Molecular mechanisms in allergy and clinical immunology

Series editors: William T. Shearer, MD, PhD, Lanny J. Rosenwasser, MD, and Bruce S. Bochner, MD

Chemokines: Roles in leukocyte development, trafficking, and effector function

Santa Jeremy Ono, PhD, Takao Nakamura, MD, PhD, Dai Miyazaki, MD, PhD, Masaharu Ohbayashi, PhD, Maria Dawson, MSc, and Masako Toda, PhD *London, United Kingdom*

This activity is available for CME credit. See page 37A for important information.

Chemokines, representing a large superfamily of 8- to 15-kd proteins, were originally discovered through their ability to recruit various cell types into sites of inflammation. It is now clear that these molecules play a much wider role in immune homeostasis, playing key roles in driving the maturation, homing, and activation of leukocytes. In this review we analyze the roles chemokines play in the development, recruitment, and activation of leukocytes. Because signaling from the receptors drives these processes, signal transduction from chemokine receptors will also be reviewed. Taken together, we highlight the various points at which chemokines contribute to allergic inflammation and at which their targeting might contribute to new therapies for type I hypersensitivity reactions. (J Allergy Clin Immunol 2003;111:1185-99.)

Key words: *Chemokines, receptors, allergy, asthma, signal transduction*

Abbreviations used

BMMC: Bone marrow–derived mast cell
GPCR: G protein–coupled receptors
mMCP-6: Mouse mast cell protease 6
ODN: Oligodeoxynucleotide
PI3K: Phosphoinositol 3 kinase
SCF: Stem cell factor
STAT: Signal transducer and activator of transcription

Various pathologic conditions, both classically defined as immune mediated (type I hypersensitivity–allergic and autoimmune diseases and allograft-xenograft rejection) and others (eg, atherosclerosis and Alzheimer disease) result in part from an inflammatory response.[1] Additional diseases (eg, Parkinson disease and age-related macular degeneration) also exhibit clear evidence of inflammation, indicating that their pathogenesis might have an immune component or that disease progression or severity might be linked to inflammation.[2] Both the initial stages of these diseases and downstream sequelae (eg, tissue remodeling and smooth muscle hypertrophy) depend on the recruitment to and activation of leukocytes to the inflammatory lesion.[3]

Because of the wide range of diseases with an inflammatory component, there has been a sustained effort over the past decades to identify or generate anti-inflammatory compounds.[4] Indeed, many widely used drugs target inflammation generally (eg, aspirin, glucocorticoids, and cyclosporine) or target specific inflammatory mediators (antihistamines, leukotriene inhibitors, and cyclooxygenase 2 inhibitors).[5] However, these drugs (although highly effective in specific scenarios) each have inherent flaws (eg, in their lack of specificity, limited potency, or the serious side effects that accompany their prolonged use).[6]

The discovery of chemokines, small 8- to 15-kd polypeptides that control the movement and activation of immunocytes, has provided attractive new targets for anti-inflammatory drug design.[7] The finding that the expression of chemokines and their receptors is under stringent developmental and tissue-specific control suggests that compounds targeted at these molecules might permit a level of specific immune suppression previously unattainable with existing drugs.[8] Finally, that the chemokines bind to G protein–coupled serpentine receptors, proved targets for new drug development, further support the feasibility of these targets as a platform for a new generation of anti-inflammatory drugs.[9]

From the Department of Immunology, University College London, University of London, Institutes of Ophthalmology and Child Health, and Moorfields Eye Hospital, London.

Drs Nakamura and Toda contributed equally to the preparation of this article.

Supported by grants from the National Institutes of Health, Wellcome Trust, MRC, BBSCRC, Santen Pharmaceutical Inc, GlaxoSmithKline plc, Biogen Inc, Fight for Sight, and the Japan Society for the Promotion of Science (M. Toda).

Received for publication April 8, 2003; revised April 23, 2003; accepted for publication April 24, 2003.

Reprint requests: Santa Jeremy Ono, PhD, GlaxoSmithKline Professor of Biomedical Sciences, University College London, Institute of Ophthalmology, 11-43 Bath St, London, EC1V 9EL, United Kingdom.

0091-6749/2003 $30.00 + 0
doi:10.1067/mai.2003.1594

TABLE I. The chemokine receptors and their ligands

Systematic name	Human ligand	Chemokine receptors
CXC chemokine-receptor family		
CXCL1	GROα/MGSAα	CXRX2 > CXCR1
CXCL2	GROβ/MGSAβ	CXCR2
CXCL3	GROγ/MGSAγ	CXCR2
CXCL4	PF4	Unknown
CXCL5	ENA-78	CXCR2
CXCL6	GCP-2	CXCR1, CXCR2
CXCL7	NAP-2	CXCR2
CXCL8	IL-8	CXCR1, CXCR2
CXCL9	Mig	CXCR3a
CXCL10	IP-10	CXCR3a
CXCL11	I-TAC	CXCR3a
CXCL12	SDF-1α/β	CXCR4b
CXCL13	BCA-1	CXCR5
CXCL14	BRAK/boekine	Unknown
(CXCL15)	Unknown	Unknown
CXCL16		CXCR6
C chemokine-receptor family		
XCL1	Lymphotactin/SCM1α/ATAC SCM-1β	XCR1
XCL2	SCM-1β	XCR2
CXC3C chemokine-receptor family		
CXC3CL1	Fracktalkine	CXC3CR1
CC chemokine-receptor family		
CCL1	I-309	CCR8
CCL2	MCP-1/MCAF/TDCF	CCR2
CCL3	Mip-1α/LD78α	CCR1, CCR5
CCL3L1	LD78β	CCR1, CCR5
CCL4	MIP-1β	CCR5c
CCL5	RANTES	CCR1, CCR3, CCR5
(CCL6)	Unknown	Unknown
CCL7	MCP-3	CCR1, CCR2, CCR3
CCL8	MCP-2	CCR3, CCR5c
(CCL9/10)	Unknown	CCR1
CCL11	Eotaxin	CCR3
(CCL12)	Unknown	CCR2
CCL13	MCP-4	CCR2, CCR3
CCL14	HCC-1	CCR1, CCR5
CCL15	HCC-2/Lkn-1/MIP-1δ	CCR1, CCR3
CCL16	HCC-4/LEC/LCC-1	CCR1, CCR2
CCL17	TARC	CCR4
CCL18	DC-CK1/PARC/AMAC-1	Unknown
CCL19	MIP-3β/ELC/exodus-3	CCR7d
CCL20	MIP-3α/LARC/exodus-1	CCR6
CCL21	6Ckine/SLC/exodus-2	CCR7d
CCL22	MDC/STCP-1	CCR4
CCL23	MPIF-1/CKβ8/CKβ8-1	CCR1
CCL24	Eotaxin-2/MPIF-2	CCR3
CCL25	TECK	CCR9
CCL26	Eotaxin-3	CCR3
CCL27	CTACK/ILC	CCR10
CCL28	MEC	CCR3/CCR10

GRO, Growth related oncogene; *MGSA,* melanoma growth stimulatory activity; *PF,* platelet factor; *ENA,* epithelial neutrophil activating; *GCP,* granulocyte chemotactic protein; *NAP,* neutrophil-activating peptide; *Mig,* monokine-induced by IFN-γ; *IP,* IFN-g inducible protein; *I-TAC,* IFN-inducible T-cell a chemoattractant; *SDF-1,* stromal cell–derived factor 1; *BCA,* B-cell attracting chemokine; *BRAK,* breast and kidney-expressed chemokine; *SCM,* Single C motif; *ATAC,* activation-induced, chemokine-related molecule; *I-309,* a nameless human chemokine; *MCP,* monocyte chemoattractant protein; *MCAF,* monocyte chemotactic and activating factor; *TDCF,* tumor-derived chemotactic factor; *MIP,* macrophage inflammatory protein; *LD78,* macrophage inflammatory protein-1l; *HCC,* human CC chemokine; *Lkn,* leukotactin; *LEC,* liver-expressed chemokine; *TARC,* thymus- and activation-regulated chemokine; *DC-CK1,* dendritic cell–derived CC chemokine; *PARC,* pulmonary and activation-regulated chemokine; *AMAC,* alternative macrophage activation-associated CC chemokine; *ELC,* (Ebl-1), EBL-l-ligand chemokine; *LARC,* liver- and activation-regulated chemokine; *SLC,* secondary lymphoid tissue chemokine; *MDC,* macrophage-derived chemokine; *STCP,* stimulated T-cell chemoattractant protein; *MPIF,* myeloid progenitor inhibitory factor; *CK,* chemokine; *TECK,* thymus-expressed chemokine; *CTACK,* cutaneous T cell–activating chemokine; *ILC,* IL-11 receptor alpha-locus chemokine; *MEC,* mucosae-associated epithelial chemokine.

It is now clear that both chemokines and their receptors are expressed on a myriad of cell types (albeit with specificity).[10] The expression of chemokines and their receptors is also inducible on several cell types (during the early stages of an inflammatory response).[11] Several other reviews have surveyed the range of cells that express chemokines and their receptors.[12-14] In this review, although touching on basic features of the chemokine system, we will focus largely on the expression of chemokines and their receptors on cells relevant to type I hypersensitivity reactions and in certain autoimmune diseases. Particular emphasis will be placed on the expression of chemokines and their receptors during mast cell development and during acute inflammation. This information provides clues as to the role the chemokine system plays in mast cell development and homing and for the specificity and efficacy of pharmaceuticals targeted at this system.

CHEMOKINES AND THEIR RECEPTORS

Chemokines

The chemokines are a superfamily of low-molecular-weight, secreted, heparin-binding molecules that serve as potent chemoattractants for cells of the immune system.[12-14] There are, at the time of the writing of this review, greater than 50 chemokines, and it is likely that more will be identified through genome sequence mining or classical recombinant DNA methods (Table I). The chemokines are clearly distinct from other chemoattractants, such as the complement components C3a and C5a and the lipid breakdown products leukotriene B4 and platelet-activating factor. Although there is limited overall sequence homology between chemokines, they are grouped within a family because they are predicted to have a similar 3-dimensional structure (a monomeric fold generated by a floppy N-terminus, 3 internal β-strands, and a C-terminal α-helix).[15] The fold is held together by 2 disulphide bridges that are found in 4-cysteine chemokines, although this is variant in CCL21 and XCL1 (Fig 1).[16] The floppy N-terminus is essential for receptor activation. These 50 chemokines bind 19 functional receptors, with 10 receptors exhibiting promiscuity for 2 or more chemokines (Table I).

The chemokines are thought to derive from 3 or 4 ancestral genes and can be categorized into 4 supergene families. This classification has been based on the number and spacing of conserved cysteine residues found in the N-terminus of the polypeptides. The major chemokine families are termed the CC and CXC chemokines (with 50 or more members represented in the 2 families). The C and CX3C chemokines are much smaller families, with one or a few members per family.

