

Molecular mechanisms in allergy and clinical immunology

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Chemokines and allergic disease

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Understanding the chemokine network has become one of the great challenges for researchers interested in inflammatory mechanisms and inflammation-based diseases. The complexity and diversity of the system provide not only a daunting task for its comprehension but also numerous opportunities for development of new, targeted therapies. It is now certain that chemokines are involved as important mediators of allergic inflammation; the fine details and scope of their roles are now under investigation. Presumably, because of distinct pressures on the immune systems of people living in different geographic regions, genetic variation of ligands, receptors, and regulatory regions in the network have emerged. Establishing the roles of these polymorphisms in determining disease susceptibility or progression among individuals and in distinct ethnic groups will provide a basis for improved understanding and treatment of allergic diseases. (*J Allergy Clin Immunol* 1999;104:723-42.)

Key words: Allergic disease, angiogenesis, chemokine, chemotaxis, cytokines, eosinophil, glucocorticoids, hematopoieses, mediators, transendothelial migration

A tremendous body of information has recently accumulated regarding the role of chemokines and their receptors in a variety of homeostatic and disease processes. Chemokines have been implicated in such diverse conditions as atherosclerosis, AIDS, and asthma. Although chemokines were initially appreciated to be chemotactic cytokines, it is now apparent that their spectrum of activities is broad and encompasses effects on tissue morphogenesis, proliferation, and apoptosis of a variety of cell types, recruitment and activation of inflammatory cells, and angiogenesis. There are now more than 40 chemokines, and more than 20 distinct receptors have been identified. Diverse chemokines and receptors can direct the traffic of distinct cell types in the immune system (eg, dendritic cells, T_H1 and T_H2 cells, eosinophils, neutrophils, etc) to distinct anatomic locations (eg, subregions of lymph nodes, skin, gut mucosa, etc). The purpose of this review is to discuss chemokine biology in general terms as well as in the specific context

Abbreviations used

AP-1:	Activator protein-1
AURE:	Adenosyl uridyl-rich element
BAL:	Bronchoalveolar lavage
BDP:	Beclomethasone dipropionate
CREB:	Cyclic AMP-responsive element binding protein
DARC:	Duffy antigen/receptor for chemokines
EBI-1:	EBV-induced molecule
ELC:	EBI-1 ligand chemokine (MIP-3)
ENA-78:	Epithelial-derived neutrophil-activating peptide
FIC:	Fibroblast-induced chemokine (MCP-3)
GCP:	Granulocyte chemotactic protein
GR:	Glucocorticoid receptor
GRE:	Glucocorticoid response element
GRO:	Growth-regulated oncogene
HCC:	Hemofiltrate CC chemokine
ICAM:	Intercellular adhesion molecule
IP-10:	IFN- γ -inducible protein-10
I-TAC:	IFN-stimulated T cell α chemoattractant
LARC:	Liver and activation-regulated chemokine (MIP-3)
LFA-1:	Leukocyte function associated antigen-1
LPTN=LTN:	Lymphotactin
MCP:	Monocyte chemoattractant protein
MDC:	Macrophage-derived chemokine
MIG:	Monokine induced by IFN- γ
MIP:	Macrophage inflammatory protein
mRNA:	Messenger RNA
m:	Murine
NAP:	Neutrophil-activating peptide
NF- κ B:	Nuclear factor- κ B
PF4:	Platelet factor 4
PPBP:	Proplatelet basic protein
RV:	Rhinovirus
SCM:	Single c motif
SDF-1:	Stromal cell-derived factor
STAT:	Signal transducer and activator of transcription
TARC:	Thymus and activation-regulated chemokine
TCA1-3:	T-cell activation gene
β -TG:	β -Thromboglobulin
3' UTR:	3' Untranslated region
VCAM:	Vascular cell adhesion molecule
VLA-4:	Very late antigen

of allergic diseases. Special attention will be paid to the role of genetic variability in chemokine genes and how this may influence the susceptibility to allergic disease among individuals of different ethnic groups.

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CHEMOKINE SUBFAMILIES

Chemokines are primarily 8- to 15-kd proteins that can be subdivided into 4 structural branches or subfamilies on the basis of the number and appearance of conserved cysteine residues in the primary sequence: CC, CXC, C, and CX3C. A simple schematic of representatives of the chemokine subfamilies is shown in Fig 1. The first 2 subfamilies (CXC [or α] and CC [or β]) are characterized by the presence or absence of an intervening amino acid between the first 2 of 4 conserved cysteines.¹ The C (or γ) subfamily includes lymphotactin (LPTN=LTN) and single c motif-1 (SCM-1), which have only 2 conserved cysteines.² The CX3C chemokine subfamily has only 1 member, fractalkine (also known as neurotactin), which possesses a transmembrane domain that is joined to the chemokine-like domain by a long mucin-rich region.³ A comprehensive list of the members of each subfamily, the receptors that they interact with, cell sources, target cells, and biologic effects is provided in Table I. It can be seen in Table I that the members of the CC family include the majority of the chemokines that stimulate movement of cells associated with the allergic response (including eosinophils, basophils, T cells, and monocytes). For this reason, subsequent sections will focus primarily on the roles of CC chemokines and their receptors. For reference, gene symbols and chromosomal locations for chemokines and their receptors are provided in Table II.

