. nuc, Nucleus. From Davis B, Walter R, Pearson C *et al*. (1982) Membrane activity and topography of F-Met-Leu-Phe-treated polymorphonuclear leukocytes. Acute and sustained responses to chemotactic peptide. *American Journal of Pathology* **108**: 206–216, by permission of the *American Journal of Pathology*.

detail but the chemoattractant-induced formation of lamellipodia correlates temporally and spatially with polymerization of actin. Myosin proteins probably contribute by providing the contractile forces on actin networks in the lamellae.

Cellular Responses

Chemoattractant receptors, like other members of the G protein-coupled receptor family, are functionally linked to phospholipases via G proteins (**Figure 1**). The model for chemoattractant-induced signal transduction involves a receptor whose affinity is increased by conformational changes induced by association with the guanosine diphosphate (GDP)-bound state of heterotrimeric G proteins. Many chemoattractant-induced signalling events are inhibited by *Bordetella pertussis* toxin, suggesting that chemokine receptors are linked to G proteins of the G_i class. Upon ligand binding, the activated receptor catalyses exchanges of GDP from guanosine triphosphate (GTP) by the G protein α subunit, resulting in dissociation of α from $\beta\gamma$ subunits. In turn, $\beta\gamma$ activates a phosphoinositide-specific phospholipase C (PLC), leading to the accumulation of IP_3 and diacylglycerol in the cytoplasm. IP_3 mobilizes calcium from intracellular stores, and diacylglycerol in conjunction with calcium activates various isoforms of protein kinase C. Activation of protein kinase C, as well as calcium-sensitive protein kinases, catalyses phosphorylation and accounts for some of the signal activated by chemoattractants. Phospholipase A_2 and D and phosphatidylinositol 3-kinase (PI-3K) activity are also rapidly stimulated in cells by chemoattractants. PI-3K catalyses the phosphorylation of phosphatidylinositol bisphosphate (PIP_2) to generate phosphatidylinositol trisphosphate (PIP_3). Chemoattractant receptor signalling also activates small GTP-binding proteins of the Ras, Rac and Rho families (Laudanna *et al*., 1996). Ras activation leads to the stimulation of a cascade of protein kinases that ultimately phosphorylate and activate MAPK or extracellular signal-regulated kinase (ERK). Rac and Rho are involved in cell motility through regulation of actin-dependent processes, such as membrane ruffling, pseudopod formation, and assembly of focal adhesion complexes. Although the precise role and interrelationship of these multiple pathways are not entirely clear, chemoattractant receptors activate multiple intracellular signalling pathways that regulate the intracellular machinery necessary to propel the cell in its chosen direction.

Leucocyte extravasation from the blood into the tissues is a regulated multistep process involving a series of coordinated leucocyte–endothelial cell interactions. Several families of molecular regulators such as the selectins, the integrins and the chemokines are thought to control different aspects of the process. The selectins facilitate the movement of leucocytes along the surface of endothelial cells ('rolling'). Chemoattractants also provide the signals that convert the low-affinity selectin-mediated interaction into a higher-affinity integrin-mediated interaction that leads to leucocyte firm adhesion and extravasation.

Measurement of Chemotaxis

Although the directed migration of leucocytes was appreciated in the nineteenth century, chemotaxis of neutrophils was first observed under the microscope approximately 40 years ago by means of a photographic trace technique. However, it was not until the quantification of leucocyte chemotaxis *in vivo* became possible in 1962 that the field began to develop. At this time, a method was developed by Boyden for the analysis of leucocyte migration through microporous filters across which a

chemical gradient could be established. In initial studies using this technique it was shown that immune complexes, when added to fresh but not heat-inactivated serum, led to the production of chemotactic activity, which was later identified as C5a. It was the development of the Boyden chemotaxis chamber in 1962 that led to the rapid characterization of the chemokine superfamily which has catapulted chemotaxis into the mainstream of immunology.

In general, methods of assessing chemotaxis *in vivo* fall into two broad categories: (1) microscopic observation of leucocytes directly or by time-lapse cinematography, (2) measurement of changes in distribution of leucocytes in the presence of a chemotactic stimulus. This latter method is quantitative and measures the distribution of leucocytes between two chambers or their migration under agarose. In the method first described by Boyden and most widely used today, leucocytes are separated from the chemotactic substance by a micropore filter, which has pores of a size such that the leucocytes are able to squeeze through by active migration, but not by falling through passively. The leucocytes are allowed to settle on the filter in the upper compartment. The chemotaxin in the lower compartment then diffuses through to the top of the filter. The leucocytes are chemoattracted towards the higher concentration of the chemotactic gradient. Migrated leucocytes can be assessed by removing the filter, and fixing and staining them for direct microscopic counting or automated biochemical quantitation. Alternatively, leucocytes can be labelled with fluorescent dyes or radioactivity, and then migrated leucocytes can be counted automatically. The most commonly used filters are made from nitrocellulose of about 150 μm in thickness with pore sizes of 0.4–12 μm. This assay can also be modified so that leucocytes crawl through an endothelial cell monolayer instead of a bare filter. Endothelial cells are grown on plastic inserts that contain different pore sizes, and migrated leucocytes fall to the bottom of the well where they can be quantitated and even phenotypically analysed by flow cytometry. The migration assay under agarose is performed with the chemotactic factor and buffer placed in reservoirs directly opposite each other in an agarose plate, with cells in a third reservoir in a line equidistant between the other two. The difference in the extent of migration between cells exposed to chemotaxin and to buffer is a measure of the extent of stimulated locomotion. Another assay is the leading front technique, in which the distance that the leading leucocytes have moved into the filter in a given period of time is determined.

In vivo chemotaxis can be measured following the local injection of a chemotactic factor into tissue, such as the peritoneum or dermis, or its application to a mucosal surface, such as the respiratory tree. Leucocytes can then be quantitated following lavage or histological examination of tissue. Alternatively, leucocytes can be labelled radioactively and injected into an animal before instillation of the chemotactic factor, and extravasated leucocytes can then be quantitated. *In vivo* techniques have the advantage of testing many cell types at once, but the effects of an agent can be indirect and therefore need to be confirmed on purified cells *in vivo*.

Summary

Although initially identified as chemotactic factors profoundly induced by proinflammatory stimuli that mobilize the innate immune system and control the recruitment of leucocytes into inflammatory foci, chemoattractants, and in particular the chemokines, are emerging as important regulators of cellular trafficking in development and homeostasis. In addition, they seem to be critical for the coordinated movement of dendritic cells and lymphocytes necessary to link the acquired immune response to the innate response and to generate long-lasting antigen-specific immunity.

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