Chemokine receptors

Each family of chemokines interacts with a reciprocal family of heterotrimeric, 7-transmembrane, G protein–coupled receptors (GPCRs) expressed almost exclusively on leukocytes. For example, there are 11 CC chemokine receptors, 5 CXC receptors, 1 CX3C receptor, and 1 C chemokine receptor identified thus far (Table I). Chemokine receptors are approximately 350 amino acids in length and have a short extracellular N-terminus and short intracellular C-terminus. The intracytoplasmic tails contain conserved serine and threonine residues that become phosphorylated on receptor occupancy. The 7-transmembrane domains are α-helical, and 3 intracellular and 3 extracellular loops exist between the transmembrane domains (Fig 1). A disulphide bridge between extracellular loops 1 and 2 confers rigidity to the receptor within the lipid bilayer, and the ligand binding domain is formed by a pocket generated between the N-terminal tail and the extracellular loop 3. Certain recep-

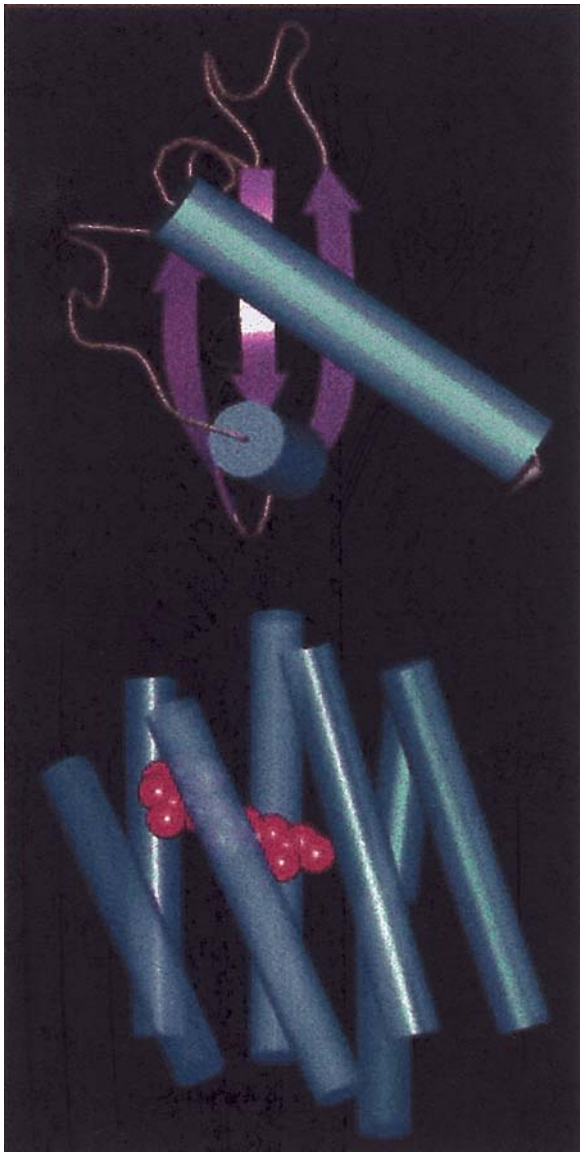

FIG 1. Three-dimensional structure of a generic chemokine receptor and its ligand.[16] A chemokine fold is shown with N- and C-terminal α helixes and internal β strands in magenta. The 7-transmembrane GPCR is shown beneath.

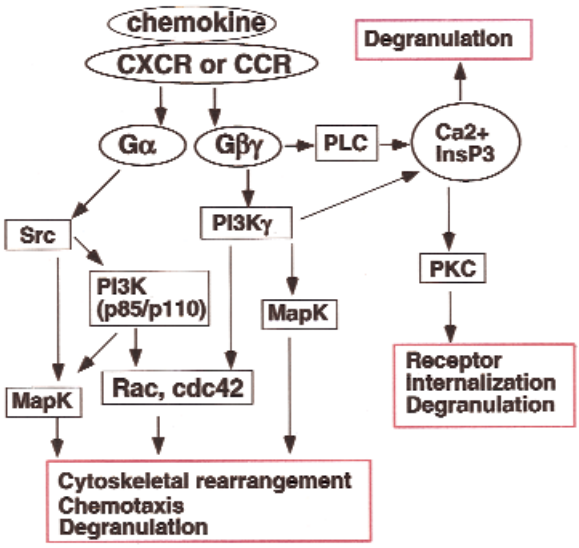

FIG 2. Signaling from chemokine receptors. After ligand binding, the heterotrimeric Gβγα proteins dissociate into Gα and Gβγ subunits. Gβγ can directly activate the PI3Kγ and PLC. The Gα subunit can induce Src family activation and the p85/p110 PI3K pathway. These cascades can induce various downstream events. There is some redundancy in the pathways used for specific downstream events.

tors, such as CCR1, will bind multiple chemokines (eg, CCL3, CCL5, CCL7, and CCL8), although this rarely occurs between chemokine families. One exception involves the Duffy antigen receptor, which can bind multiple chemokines from different families. Others, such as CXCR1, bind fewer molecules, such as CXCL8 and CXCL6. This relative specificity and the unique chemokine receptor expression patterns on leukocyte subsets account for much of the leukocyte subset trafficking observed in inflammatory lesions. These same characteristics (albeit complicated by chemokine interactions with multiple receptors and chemokine redundancy) are also what make the system an attractive one for targeted immune suppression.

This being said, it is also important to note that the chemokine receptor phenotype of inflammatory cells is known to fluctuate during their differentiation and exposure to external stimuli (both from secreted molecules and from cell-cell contact).[17,18] Thus chemokine receptor expression varies on mast cells, neutrophils, and eosinophils depending on their stage of differentiation and activation status.[19,20] Finally, it is also relevant to note that many other cell types (eg, epithelial cells, endothelial cells, and muscle cells) also express both chemokines and their receptors.[21-24] This makes the identification of the cell source or sources of chemokines at an inflammatory lesion complex. The expression of chemokine receptors on other cell types helps explain changes in gene expression that occur in these cell types during an inflammatory response and also suggests that chemokine-receptor antagonists might have broader anti-inflammatory properties than the blockade of leukocyte trafficking.

Because a focus of this review is the mast cell, we will pay most attention on the CCR1, CCR3, CCR4, CCR5, and CXCR2 receptors that have been identified on mast cells (Fig 2). These receptors confer on human primary mast cells the ability to respond to CXCL8, CCL11, stromal cell-derived factor 1, and CCL3, respectively.[25-28] Mast cells expressing these receptors are both able to chemotax to appropriate ligand gradients and to exhibit calcium fluxes on receptor engagement. In the case of CXCR2, there is evidence that $CXCR2^+$ mast cells might play an important role in protection from fungal airway infection.[29] A role of $CCR3^+$ mast cells in an inflammatory response comes from the analysis of $CCR3^{-/-}$ mice challenged with aerosolized ovalbumin.[30]

Signaling from chemokine receptors

Chemotaxis of inflammatory cells toward a chemokine gradient requires signals coming from the short intracytoplasmic tails of the chemokine GPCRs.[31,32] The conserved serine and threonine residues within these tails are important for this signaling because mutagenesis of these residues impairs receptor signaling. Studies of signaling from CCR1 indicate that phosphoinositide 3-kinase γ is critical for receptor signaling by using the protein kinase C pathway.[33] This is true for both leukocyte movement (although other isoforms-pathways might also be involved in cell movement) and costimulation during leukocyte activation-degranulation. Other changes that occur after receptor activation include classical calcium fluxes within the cell and the generation of both oxygen radicals and lipid mediators.[34] Signaling from the chemokine receptor also leads to dramatic changes in cell shape within seconds of receptor occupancy. Membrane ruffling is observed in scanning electron micrographs of stimulated cells, and actin polymerization and breakdown accounts for the extension and retraction of lamellipodia that propel the cell toward the chemokine gradient.[35,36] Chemokine signaling also induces the expression of multiple integrins on the surface of the leukocyte, allowing for the firm adherence of the leukocyte to the endothelial cell wall near a site of inflammation.[37] There is also integrin recycling at the leading edge of the migrating leukocyte.[38] This is followed by transendothelial migration of the cells and their accumulation within the mucosa tissue. Other events that are driven by chemokine receptor signaling include early differentiation of hematopoietic precursors and trafficking within the immune system.[39]

There is now a great deal known about the molecular events that mediate chemokine receptor signaling and downstream events.[40] In addition to cell movement, chemokine receptor signaling activates gene expression and can induce intracellular events, such as mast cell degranulation (Fig 3). Signaling from chemokine receptors is thought to involve a shift in a steady state equilibrium between an active and inactive conformational form of the receptor toward one predominated by the active form. This is postulated to initiate with receptor dimerization and activation of the JAK/signal transducer and activator of transcription (STAT) pathway mediated by a

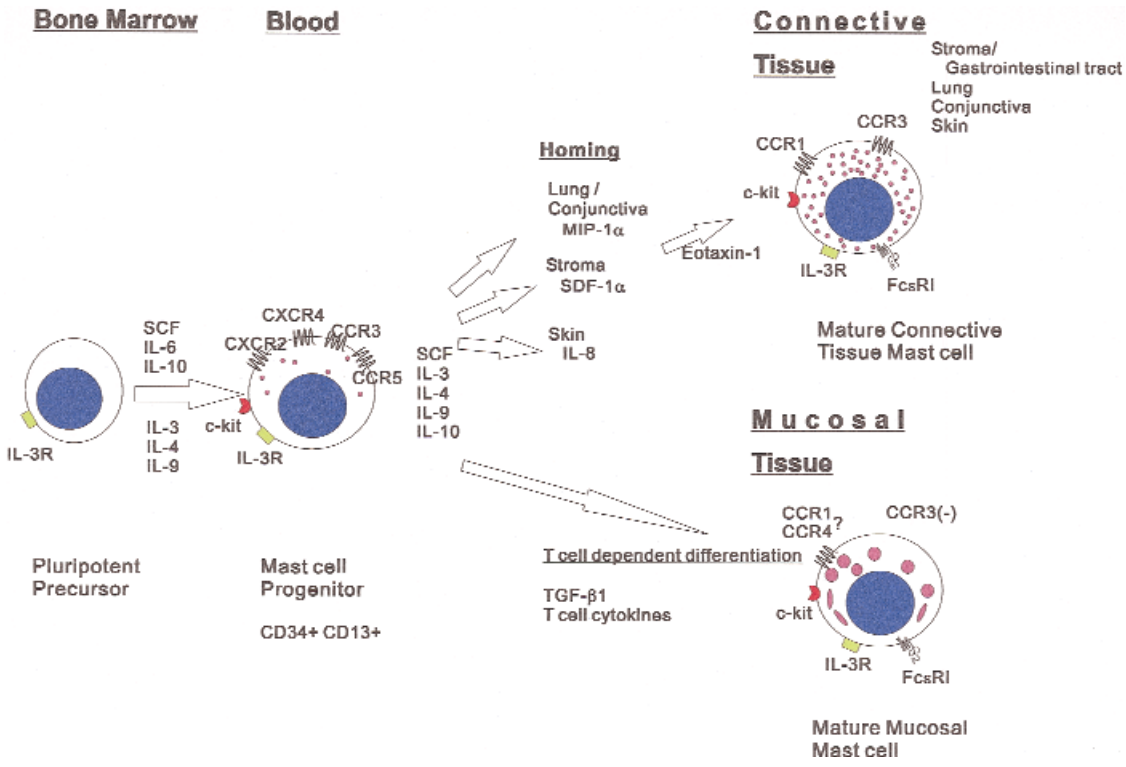

FIG 3. Chemokine receptor expression during mast cell differentiation and homing. Changes in chemokine receptor expression during mast cell development (pluripotent precursor to mast cell progenitor to mature mast cells) are shown, as well as key cytokines involved in driving the differentiation process. Mast cell heterogeneity is shown, with differences in chemokine receptor expression shown on mucosal and connective-type mast cells. Some of the chemokines essential for the homing of mast cells to specific tissues are also shown.

conserved DRY motif found in the intracytoplasmic tails of the several members of the CCR and CXCR receptors.[41] Receptor dimerization leads to phosphorylation of the conserved residues on the DRY motif and recruitment of JAK1 to the receptor (tyrosine 139 in CCR2). This phosphorylation event is not required for receptor homodimerization but is essential for Gαi protein coupling, calcium fluxes, and cell movement. STAT5b (in the case of CCL5 interaction with CCR5) is then recruited to the receptor and is in turn activated.[42,43] JAK2 is also recruited to CXCR4 dimerized after stromal cell–derived factor 1α addition, and multiple other STAT molecules (STAT1, STAT2, and STAT3) can be recruited to various other chemokine receptors after JAK recruitment.[44] These activated STAT molecules can then translocate into the nucleus of the chemokine-stimulated cell and directly activate (and sometimes repress) gene expression. Because the chemokine receptors are often coexpressed (eg, CCR3 and CCR1 on conjunctival mast cells), there is also a possibility that different chemokine receptors might heterodimerize on the surface of single- or multiple-ligand treated cells. This might explain the synergistic or unusual responses cells exhibit when treated with multiple chemokines. This might also explain how signaling from chemokine receptors might influence other immune receptors (eg, FcεRI or cytokine receptors).

Signaling from chemokine receptors is typically inhibited by pertussis toxin, suggesting that Gi proteins are key to the transduction of signals. Gαi physically associates with multiple chemokine receptors, but there is also evidence that other pertussis toxin–resistant Gi proteins, such as Gq or G16, might also associate with certain receptors. The coupled Gi protein might also differ in distinct cell types. Ligand binding results in the inhibition of adenylyl cyclase and release of intracellular calcium stores, as would be expected for Gαi-coupled GPCRs.[45] Although Gi-coupled chemokine receptors do not appear to be competent for cell movement, other downstream processes appear to be Gi dependent. The precise nature of G protein subunits required for different signaling events awaits further study. Activation of the receptor then results in the disassociation of Gαβγ bound to the receptor into Gβγ and GTP-bound Gαi. Janus kinase recruitment to the receptor and tyrosine phosphorylation of the receptor then recruits Gαi to at least one intracellular loop, triggering multiple signaling pathways.

Phospholipase C activation is triggered through Gβγ and yields inositol (1,4,5) triphosphate and diacylglycerol (Fig 3). Inositol triphosphate in turn mobilizes release of calcium from intracellular stores. This calcium, together with diacylglycerol, then activates multiple protein kinase C isoforms that ultimately drive down-

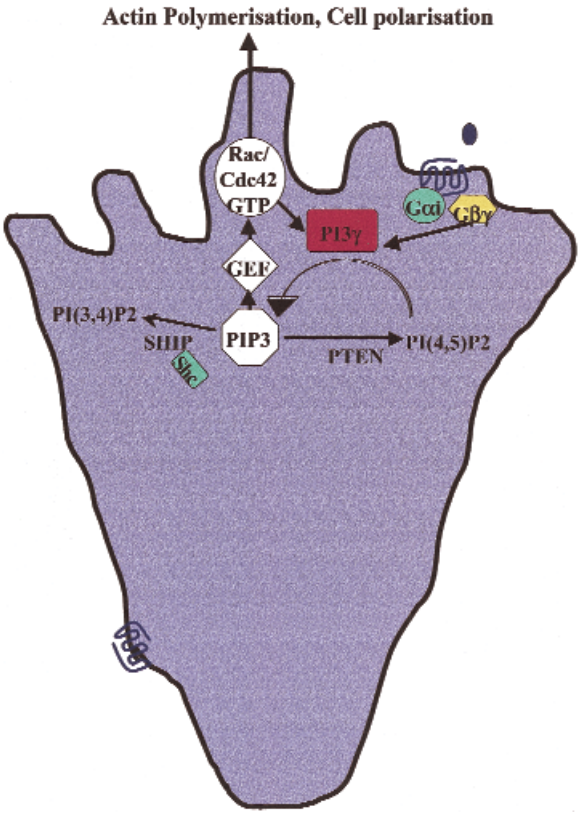

FIG 4. Molecular basis of chemokine-directed cell movement. A chemokine receptor–expressing cell responds to a chemokine gradient by establishing cell polarity. One feature of this is the accumulation of the PI3K product PIP3 at the leading edge of the migrating cell. PIP3 is essential for the activation of Rac/Cdc42. GEF catalyzes the conversion of inactive GDP-bound Rac/Cdc42 to the active GTP form. This leads to actin polymerization and chemotaxis. Two negative phosphatase regulators of PIP3 accumulation are SH domain-containing inositol phosphatase and PTEN, which generate PI (3,4) P2 and PI (4,5) P2, respectively.

stream events throughout the cell. Eventual receptor desensitization appears to operate through G protein–coupled kinases that associate with Gβγ.[46]

Phospholipase A2 and D can also be activated on chemokine receptor activation.[47] Arachidonic acid release driven by phospholipase A2 is important for optimal cell movement toward a chemokine gradient. The roles for activated phospholipase D are as of yet unclear.

Recent studies have clearly demonstrated a key role for phosphoinositol 3 kinase (PI3K) in chemokine receptor signaling.[48,49] Indeed, we find that CCR1-mediated signaling in the mast cell–like cell line RBL-2H3 is inhibited by wortmannin.[50] PI3K activity is rapidly enhanced on ligand binding and mediates p85 association with multiple CXCRs. This association in turn generates lipid mediators that are second messengers in the protein kinase C, AKT (also called PKB or Rac), and Ras pathways (Fig 4). As stated earlier, PI3Kγ is important in both cell movement and the degranulation response (as assessed with PI3Kγ-deficient mice). However, inhibition of cell movement in these mice is only partial, suggesting that other PI3K isoforms, other molecules (eg, the p85/p110 heterodimer), or both might also participate in cell migration. The clarification of the potential roles of PI3Kα and PI3Kδ in leukocyte migration and activation requires ongoing analysis in PI3Kα- and PI3Kδ-deficient mice.[51] This work also needs to be performed with animal models of allergic or autoimmune inflammation to recreate as closely possible what occurs in vivo in specific tissues.[52,53] Alternatively, existing dominant-negative forms of these molecules might be expressed in specific cells, and their ability to affect chemokine-directed movement can be assessed. This is, however, difficult because it is often not easy to generate stable cell lines expressing such mutant signaling molecules. In this case the respective roles of particular PI3K isoforms might be amenable to dissection by using specific small-molecule inhibitors. One other scenario that must be considered is that other molecules (aside from the p110 isoforms) might also play an important role in cell movement. For example, p125FAK or Pyk2 can bind to receptor-bound p85 and become activated on ligand binding.[54] This, in turn, can mediate the association of the chemokine receptor with cytoskeletal proteins, such as paxillin.[55]

Through one or more of these pathways, PI3K is clearly important for cell polarization and movement. Future work will need to dissect the relative contributions of PI3K-generated PIP3 (phosphatidylinositol 3,4,5-triphosphate) and other PI3K effectors, such as Rho, Rac, and Cdc42. All 4 of these intracellular messengers are known to mediate changes in the actin cytoskeleton and would be expected to participate in cell migration.[56] Finally, it should be noted that the mitogen-activated protein kinase cascade is also activated on chemokine binding to their receptors.[57] However, although this is likely involved in the activation of chemokine-dependent gene expression, it does not appear to have a major role in chemotaxis or chemokinesis.

The intracellular messengers generated from the activation of the PI3K pathway might also play roles in chemokine-mediated changes in leukocyte differentiation, activation, and effector function. The fine dissection of these signal transduction pathways will hopefully permit the design of highly specific small molecules with potential as new anti-inflammatory compounds.

CHEMOKINES IN LEUKOCYTE RECRUITMENT

The accumulation of immune cells at a site of allergic or autoimmune inflammation is a hallmark of these diseases. This typically occurs between 6 and 48 hours after allergen exposure or breakdown of immunologic tolerance.[58] The portfolio of cells that infiltrate the tissue-organ vary significantly between allergic and autoimmune diseases, between tissues (eg, in asthma versus conjunctivitis), and even within a single tissue (eg, within the conjunctiva, depending on the nature of allergen or sensitization scheme). Although several chemoattractants exist in nature

(as mentioned earlier), the chemokines play a key role in the recruitment of leukocytes to an inflammatory lesion. The profound effect of chemokine deficiency in specific leukocyte recruitment in certain animal models underscores the importance of chemokines in this process. The accumulation of these cells in late-phase allergic disease or in autoimmunity is highly relevant to the disease phenotype because these cells (often activated) contribute to the disease process, such as tissue (subepithelial) remodeling, goblet cell proliferation or hyperplasia, and airway hyperreactivity (in asthma).

Recruitment of monocytes-macrophages

Most of the mucosa tissues affected by an inflammatory response have pre-existing monocytes-macrophages. These cells are highly important for the progression of allergic-autoimmune diseases but might also play an essential role in initiation of disease. The mononuclear cells found in several tissues (and in peripheral blood) can express Fc receptors, both the low-affinity and high-affinity IgE receptors, and are also capable of presenting antigen. Indeed, these cells are able to express low levels of chemokines, which can be induced further on receptor cross-linking.[59] In type I hypersensitivity the production of chemokines by these cells and the subsequent binding of these chemokines to chemokine receptors on allergic effector cells (mast cells and eosinophils) has been hypothesized by us and others to constitute an obligatory costimulatory signal in the in vivo activation of these cells.[60,61] In autoimmunity the antigen-presenting capacity of these cells can clearly amplify the autoimmune response. They can take up and present initial and secondary tissue-specific antigens released at an inflammatory lesion (eg, in insulitis), thus amplifying and broadening the immune response. The phenotype of the mononuclear phagocytes operative at the early stages of disease is also key (based on the nature of cell-surface molecules and cytokines-chemokines expressed on the cells), helping to shape the nature of the ensuing immune response (their regulatory capacity). As reservoirs of mediators (eg, histamine, prostaglandins, cytokines, and chemokines), they can also directly contribute to tissue injury (eg, in β-cell death in type I diabetes).

Because the number of monocytes and macrophages can increase 3- to 100-fold at a site of inflammation, the aforementioned regulatory and effector roles of these cells become even more prominent after an inflammatory response has begun. The key chemokines that attract these cells to a site of inflammation include the following: CCL2, CCL3, CCL5, CCL7, CCL8, CCL13, CCL17, and CCL22.[62]

Recruitment of T lymphocytes

In both allergic inflammation and autoimmunity, one can frequently identify antigen-specific, pathogenic $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, or both in the inflammatory lesion. Although the number of T lymphocytes detected in a site of allergic inflammation are relatively small (when compared with esoinophils and neutrophils), they likely play an important role in type I hypersensitivity reactions. The evidence for a role for T lymphocytes in allergic disease is comparatively strong.[63] T subset–specific cytokines are detected in biopsy specimens and lavage fluid of inflamed tissues-organs and correlate with disease severity. There is also, perhaps not surprisingly, a rough correlation between the frequency of T cells detected in an inflammatory lesion and disease severity.[64] Depletion of pan-T cells or specific subsets with neutralizing antibodies or genetically deficient mice can abrogate the development of allergic disease in these animal models.[65] In human subjects there is a good correlation between the levels of T_H2 cytokines produced on T-cell activation and serum IgE levels and relative risk for asthma.[66] A similar situation exists in many autoimmune diseases. Once again, both $CD4^+$ and $CD8^+$ T lymphocytes are frequently detected in autoimmune lesions, and some of these cells exhibit antigen specificity and the ability to induce disease on transfer to naive hosts.[67] For these reasons, it is clearly important to understand how pathogenic T cells are recruited to a site of inflammation.

The accumulated evidence suggests that 4 chemokines, CCL2, CCL, CCL22, and CCL17, play a role in T-cell polarization, recruitment, or both into an inflammatory lesion.[68,69] The evidence for an important role for CCL2 comes from in vitro T-cell differentiation assays that indicate that CCL2 skews in vitro T-cell differentiation toward the T_H2 phenotype and studies with CCL2-deficient mice that exhibit a profound block in T_H2 responses.[70] CCL2-deficient mice show a clear deficit in T_H2 cells recruited to the allergen-challenged lung and have reduced airway hyperreactivity.

CCL11, CCL22, and CCL17 appear to selectively recruit T_H2 lymphocytes to sites of inflammation.[71] This chemotaxis stems from the expression of CCR3 and CCR4 on T_H2 lymphocytes.[72] CCR8 is also selectively expressed on T_H2 cells and might play a role in their recruitment into allergen-inflamed tissues through CCL17.[73] All 3 ligands are expressed from the epithelium of inflamed lung tissue, and immunohistochemical studies indicate that the T_H2^+ cells in the lung are either $CCR4^+$ or, to a lesser extent, $CCR8^+$. Neutralization of CCL22 or CCL17 in the murine model decreased the levels of T_H2 cytokines detected in bronchoalveolar lavage fluid, decreased airway hyperreactivity, and also resulted in a general decrease in the cellularity of the late-phase infiltrate.[74] Finally, adoptive transfer studies with allergen-specific T_H2 cells strongly suggest that CCL11 plays an important role in the initial stages of T_H2 cell recruitment into the inflammatory lesion.

Recruitment of mast cells

There has been comparatively little work performed investigating the chemokines involved in mast cell recruitment and activation during allergic inflammation (Fig 5). However, it is known that mast cells have the capacity to express the CCR1, CCR2(?), CCR3, CCR4, CCR5, CXCR2, and CXCR4 receptors.[75] In vivo studies

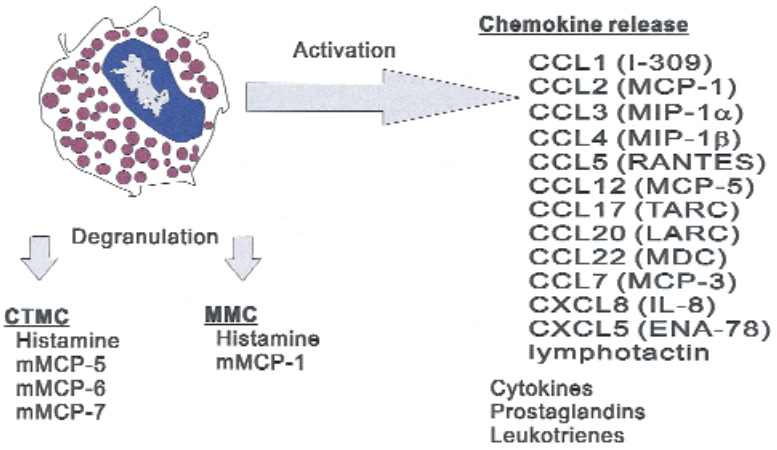

FIG 5. Chemokine production from activated mast cells. The production of different sets of mast cell proteases during degranulation are also shown. These protease profiles help define mast cell subtypes and have functional consequences caused by the chemoattractant capacities of the proteases.

with recombinant ligands indicate that CCL2 and perhaps CCL5 play an important role in mast cell recruitment and activation in the lung.[76] Because CCL2 binds to CCR2, it is not clear whether CCL2's effects are directly through CCR2 (CCR2 expression on mast cells is equivocal) or through another chemokine receptor. CCL5 might recruit mast cells to the inflammatory lesion through CCR1, CCR3, or CCR5. There is also evidence that CXCL8 might be inhibitory to mast cell recruitment and activation.[77] It is known that CXCL8 can inhibit chemokine-dependent histamine release from mast cells and basophils and that the levels of CXCL8 decrease in nasal secretions from patients undergoing allergic responses.[78] In the conjunctiva we have found with CCL11-deficient mice that the recruitment of mast cells in the late-phase reaction is partially impaired, suggesting that this chemokine plays a role in the recruitment of mast cells to the inflamed eye during an allergic response. Paradoxically, these same mice (although exhibiting partial reduction in eosinophil recruitment in the lung) showed significant increases in the recruitment of mast cells into the trachea and heightened airway hyperresponsiveness to methacoline.[79] These results might reflect an alternative pathway of mast cell (and eosinophil) recruitment in the chronic ovalbumin model (perhaps depending more on CXCR4) than CCR3/CCL11.

An important role for CCR3/CCL11 in mast cell homing is supported by data obtained from the analysis of CCR3-deficient mice.[80] Gerard et al found increased numbers of intraepithelial mast cells in the trachea of CCR3-deficient mice.[80] This increase in the numbers of mast cells was tissue specific, with no evidence for a similar increase in other tissues (eg, in the skin). Studies are in progress to probe the molecular basis of this tissue-specific accumulation of mast cells in CCR3-deficient mice.

Eosinophil recruitment

In allergic inflammation eosinophil recruitment from the bone marrow and circulation to the inflamed tissue is the most dramatic event associated with the late-phase reaction.[81] These activated eosinophils are also clearly pathogenic, releasing multiple mediators that contribute to chronic disease.[82] In human subjects 7 chemokines (CCL11, CCL24 and CCL26, CCL5, CCL7, CCL13, and CCL3) and the cytokine IL-5 have been implicated in the recruitment of eosinophils into various tissues.[83] GM-CSF can also stimulate the recruitment of eosinophils into tumor masses in vivo when transduced into a tumor during gene therapy.[84] Several of these chemokines can be detected in biologic fluids and homogenates obtained after allergen challenge. In each case a low level of the chemokine can be detected by means of ELISA in the uninflamed tissue, and this is increased during the first 4 to 6 hours after allergen challenge. Of the chemokines-cytokines mentioned, the strongest evidence for a direct role in eosinophil recruitment exists for CCL11, IL-5, CCL5, and CCL7.[85] In addition to increased levels in allergen-inflamed tissues, there is also considerable evidence with either neutralizing antibodies or in gene-deficient animals that these chemokines are important for eosinophil recruitment in the lung, skin, gut, and eye.[86] The often partial decrease in eosinophilia when one of these chemokines-cytokines (or their receptor) is targeted suggests that 2 or more of these molecules cooperate in eosinophil recruitment in vivo. For example, the data suggest that IL-5 plays an important role in eosinophil exit from the bone marrow; CCL11 plays an essential role in the initial recruitment of eosinophils to the lesion, and IL-5 (again) plays an important role for the maintenance of eosinophilia in chronic disease.

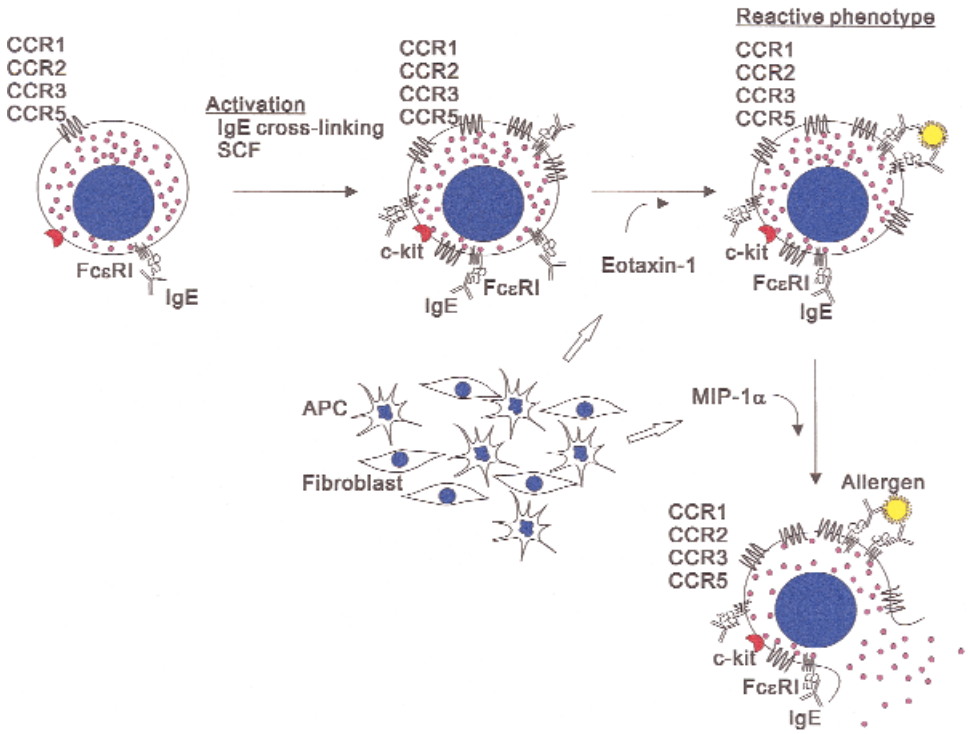

FIG 6. Chemokine receptor expression during acute-phase inflammation. Dynamic changes in chemokine receptor expression on mast cells during mast cell sensitization and activation are shown. Some key cytokines driving these changes, such as SCF and CCL11, are also shown. Chemokines are also produced from nearby cells, such as antigen-presenting cells, endothelial cells, and fibroblasts, and these likely have paracrine effects on mast cell expression of chemokine receptors, such as CCR3, CCR1, and CCR5.

Analysis of CCR3-deficient mice has shown clearly that CCR3 expression on eosinophils is essential for skin and lung eosinophilia.[87] The deficit in eosinophils observed in the lungs of these mice is also paralleled with a failure of airway hyperreactivity to develop to methacoline after allergen challenge.

Molecular basis of chemokine-directed cell movement

The previous sections of this article have discussed how signals are transduced from activated chemokine receptors and what chemokines are important for the recruitment of specific leukocyte subsets to an inflammatory lesion. A frontier in understanding cell movement involves the question of how cell polarity is acquired after receptor engagement and how membrane proteins (including chemokine receptors) are sorted to the leading edge of the migrating cell (Fig 6). This is key because cell motility requires that polarity is established, with chemokine receptors and integrins focused on the leading edge (and occasionally lagging edge) of the cell.[88] Because cell motility (and not signaling per se) is disrupted when cholesterol is depleted from the membranes of chemokine-treated cells, a working model is that chemokine receptors (along with other lipid raft-associated proteins), through associations with membrane raft microdomains, are sorted to what will become the leading edge of a cell.[89] This is thought to be achieved by association of chemokine receptors with glycosphingolipids and cholesterol-enriched raft precursors in the trans-Golgi network. These rafts are then delivered to the leading edge in carrier vesicles. Much more work needs to be done to understand how the chemokine receptor–containing rafts are properly positioned at the leading edge of the cell. This likely is linked to the rapid change in the distribution of F-actin that follows chemokine receptor activation. This change from a radial array to one of specific accumulation within the cell is thought to help propel the cell toward the source of chemokine. It will also be important to study whether there is heterogeneity in chemokine receptor representation within the leading edge (aside from that which can be explained by expression differences) and whether this model holds for different leukocyte subsets. It is particularly striking that although this process has received a good deal of attention with respect to T and B lymphocytes, natural killer cells, antigen-presenting cells, and neutrophils, very little has been done to understand mast cell chemotaxis at this level.[90-92]

CHEMOKINES IN MAST CELL DEVELOPMENT

Chemokine production from mast cells

As has been pointed out earlier, several cell types are capable of expressing chemokines constitutively or in response to receptor engagement. In this review we focus on the role of the chemokine system on mast cell growth

and effector function. Chemokine production from mast cells has been analyzed in view of the key role this cell plays in type I hypersensitivity reactions. This role stems from it being a source of both preformed and newly formed mediators of inflammation (eg, histamine, heparin, proteases, prostaglandin D2, and leukotriene C4) that both directly provoke clinical symptoms during the acute-phase reaction and potentiate the late-phase reaction.[93] Because mast cells were already known to express certain cytokine genes on allergen-mediated FcεRI cross-linking or stem cell factor (SCF)–driven mast cell differentiation, it was expected that mast cells might also express chemokines during development or on activation.[94]

Initial analyses focused on the effect of SCF or c-kit receptor ligand addition to bone marrow–derived mast cells (BMMCs).[95-97] SCF binds to the c-kit receptor and is essential for mast cell differentiation, survival, and activation. SCF stimulation of BMMCs releases the chemokines CCL22, CCL17, and CCL2. Murine mast cells activated through FcεRI expressed CCL3, CCL4, and CCL2. Each of these chemokines has the potential to both initiate and amplify inflammatory responses in multiple tissues. Concomitant engagement of the c-kit receptor and FcεRI resulted in cooperative activation of the genes encoding CCL2, CCL17, and CCL22.

A very recent exhaustive study of IgE-dependent gene activation in both BMMCs and human cord blood–derived mast cells confirms that the genes encoding CCL3, CCL2, and CCL4 are expressed at high levels in activated mast cells and found that CCL1 and the 4-1BB (CD137) costimulatory molecule are among the most highly expressed transcripts in human and mouse activated mast cells.[98]

Because chemokine expression from activated mast cells has the potential of exerting both an autocrine effect and a paracrine effect on nearby cells during an inflammatory response, it will be important to dissect the signal transduction pathways emanating from FcεRI and the c-kit receptor that result in induction of specific chemokine genes in mast cells and basophils.

Chemokine receptor expression on developing mast cells

It is now clear that there is considerable mast cell heterogeneity at the biochemical and functional levels in vivo.[99] This heterogeneity is introduced during mast cell differentiation and is most apparent when one analyzes terminally differentiated mast cells. The link between phenotypic diversity and functional differences comes from the analysis of mast cell products, such as proteases, TNF-α, and the eicosanoids. These markers differentiate mast cell subsets and are now known to directly contribute to mast cell effector function.

The example of mast cell proteases is quite well defined and is used here as an example of mast cell heterogeneity. In human subjects there are differences in the substrate specificities of tryptase α and βII tryptase, and mouse mast cell protease (mMCP) 6 and mMCP-7 have very different abilities to chemoattract leukocytes in vivo.[100-102] mMCP-6, but not mMCP-7, acts as a neutrophil chemoattractant in the mouse trachea, peritoneal cavity, and conjunctiva. mMCP-7, on the other hand, has a unique ability to cleave fibrinogen in vivo, presumably inhibiting excessive clot formation. Because these proteases are expressed on submucosal or dermal mast cells, they are lacking in intraepithelial mast cells. Thus mast cell heterogeneity defined at the level of protease expression clearly affects these parameters of mast cell effector function.

Mast cell heterogeneity also has consequences for innate immunity. One of the most dramatic demonstrations of the mast cell's role in innate immunity was the finding that mast cell–deficient (W/Wv strain) mice are highly susceptible to death from microbial insult after cecal ligation-puncture.[103] This was proved to be directly linked to the lack of mast cells because protection could be conferred by means of adoptive transfer of wild-type, but not TNF-α–deficient, mast cells. This has been interpreted to suggest that TNF-α, derived from the mast cell, acts as an important neutrophil chemoattractant, aiding in the clearance of microbial pathogens. Because SCF administration to TNF-α–deficient mice could confer partial protection from microbial insult, this is suggestive that other mast cell mediators (possibly mMCP-6) might substitute for TNF-α in the indirect recruitment of neutrophils through CXCL8 production from bystander cells. Thus the mMCP-6$^+$ subset of mast cells would participate in this facet of innate immunity. In contrast, mMCP-6$^-$ mast cells are important for protection from infection with *Trichinella spiralis* and *Nippostrongylus brasiliensis*.

The finding of clear phenotypic and functional differences between mast cell subsets underscores the importance of defining ligand receptors that drive the differentiation of mast cells either directly or through the activation of lineage commitment factors. The chemokine-receptor system is likely to play an important role in the generation of mast cell heterogeneity, defined both at the level of other gene products (eg, mast cell proteases) and the chemokines-receptors they express (Fig 2).

Studies have already begun on the expression of chemokines and chemokine receptors during mast cell differentiation.[104-113] These experiments make use of both BMMCs and human in vitro–derived mast cells, with cord blood or peripheral blood as starting material. Chemokine and chemokine receptor expression has been studied in both systems. In BMMCs transcripts for CCR1, CCR2, CCR3, CCR4, and CCR5 have been detected in SCF-treated cells. Direct analysis of murine mast cells isolated from various mouse strains indicates that transcripts for CCR1, CCR2, CCR3, and CCR6 are expressed on activation of mast cells (at least in certain strains). Clearly, more analysis in specific tissues and an extension to studies of chemokine receptor expression at the level of protein expression are required to obtain a clear picture of what chemokine receptors are expressed during mast cell differentiation and in fixed tissue mast cells.

In the human cord blood system, cells reminiscent of both progenitor mast cells and mature mast cells can be generated on culture with SCF, IL-6, and IL-10. The cells transit from a c-kit^{lo}/CD13^{+}/FcεRI^{lo}/IL-3Rα^{+}/integrin β3^{-} phenotype (at 4 weeks) to a c-kit^{hi}/CD13^{+}/FcεRI^{+}/integrin β3^{+} phenotype (at 8 weeks). The human progenitor mast cells (4 weeks' culture) express CXCR2, CCR3, CXCR4, and CCR5 and respond (calcium fluxes and in vitro migration assays) to the ligands CXCL8, CCL11, CCL12, and CCL3. Only CCR3 expression is maintained in mature human mast cells in this in vitro system. Because other chemokine receptors have been observed on mature human mast cells in situ (once again exhibiting heterogeneity between and within mucosal tissues), this diversity likely reflects the specific recruitment of mast cell subsets to particular tissues (perhaps dictated in part by the chemokine receptors they express), an effect of the specific microenviroment (both secreted and through cell-cell contact), or both on the eventual phenotype of the mast cell. This has been found to vary once again between mast cells analyzed at different anatomic sites and within different layers of the same tissue.

The finding that mast cell heterogeneity extends to the chemokine-receptor system and that this heterogeneity likely has both indirect (eg, during differentiation) and direct (eg, in mast cell activation) functional consequences indicates the importance of obtaining further information on the roles the chemokine-receptor system play in mast cell development and effector function.

CHEMOKINES IN LEUKOCYTE ACTIVATION

Acute-phase reaction

In a previous section we have discussed experiments that indicate that chemokine gene expression is induced in BMMCs on FcεRI or c-kit receptor engagement. Because these receptors are engaged during the acute-phase reaction in vivo, it is hypothesized that in vivo mast cells might also express chemokines during the acute-phase reaction. Although this appears reasonable, our own analysis of chemokine gene expression during the acute-phase reaction in the conjunctiva indicates that mast cells are not a primary source of allergen-induced chemokine during the acute phase. In situ hybridization analysis suggests that the primary source of allergen-induced CCL11 and CCL3 in the conjunctiva is in fact mononuclear cells.[114] In the skin early chemokine production has been mapped to elastase-positive neutrophils and BB-1^{+} basophils and at later stages to CD68^{+} macrophages.[115] There was in fact an inverse correlation between CCL3^{+} cells and tryptase-positive mast cells. In addition, the production of an acute wheal-and-flare reaction after intradermal injection of CCL11 and CCL24 has suggested that the chemokines (produced by other cells) might induce mast cell degranulation through CCR3.

Our own experiments with CCL11$^{-/-}$ and CCL3$^{-/-}$ mice are supportive of such a scenario. Although we do not see rapid induction of chemokine gene expression in mast cells during the acute-phase reaction, we do see rapid induction from mononuclear cells. These are likely to be analogous to the CD68^{+} macrophages observed in the skin. Our observation of a profound decrease in clinical symptoms during the acute phase in these chemokine-deficient mice and a parallel decrease in the mast cell degranulation index on specific allergen challenge supports the view that chemokines (produced from nearby cells) provide obligate costimulatory signals for mast cell activation in vivo. The cell source and nature of chemokines required for mast cell activation in the acute phase might vary depending on mucosa tissue (ie, skin vs conjunctiva), but at least in these 2 examples, the chemokine does not appear to work through a paracrine mechanism.

Chemokine receptor expression on mast cells during acute inflammation

Because there is evidence that both CCL3 and CCL11 might prime or costimulate IgE-dependent mast cell activation, it is also important to understand the state of chemokine receptor expression on mast cells during the acute-phase reaction. As has been stated earlier, mast cells have been shown to express CCR3 and, in certain circumstances, CCR1, CCR4, CCR5, and CXCR2.[115-120] Using BMMCs as a model, one group has reported that engagement of c-kit receptor, FcεRI, or both has been shown to increase the abundance of CCR1, CCR2, CCR3, and CCR5 transcripts. Such activated BMMCs were argued to likely express functional CC receptors on their cell surface because the activated cells exhibited specific chemotaxis to their ligands: CCR1 (CCL3), CCR2 (CCL2), CCR3 (CCL11), and CCR5 (CCL4). No chemotaxis was observed with CCL22 and CCL17 (CCR4). In contrast, a second group performing similar studies with BMMCs and cord blood–derived human mast cells failed to detect appreciable induction of any chemokine receptor (with the possible exception of CCR1 and CXCR3) in BMMCs and (CCR1, CCR6, and CXCR3) in human cord blood–derived mast cells. Clearly, additional work needs to be done to resolve whether mast cell differentiation or activation in vitro is accompanied by changes in chemokine receptor expression and whether this is relevant to the sensitivity of the mast cell to FcεRI-mediated activation. There is, of course, also a need to ask this question with respect to the intact mast cell during an inflammatory response.

Nevertheless, the observation of upregulation of certain chemokine receptors (with particular attention to CCR1) on SCF or IgE receptor engagement indicates that mast cells at the site of inflammation might also exhibit enhanced levels of chemokine receptor and thus become more susceptible to chemokine stimulation. This might play both an obligate initial role in mast cell activation and acute disease (as observed in the skin and eye) or might amplify or prolong inflammation after exposure to allergen. It is clearly important to carefully probe changes in the levels of chemokine receptor expression on mast cells during both the sensitization and acute phases of allergic disease.

CHEMOKINES AND CHEMOKINE RECEPTOR AS TARGETS IN THE TREATMENT OF ALLERGIC DISEASES

The specificity of chemokines and the tissue-specific expression of chemokine receptors on leukocytes render them as perhaps the leading targets for anti-inflammatory drug design. Their targeting also appears to be feasible because chemokine-deficient or chemokine receptor–deficient mice are generally healthy. This being said, there is still the possibility that individuals treated with chemokine receptor antagonists (of which there are already scores) or humanized neutralizing antibodies will experience unwanted side effects. The finding that CCR1$^{-/-}$ mice have a decreased resistance to *Toxoplasma gondii* infection is one example that we must consider carefully because of potential negative consequences of therapies directed at this system.[121] The other concern is that there might be a heterogeneous response both between species and within human subjects. Thus targeting of a particular chemokine receptor might exhibit no side effects in a murine model but might do so in a human model. Because CCR2 or CCR3 deficiency has been associated with increased susceptibility to airway hyperreactivity in mice, there is clearly reason to proceed with care, despite the attractiveness of the system.

One other clear problem is that many chemokines can act through several receptors. Thus blockade of one chemokine receptor might not substantially reduce leukocyte recruitment and inflammation if the inflammatory cell also expresses alternate receptors for the chemokine. Similarly, the frequent redundancy in chemokines expressed at a site of inflammation might hamper attempts to stem leukocyte recruitment through blockade of the chemokine. For example, blockade of CCL11 might be ineffective if CCL5 is also expressed at a site of inflammation. Despite these problems, there are sufficient positive data coming from in vivo tests of chemokine-receptor antagonists to indicate that the approach is likely to have merit in specific settings.

Chemokines

Although most companies are targeting the chemokine receptors, it is also clearly possible to target chemokines directly. The feasibility of this has been demonstrated by us and others in rodent models, in which neutralizing antibodies to CCL3 (as one example) have been able to inhibit type I hypersensitivity reactions in vivo.[122] Other reagents that target human chemokines have either been generated or are in the process of being developed, and these also hold promise for the therapy of inflammatory diseases. One example of such an antibody is CAT213, an antibody genetically engineered by Cambridge Antibody Technologies plc (Cambridge, United Kingdom), with specificity for CCL11.[123] It will be of interest to determine how this and other chemokine-directed antibodies perform in preclinical and clinical trials.

An alternative approach to target chemokines is at the level of their production. For example, it is known that treatment of rodents (systemically or locally) with immunostimulatory oligodeoxynucleotides (CpG-ODNs among other things) can inhibit the inducible expression of chemokine genes. This has also been observed when specific cell types (eg, antigen-presenting cells) are treated with CpG-ODNs in vitro. Indeed, we have found that CpG-ODN–treated mice have lost the capacity to express chemokines in the conjunctiva after specific allergen challenge and that these mice are protected from acute- and late-phase inflammation.[124]

Chemokine receptors

Clearly most of the effort in generating new drugs targeted at the chemokine-receptor system has been placed on the identification, synthesis, or both of chemokine receptor antagonists.[125,126] This effort has already yielded dozens of candidate inhibitors that are already in the public domain and in various phases of preclinical trials, human trials, or both. The relative specificity of chemokine receptors as targets (in comparison to second messengers, for example) has made the antagonism of the receptors a major priority in next-generation immunotherapeutics. The known and in-house compounds have tremendous specificity and affinity and in general have exhibited few adverse reactions.

There is already clear evidence that chemokine receptor antagonism through small-molecule inhibitors holds promise. CXCR2 antagonism profoundly affects neutrophil recruitment and might be beneficial for arthritis and chronic obstructive pulmonary disease.[127] CCR2 antagonism inhibits monocyte recruitment in vivo and might be useful in the treatment of atherosclerosis.[128] CCR1 antagonism (through multiple inhibitors; eg, BX471 [Berlex]) has potential indications in renal fibrosis and allergic inflammation.[129] CCR5 antagonists (interfering with macrophage development and granulocyte activation) might be useful in the treatment of multiple sclerosis and seasonal allergy.[130] CX3CR1 antagonism is expected to have an effect on monocyte effector function and might therefore have indications in transplantation and coronary artery disease.[131] Finally, CCR3 antagonism is hoped to be useful in the treatment of type I hypersensitivity reactions. Clear data on the effects of CCR3 antagonists in in vivo models of allergic disease are pivotal because there is reason to believe that such compounds can either ameliorate or exacerbate disease.[132-134]

CONCLUSIONS

Within the past decade, since the discovery of chemokines, there has been tremendous progress in our understanding of this remarkable superfamily of immune mediators. Although these products first attracted attention as chemoattractants for immune cells, they are now known to play key roles in immune cell differentiation and activation. The expression of chemokines also has roles in downstream events, such as tissue remodeling. Because of the clear role this superfamily plays in allergic and autoimmune inflammation (not to mention transplantation and viral infection), it is well recognized that

targeting the system has considerable potential with respect to new drug design. Several steps can be targeted in the design of such drugs: the chemokines themselves (both before and after synthesis), their receptors, chemokine signaling, and perhaps intracellular mechanisms of leukocyte movement. We are currently at the stage at which a large panel of antagonists directed at each of these steps has been generated and at which some promising preclinical data have emerged. In the next several years, data from clinical trials will also emerge, delivering key verdicts on whether and in what settings such new compounds will have therapeutic value.

REFERENCES

1. Von Herrath MG, Harrison LC. Regulatory lymphocytes: antigen-induced regulatory T cells in autoimmunity. Nat Rev Immunol 2003;3:223-32.
2. Hageman GS, Luthert PJ, Victor Chong NH, Johnson LV, Anderson DH, Mullins RF. An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. Prog Retin Eye Res 2001;20:705-32.
3. Davies DE, Wicks J, Powell RM, Puddicombe SM, Holgate ST. Airway remodeling in asthma: new insights. J Allergy Clin Immunol 2003;111:215-25
4. Flower RJ. The development of COX2 inhibitors. Nat Rev Drug Discovery 2003;2:179-91.
5. Schultz A, Stuck BA, Feuring M, Hormann K, Wehling M. Novel approaches in the treatment of allergic rhinitis. Curr Opin Allergy Clin Immunol 2003;3:21-7.
6. Furue M, Terao H, Rikihisa W, Urabe K, Kinukawa N, Nose Y, et al. Clinical dose and adverse effects of topical steroids in daily management of atopic dermatitis. Br J Dermatol 2003;148:128-33.
7. Wolpe SD, Cerami A. Macrophage inflammatory proteins 1 and 2: members of a novel superfamily of cytokines. FASEB J 1989;3:2565-73.
8. D'Ambrosio D, Panina-Bordignon P, Sinigaglia F. Chemokine receptors in inflammation: an overview. J Immunol Methods 2003;273:3-13.
9. Root AW, Root MJ. Clinical pharmacology of human growth hormone and its secretagogues. Curr Drug Targets Immune Endocr Metabol Disord 2002;2:27-52.
10. Wong MM, Fish EN. Chemokines: attractive mediators of the immune response. Semin Immunol 2003;15:5-14.
11. Campbell JD, Stinson MJ, Simons FE, HayGlass KT. Systemic chemokine and chemokine receptor responses are divergent in allergic versus non-allergic humans. Int Immunol 2002;14:1255-62.
12. Zimmermann N, Hershey GK, Foster PS, Rothenberg ME. Chemokines in asthma: cooperative interaction between chemokines and IL-13. J Allergy Clin Immunol 2003;111:227-42.
13. Borish LC, Steinke JW. Cytokines and chemokines. J Allergy Clin Immunol 2003;111(suppl 2):460-75.
14. Gordon S. Viral mimicry of cytokines, chemokines and their receptors. Nat Rev Immunol 2003;3:36-50.
15. Alexander JM, Nelson CA, van Berkel V, Lau EK, Studts JM, Brett TJ, et al. Structural basis of chemokine sequestration by a herpes virus decoy receptor. Cell 2002;111:343-56.
16. Janeway CA, Ytavers P, Walport M, Shlomchik M. Immunobiology 5: the immune system in health and disease. New York: Garland; 2002.
17. Langenkamp A, Nagata K, Murphy K, Wu L, Lanzavecchia A, Sallusto F. Kinetics and expression patterns of chemokine receptors in human CD4+ T lymphocytes primed by myeloid or plasmacytoid dendritic cells. Eur J Immunol 2003;33:474-82.
18. Price KS, Friend DS, Mellor EA, De Jesus N, Watts GF, Boyce JA. CC chemokine receptor 3 mobilizes to the surface of human mast cells and potentiates immunoglobulin E-dependent generation of interleukin 13. Am J Respir Cell Mol Biol 2003;28:420-7.
19. Lang K, Hatt H, Niggemann B, Zaenker KS, Entschladen F. A novel function for chemokines: downregulation of neutrophil migration. Scand J Immunol 2003;57:350-61.
20. Mariani M, Panina-Bordignon P. Analysis of homing receptor expression on infiltrating leukocytes in disease states. J Immunol Methods 2003;273:103-14.
21. Wuyts A, Struyf S, Gijsbers K, Schutyser E, Put W, Conings R, et al. The CXC chemokine GCP-2/CXCL6 is predominantly induced in mesenchymal cells by interleukin-1beta and is down-regulated by interferon-gamma: comparison with CXCL8/CXCL8. Lab Invest 2003;83:23-34.
22. Combadiere C, Potteaux S, Gao JL, Esposito B, Casanova S, Lee EJ, et al. Decreased atherosclerotic lesion formation in CX3CR1/apolipoprotein E double knockout mice. Circulation 2003;107:1009-16.
23. Mancardi S, Vecile E, Dusetti N, Calvo E, Stanta G, Burrone OR, et al. Evidence of CXC, CC and C chemokine production by lymphatic endothelial cells. Immunology 2003;108:523-30.
24. Savinov AY, Wong FS, Stonebraker AC, Chervonsky AV. Presentation of antigen by endothelial cells and chemoattraction are required for homing of insulin-specific CD8(+) T cells. J Exp Med 2003;197:643-56.
25. Coward WR, Okayama Y, Sagara H, Wilson SJ, Holgate ST, Church MK. NF-kappa B and TNF-alpha: a positive autocrine loop in human lung mast cells? J Immunol 2002;169:5287-93.
26. Woo CH, Jeong DT, Yoon SB, Kim KS, Chung IY, Saeki T, et al. Eotaxin induces migration of RBL-2H3 mast cells via a Rac-ERK-dependent pathway. Biochem Biophys Res Commun 2002;298:392-7.
27. Juremalm M, Hjertson M, Olsson N, Harvima I, Nilsson K, Nilsson G. The chemokine receptor CXCR4 is expressed within the mast cell lineage and its ligand stromal cell-derived factor-1alpha acts as a mast cell chemotaxin. Eur J Immunol 2000;30:3614-22.
28. Carollo M, Hogaboam CM, Kunkel SL, Delaney S, Christie MI, Perretti M. Analysis of the temporal expression of chemokines and chemokine receptors during experimental granulomatous inflammation: role and expression of MIP-1alpha and CCL2. Br J Pharmacol 2001;134:1166-79.
29. Schuh JM, Blease K, Hogaboam CM. CXCR2 is necessary for the development and persistence of chronic fungal asthma in mice. J Immunol 2002;168:1447-56.
30. Humbles AA, Lu B, Friend DS, Okinaga S, Lora J, Al-Garawi A, et al. The murine CCR3 receptor regulates both the role of eosinophils and mast cells in allergen-induced airway inflammation and hyperresponsiveness. Proc Natl Acad Sci U S A 2002;99:1479-84.
31. Youn BS, Yu KY, Alkhatib G, Kwon BS. The seventh transmembrane domain of cc chemokine receptor 5 is critical for MIP-1beta binding and receptor activation: role of MET 287. Biochem Biophys Res Commun 2001;281:627-33.
32. Han KH, Green SR, Tangirala RK, Tanaka S, Quehenberger O. Role of the first extracellular loop in the functional activation of CCR2. The first extracellular loop contains distinct domains necessary for both agonist binding and transmembrane signaling. J Biol Chem 1999;274:32055-62.
33. Fan J, Malik AB. Toll-like receptor-4 (TLR4) signaling augments chemokine-induced neutrophil migration by modulating cell surface expression of chemokine receptors. Nat Med 2003;9:315-21.
34. Nardelli B, Tiffany HL, Bong GW, Yourey PA, Morahan DK, Li Y, et al. Characterization of the signal transduction pathway activated in human monocytes and dendritic cells by MPIF-1, a specific ligand for CC chemokine receptor 1. J Immunol. 1999;162:435-44.
35. Lepidi H, Zaffran Y, Ansaldi JL, Mege JL, Capo C. Morphological polarization of human polymorphonuclear leucocytes in response to three different chemoattractants: an effector response independent of calcium rise and tyrosine kinases. J Cell Sci 1995;108(suppl):1771-8.
36. Tenscher K, Metzner B, Schopf E, Norgauer J, Czech W. Recombinant human eotaxin induces oxygen radical production, Ca(2+)-mobilization, actin reorganization, and CD11b upregulation in human eosinophils via a pertussis toxin-sensitive heterotrimeric guanine nucleotide-binding protein. Blood 1996;88:3195-9.
37. Shimizu Y. Signal transduction events regulating integrin function and T cell migration: new functions and complexity. Immunol Res 2003;27:107-28.
38. Lawson MA, Maxfield FR. Ca(2+)- and calcineurin-dependent recycling of an integrin to the front of migrating neutrophils. Nature 1995;377:75-9.
39. Vulcano M, Struyf S, Scapini P, Cassatella M, Bernasconi S, Bonecchi R, et al. Unique regulation of CCL18 production by maturing dendritic cells. J Immunol 2003;170:3843-9.
40. Proudfoot AE, Power CA, Rommel C, Wells TN. Strategies for chemokine antagonists as therapeutics. Semin Immunol 2003;15:57-65.
41. Rodriguez-Frade JM, Mellado M, Martinez-A C. Chemokine receptor dimerization: two are better than one. Trends Immunol 2001;22:612-7.

42. Monteclaro FS, Charo IF. The amino-terminal extracellular domain of the CCL2 receptor, but not the CCL5/MIP-1alpha receptor, confers chemokine selectivity. Evidence for a two-step mechanism for CCL2 receptor activation. J Biol Chem 1996;271:19084-92.
43. Schier AF. Chemokine signaling: rules of attraction. Curr Biol 2003;13:R192-4.
44. Rochdi MD, Parent JL. Galpha q-coupled receptor internalization specifically induced by Galpha q signaling: regulation by EBP50. J Biol Chem 2003;278:17827-37.
45. Preobrazhensky AA, Dragan S, Kawano T, Gavrilin MA, Gulina IV, Chakravarty L, et al. Monocyte chemotactic protein-1 receptor CCR2B is a glycoprotein that has tyrosine sulfation in a conserved extracellular N-terminal region. J Immunol 2000;165:5295-303.
46. Okkenhaug K, Vanhaesebroeck B. PI3K in lymphocyte development, differentiation and activation. Nat Rev Immunol 2003;3:317-30.
47. Fenard D, Lambeau G, Valentin E, Lefebvre JC, Lazdunski M, Doglio A. Secreted phospholipases A(2), a new class of HIV inhibitors that block virus entry into host cells. J Clin Invest 1999;104:611-8.
48. Suzuki Y, Rahman M, Mitsuya H. Diverse transcriptional response of CD4(+) T cells to stromal cell-derived factor (SDF)-1: cell survival promotion and priming effects of SDF-1 on CD4(+) T cells. J Immunol 2001;167:3064-73.
49. Curnock AP, Ward SG. Development and characterisation of tetracycline-regulated phosphoinositide 3-kinase mutants: assessing the role of multiple phosphoinositide 3-kinases in chemokine signaling. J Immunol Methods 2003;273:29-41.
50. Toda M, Nakamura T, Richardson RM, Ono SJ. Costimulation with MIP-1a and IgE antigen enhances degranulation but inhibits chemotaxis in RBL-2H3 cells expressing human CCR1 [abstract]. J Allergy Clin Immunol 2003;111:S117.
51. Fukao T, Yamada T, Tanabe M, Terauchi Y, Ota T, Takayama T, et al. Selective loss of gastrointestinal mast cells and impaired immunity in PI3K-deficient mice. Nat Immunol 2002;3:295-304.
52. Miyazaki D, Liu G, Clark L, Ono SJ. Prevention of acute allergic conjunctivitis and late-phase inflammation with immunostimulatory DNA sequences. Invest Ophthalmol Vis Sci 2000;41:3850-5.
53. Lahn M, Kanehiro A, Takeda K, Terry J, Hahn YS, Aydintug MK, et al. MHC class I-dependent Vgamma4+ pulmonary T cells regulate alpha beta T cell-independent airway responsiveness. Proc Natl Acad Sci U S A 2002;99:8850-5.
54. Maguire JE, Danahey KM, Burkly LC, van Seventer GA. T cell receptor- and beta 1 integrin-mediated signals synergize to induce tyrosine phosphorylation of focal adhesion kinase (pp125FAK) in human T cells. J Exp Med 1995;182:2079-90.
55. Sakai T, Li S, Docheva D, Grashoff C, Sakai K, Kostka G, et al. Integrin-linked kinase (ILK) is required for polarizing the epiblast, cell adhesion, and controlling actin accumulation. Genes Dev 2003;17:926-40.
56. Sachdev P, Zeng L, Wang LH. Distinct role of phosphatidylinositol 3-kinase and Rho family GTPases in Vav3-induced cell transformation, cell motility, and morphological changes. J Biol Chem 2002;277:17638-48.
57. Fernandis AZ, Cherla RP, Ganju RK. Differential regulation of CXCR4-mediated T-cell chemotaxis and mitogen-activated protein kinase activation by the membrane tyrosine phosphatase, CD45. J Biol Chem 2003;278:9536-43.
58. Solley GO, Gleich GJ, Jordon RE, Schroeter AL. The late phase of the immediate wheal and flare skin reaction. Its dependence upon IgE antibodies. J Clin Invest 1976;58:408-20.
59. Dombrowicz D, Quatannens B, Papin JP, Capron A, Capron M. Expression of a functional Fc epsilon RI on rat eosinophils and macrophages. J Immunol 2000;165:1266-71.
60. Ono SJ. Vernal keratoconjunctivitis: evidence for immunoglobulin E-dependent and immunoglobulin E-independent eosinophilia. Clin Exp Allergy 2003;33:279-81.
61. Sayama K, Diehn M, Matsuda K, Lunderius C, Tsai M, Tam SY, et al. Transcriptional response of human mast cells stimulated via the FceRI and identification of mast cells as a source of IL-11. BMC Immunol 2002;3:1-5.
62. Clemetson KJ, Clemetson JM, Proudfoot AE, Power CA, Baggiolini M, Wells TN. Functional expression of CCR1, CCR3, CCR4, and CXCR4 chemokine receptors on human platelets. Blood 2000;96:4046-54.
63. Larche M, Robinson DS, Kay AB. The role of T lymphocytes in the pathogenesis of asthma. J Allergy Clin Immunol 2003;111:450-63.
64. Lewis DB. Allergy immunotherapy and inhibition of Th2 immune responses: a sufficient strategy? Curr Opin Immunol 2002;14:644-51.
65. Chapoval SP, Marietta EV, Smart MK, David CS. Requirements for allergen-induced airway inflammation and hyperreactivity in CD4-deficient and CD4-sufficient HLA-DQ transgenic mice. J Allergy Clin Immunol 2001;168:245-50.
66. Brown V, Warke TJ, Shields MD, Ennis M. T cell cytokine profiles in childhood asthma. Thorax 2003;58:311-6.
67. Parfrey NA, Prud'homme GJ, Colle E, Fuks A, Seemayer TA, Guttmann RD, et al. Immunologic and genetic studies of diabetes in the BB rat. Crit Rev Immunol 1989;9:45-65.
68. Bonecchi R, Bianchi G, Bordignon PP, D'Ambrosio D, Lang R, Borsatti A, et al. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J Exp Med 1998;187:129-34.
69. Lloyd CM, Delaney T, Nguyen T, Tian J, Martinez-A C, Coyle AJ, et al. CC chemokine receptor (CCR)3/eotaxin is followed by CCR4/monocyte-derived chemokine in mediating pulmonary T helper lymphocyte type 2 recruitment after serial antigen challenge in vivo. J Exp Med 2000;191:265-74.
70. Kim Y, Sung SS, Kuziel WA, Feldman S, Fu SM, Rose CE Jr. Enhanced airway Th2 response after allergen challenge in mice deficient in CC chemokine receptor-2 (CCR2). J Immunol 2001;166:5183-92.
71. Sallusto F, Mackay CR, Lanzavecchia A. Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. Science 1997;277:2005-7.
72. Zingoni A, Soto H, Hedrick JA, Stoppacciaro A, Storlazzi CT, Sinigaglia F, et al. The chemokine receptor CCR8 is preferentially expressed in Th2 but not Th1 cells. J Immunol 1998;161:547-51.
73. Hirata H, Arima M, Cheng G, Honda K, Fukushima F, Yoshida N, et al. Production of CCL17 and CCL22 by naive T cells in asthmatic patients. J Clin Immunol 2003;23:34-45.
74. Kawasaki S, Takizawa H, Yoneyama H, Nakayama T, Fujisawa R, Izumizaki M, et al. Intervention of thymus and activation-regulated chemokine attenuates the development of allergic airway inflammation and hyperresponsiveness in mice. J Immunol 2001;166:2055-62.
75. Ochi H, Hirani WM, Yuan Q, Friend DS, Austen KF, Boyce JA. T helper cell type 2 cytokine-mediated comitogenic responses and CCR3 expression during differentiation of human mast cells in vitro. J Exp Med 1999;190:267-80.
76. Trautmann A, Toksoy A, Engelhardt E, Brocker EB, Gillitzer R. Mast cell involvement in normal human skin wound healing: expression of monocyte chemoattractant protein-1 is correlated with recruitment of mast cells which synthesize interleukin-4 in vivo. J Pathol 2000;190:100-6.
77. Lippert U, Artuc M, Grutzkau A, Moller A, Kenderessy-Szabo A, Schadendorf D, et al. Expression and functional activity of the CXCL8 receptor type CXCR1 and CXCR2 on human mast cells. J Immunol 1998;161:2600-8.
78. Ohkubo K, Ikeda M, Pawankar R, Gotoh M, Yagi T, Okuda M. Mechanisms of IL-6, CXCL8, and GM-CSF release in nasal secretions of allergic patients after nasal challenge. Rhinology 1998;36:156-61.
79. Hogan SP, Mishra A, Brandt EB, Foster PS, Rothenberg ME. A critical role for eotaxin in experimental oral antigen-induced eosinophilic gastrointestinal allergy. Proc Natl Acad Sci U S A 2000;97:6681-6.
80. Humbles AA, Lu B, Friend DS, Okinaga S, Lora J, Al-Garawi A, et al. The murine CCR3 receptor regulates both the role of eosinophils and mast cells in allergen-induced airway inflammation and hyperresponsiveness. Proc Natl Acad Sci U S A 2002;99:1479-84.
81. Rothenberg ME. Eosinophilia. N Engl J Med 1998;338:1592-600.
82. Gleich GJ. Mechanisms of eosinophil-associated inflammation. J Allergy Clin Immunol 2000;105:651-63.
83. Rot A, Krieger M, Brunner T, Bischoff SC, Schall TJ, Dahinden CA. CCL5 and macrophage inflammatory protein 1 alpha induce the migration and activation of normal human eosinophil granulocytes. J Exp Med 1992;176:1489-95.
84. Hung K, Hayashi R, Lafond-Walker A, Lowenstein C, Pardoll D, Levitsky H. The central role of CD4(+) T cells in the antitumor immune response. J Exp Med 1998;188:2357-68.
85. Bertrand CP, Ponath PD. CCR3 blockade as a new therapy for asthma. Expert Opin Investig Drugs 2000;9:43-52.
86. Makino Y, Cook DN, Smithies O, Hwang OY, Neilson EG, Turka LA, et al. Impaired T cell function in CCL5-deficient mice. Clin Immunol 2002;102:302-9.
87. Ma W, Bryce PJ, Humbles AA, Laouini D, Yalcindag A, Alenius H, et al. CCR3 is essential for skin eosinophilia and airway hyperresponsiveness in a murine model of allergic skin inflammation. J Clin Invest 2002;109:621-8.
88. Pinco KA, He W, Yang JT. alpha4beta1 integrin regulates lamellipodia protrusion via a focal complex/focal adhesion-independent mechanism. Mol Biol Cell 2002;13:3203-17.

89. Gomez-Mouton C, Abad JL, Mira E, Lacalle RA, Gallardo E, Jimenez-Baranda S, et al. Segregation of leading-edge and uropod components into specific lipid rafts during T cell polarization. Proc Natl Acad Sci U S A 2001;98:9642-7.
90. Salazar-Mather TP, Lewis CA, Biron CA. Type I interferons regulate inflammatory cell trafficking and macrophage inflammatory protein 1alpha delivery to the liver. J Clin Invest 2002;110:321-30.
91. Levesque JP, Hendy J, Takamatsu Y, Simmons PJ, Bendall LJ. Disruption of the CXCR4/CXCL12 chemotactic interaction during hematopoietic stem cell mobilization induced by GCSF or cyclophosphamide. J Clin Invest 2003;111:187-96.
92. Shapiro SD. Immunology: mobilizing the army. Nature 2003;421:223-4.
93. Kawakami T, Galli SJ. Regulation of mast-cell and basophil function and survival by IgE. Nat Rev Immunol 2002;2:773-86.
94. Sayama K, Diehn M, Matsuda K, Lunderius C, Tsai M, Tam SY, et al. Transcriptional response of human mast cells stimulated via the FcεRI and identification of mast cells as a source of IL-11. BMC Immunol 2002;3:5.
95. Liu ZJ, Haleem-Smith H, Chen H, Metzger H. Unexpected signals in a system subject to kinetic proofreading. Proc Natl Acad Sci U S A 2001;98:7289-94.
96. Oliveira SH, Lukacs NW. Stem cell factor and IgE-stimulated murine mast cells produce chemokines (CCL2, CCL17, CCL22) and express chemokine receptors. Inflamm Res 2001;50:168-74.
97. Yano K, Yamaguchi M, de Mora F, Lantz CS, Butterfield JH, Costa JJ, et al. Production of macrophage inflammatory protein-1alpha by human mast cells: increased anti-IgE-dependent secretion after IgE-dependent enhancement of mast cell IgE-binding ability. Lab Invest 1997;77:185-93.
98. Nakajima T, Inagaki N, Tanaka H, Tanaka A, Yoshikawa M, Tamari M, et al. Marked increase in CC chemokine gene expression in both human and mouse mast cell transcriptomes following Fcepsilon receptor I cross-linking: an interspecies comparison. Blood 2002;100:3861-8.
99. Kitamura Y. Heterogeneity of mast cells and phenotypic change between subpopulations. Annu Rev Immunol 1989;7:59-76.
100. Huang C, Friend DS, Qiu WT, Wong GW, Morales G, Hunt J, et al. Induction of a selective and persistent extravasation of neutrophils into the peritoneal cavity by tryptase mouse mast cell protease 6. J Immunol 1998;160:1910-9.
101. Huang C, Wong GW, Ghildyal N, Gurish MF, Sali A, Matsumoto R, et al. The tryptase, mouse mast cell protease 7, exhibits anticoagulant activity in vivo and in vitro due to its ability to degrade fibrinogen in the presence of the diverse array of protease inhibitors in plasma. J Biol Chem 1997;272:31885-93.
102. Ghildyal N, Friend DS, Stevens RL, Austen KF, Huang C, Penrose JF, et al. Fate of two mast cell tryptases in V3 mastocytosis and normal BALB/c mice undergoing passive systemic anaphylaxis: prolonged retention of exocytosed mMCP-6 in connective tissues, and rapid accumulation of enzymatically active mMCP-7 in the blood. J Exp Med 1996;184:1061-73.
103. Echtenacher B, Mannel DN, Hultner L. Critical protective role of mast cells in a model of acute septic peritonitis. Nature 1996;381:75-7.
104. Oliveira SH, Lukacs NW. Stem cell factor and IgE-stimulated murine mast cells produce chemokines (CCL2, CCL17, CCL22) and express chemokine receptors. Inflamm Res 2001;50:168-74.
105. Price KS, Friend DS, Mellor EA, De Jesus N, Watts GF, Boyce JA. CC chemokine receptor 3 mobilizes to the surface of human mast cells and potentiates immunoglobulin E-dependent generation of interleukin 13. Am J Respir Cell Mol Biol 2003;28:420-7.
106. Inamura H, Kurosawa M, Okano A, Kayaba H, Majima M. Expression of the CXCL8 receptors CXCR1 and CXCR2 on cord-blood-derived cultured human mast cells. Int Arch Allergy Immunol 2002;128:142-50.
107. Gurish MF, Humbles A, Tao H, Finkelstein S, Boyce JA, Gerard C, et al. CCR3 is required for tissue eosinophilia and larval cytotoxicity after infection with *Trichinella spiralis*. J Immunol 2002;168:5730-6.
108. Cosmi L, Annunziato F, Maggi E, Romagnani S, Manetti R. Chemoattractant receptors expressed on type 2 T cells and their role in disease. Int Arch Allergy Immunol 2001;125:273-9.
109. Marone G, de Paulis A, Florio G, Petraroli A, Rossi FW, Triggiani M. Are mast cells MASTers in HIV-1 infection? Int Arch Allergy Immunol 2001;125:89-95.
110. Li Y, Li L, Wadley R, Reddel SW, Qi JC, Archis C, et al. Mast cells/basophils in the peripheral blood of allergic individuals who are HIV-1 susceptible due to their surface expression of CD4 and the chemokine receptors CCR3, CCR5, and CXCR4. Blood 2001;97:3484-90.
111. de Paulis A, De Palma R, Di Gioia L, Carfora M, Prevete N, Tosi G, et al. Tat protein is an HIV-1-encoded beta-chemokine homolog that promotes migration and up-regulates CCR3 expression on human Fc epsilon RI+ cells. J Immunol 2000;165:7171-9.
112. Romagnani P, De Paulis A, Beltrame C, Annunziato F, Dente V, Maggi E, et al. Tryptase-chymase double-positive human mast cells express the eotaxin receptor CCR3 and are attracted by CCR3-binding chemokines. Am J Pathol 1999;155:1195-204.
113. Beil WJ, Schulz M, Wefelmeyer U. Mast cell granule composition and tissue location—a close correlation. Histol Histopathol 2000;15:937-46.
114. Miyazaki D, Keane-Myers A, Cheung-Chau K-W, Chau KY, Ono SJ. The roles of chemokines in the development of murine allergic conjunctivitis. Invest Ophthalmol Vis Sci 1999;40:S687.
115. Menzies-Gow A, Ying S, Sabroe I, Stubbs VL, Soler D, Williams TJ, et al. Eotaxin (CCL11) and eotaxin-2 (CCL24) induce recruitment of eosinophils, basophils, neutrophils, and macrophages as well as features of early- and late-phase allergic reactions following cutaneous injection in human atopic and nonatopic volunteers. J Immunol 2002;169:2712-8.
116. Ying, S, Meng Q, Barata LT, Kay AB. Macrophage inflammatory protein-1alpha and C-C chemokine receptor-1 in allergen-induced skin late-phase reactions: relationship to macrophages, neutrophils, basophils, eosinophils and T lymphocytes. Clin Exp Allergy 2001;31:1724-31.
117. Wakahara S, Fujii Y, Nakao T, et al. Gene expression profiles for Fc epsilon RI, cytokines and chemokines upon Fc epsilon RI activation in human cultured mast cells derived from peripheral blood. Cytokine 2001;16:143-52.
118. Juremalm M, Olsson N, Nilsson G. Selective CCL5/CCL5-induced mast cell migration through interactions with chemokine receptors CCR1 and CCR4. Biochem Biophys Res Commun 2001;50:168-74.
119. Bayce JA. Mast cells: beyond IgE. J Allergy Clin Immunol 2003;111:24-32.
120. Ono SJ. Vernal keratoconjunctivitis: evidence for immunoglobulin E-dependent and immunoglobulin E-independent eosinophilia. Clin Exp Allergy 2003;33:279-81.
121. Khan IA, Murphy PM, Casciotti L, Schwartzman JD, Collins J, Gao JL, et al. Mice lacking the chemokine receptor CCR1 show increased susceptibility to *Toxoplasma gondii* infection. J Immunol 2001;166:1930-7.
122. Osbourn JK, Earnshaw JC, Johnson KS, Parmentier M, Timmermans V, McCafferty J. Directed selection of MIP-1 alpha neutralizing CCR5 antibodies from a phage display human antibody library. Nat Biotechnol 1998;16:778-81.
123. Handy RLC, May RD, Main S, Vaughan TJ, Anderson IK. In vitro and in vivo effects of CAT-213, a human anti-eotaxin monoclonal antibody. Proc Int Symp Ocul Immunol Inflamm Transplant 2002:18
124. Miyazaki D, Liu G, Clark L, Ono SJ. Prevention of acute allergic conjunctivitis and late-phase inflammation with immunostimulatory DNA sequences. Invest Ophthalmol Vis Sci 2000;41:3850-5.
125. Agrawal L, Alkhatib G, Agrawal L. The therapeutic potential of CXCR4 antagonists in the treatment of HIV. Expert Opin Investig Drugs 2003;12:185-95.
126. Alcami A. Viral mimicry of cytokines, chemokines and their receptors. Nat Rev Immunol 2003;3:36-50.
127. Barnes PJ. Potential novel therapies for chronic obstructive pulmonary disease. Novartis Found Symp 2001;234:255-72.
128. Guo J, Van Eck M, Twisk J, Maeda N, Benson GM, Groot PH, et al. Transplantation of monocyte CC-chemokine receptor 2-deficient bone marrow into ApoE3-Leiden mice inhibits atherogenesis. Arterioscler Thromb Vasc Biol 2003;23:447-53.
129. Anders HJ, Vielhauer V, Frink M, Linde Y, Cohen CD, Blattner SM, et al. A chemokine receptor CCR-1 antagonist reduces renal fibrosis after unilateral ureter ligation. J Clin Invest 2002;109:251-9.
130. Balashov KE, Rottman JB, Weiner HL, Hancock WW. CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1alpha and IP-10 are expressed in demyelinating brain lesions. Proc Natl Acad Sci U S A 1999;96:6873-8.
131. Lesnik P, Haskell CA, Charo IF. Decreased atherosclerosis in CX3CR1-/- mice reveals a role for fractalkine in atherogenesis. J Clin Invest 2003;111:333-40.
132. Toda M, Ono SJ. Genomics and proteomics of allergic disease. Immunology 2002;106:1-10.
133. Ono SJ. Molecular genetics of allergic diseases. Annu Rev Immunol 2000;18:347-66.
134. Senechal S, Fahy O, Gentina T, Vorng H, Capron M, Walls AF, et al. CCR3-blocking antibody inhibits allergen-induced eosinophil recruitment in human skin xenografts from allergic patients. Lab Invest 2002;82:929-39.