CHEMOKINE RECEPTORS

Receptors for chemokines belong to the 7 transmembrane-spanning receptor family. Most (but not all) of these receptors are G protein-coupled receptors. Biochemical studies indicate that agonist binding to chemokine receptors can result in activation of phospholipases A2 and C, phosphatidylinositol-3-kinase, protein kinase C, signal transducer and activator of transcription (STAT) proteins, calcium flux, and cytoskeletal remodeling by a G protein-dependent process.⁴⁻⁹ Chemokine receptors can also activate members of the tyrosine kinase family. Activation of different chemokine receptors in the same cell type may produce distinct signals, however; for example, CCR2 engagement by monocyte chemoattractant protein (MCP)-1 can cause histamine release in basophils, whereas CXCR engagement by IL-8 inhibits basophil degranulation. Also associated with ligand binding is receptor internalization and, in some cases, recycling.⁵⁻⁷ Particular interest has been directed to the role of receptor internalization in the infection of susceptible cells by HIV, a process that can be mediated by CCR5, CXCR4, or other chemokine receptors in a virus strain-dependent manner. With regard to the CCR3 receptor, an important receptor in the movement of eosinophils, basophils, and possibly T_H2 cells, binding of eotaxin induces a profound and sustained receptor internalization by a Gi- and PLC-independent mechanism.⁷ There is considerable interest in the Duffy antigen/receptor for chemokines (DARC), which binds both CC and

CXC chemokines. Chemokine binding initiates rapid internalization but no signal transduction.¹⁰ A similar pattern has been observed for CCR9 (also known as CCR10 and D6).¹¹ Such promiscuous but nontriggering chemokine receptors may dampen chemokine-mediated inflammation (see below). Generation of chemokine receptor knockout animals has been instructive. For example, mice deficient in MCP-1 or its receptor, CCR2, have impairments in host defense, hematopoiesis, T_H1/T_H2 balance, monocyte-macrophage recruitment, and formation of atherosclerotic lesions.¹²⁻¹⁴ Although several other receptors or ligands have been knocked out, published information on animals deficient in receptors likely to be important in T_H1 and T_H2 development or eosinophil recruitment (eg, CXCR3, CCR4, or CCR3) is lacking.

CHROMOSOMAL ORGANIZATION OF CHEMOKINE AND CHEMOKINE RECEPTOR-ENCODING GENES

Genes encoding chemokines and their receptors most likely evolved through successive gene duplication or recombination events from a common ancestral gene, which is reflected by their high degree of DNA and amino acid sequence homology. CC chemokines (gene symbol *SCYA* for small inducible cytokine subfamily A) are clustered on chromosome 17q11.2-q23 (see Table II); exceptions are the genes encoding thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC), which are clustered with fractalkine (gene symbol *SCYD1*) on chromosome 16q13¹⁵ and eotaxin 2, which is located on chromosome 7.¹⁶ These chemokines have less homology with those located on chromosome 17. MDC and TARC bind to the same receptor (CCR4) and share similar functions (see below). The presence of distinct chromosomal locations may represent early evolutionary translocation events. It appears that evolutionarily more distant chemokine members may have unique functions.

The genes encoding CXC chemokines (gene symbol *SCYB* for small inducible cytokine subfamily B) are clustered on chromosome 4q13-q21.¹⁷ An exception is stromal cell-derived factor-1 (SDF-1), the most divergent member of the CXC chemokine family; it has been mapped to chromosome 10q11.2.¹⁸ IL-8, growth-regulated oncogene- (GRO) α , GRO- β , GRO- γ , and proplatelet basic protein (PPBP) map within a region spanning 700 kb.¹⁹ The gene encoding lymphotactin/SCM-1a (*SCYC1*) maps to human chromosome 12²⁰; the second member of the C or γ chemokine family, SCM-1b (*SCYC2*), has not yet been mapped. Fractalkine (*SCYD1*—also known as neurotactin) has been mapped to chromosome 16q13, clustered with the CC chemokine genes TARC and MDC.^{15,21} Most CC and CXC chemokine genes are composed of 2 introns and 3 exons (one exception is hemofiltrate CC chemokine [HCC]-2, which has 4 exons and 3 introns). The 3' untranslated regions (3'-UTR) of several chemokine messenger RNAs (mRNA) contain potential mRNA-destabi-

lizing adenosyl uridyl-rich elements (AUREs) (eg, eotaxin, MCP-2, liver and activation-regulated chemokine [macrophage inflammatory protein-3, LARC], IL-8).

Most of the genes encoding the CC chemokine receptors (gene symbol *CMKBR* 1-9 commonly known as CCR1-9) have been mapped to chromosome 3p21.3-p24 (Table II). CCR1, 2, 3, and 5 are clustered within a total distance of 350 kb on chromosome 3p21.3, whereas CCR4 maps more distally on 3p24.^{22,23} CCR7 was mapped to chromosome 17q12-q21. CCR9 (which is identical to CCR10 and hD6) has recently been identified and mapped to 3q21.3.²⁴ The coding region (open reading frame) of the majority of chemokine receptor genes (including DARC) resides on one single exon,²⁵ with the exception of CCR7 (EBV-induced molecule [EBI-1]), which contains 2 introns interrupting the coding region of the domain.²⁶ The DARC encoding gene (*FY*) maps to chromosome 1q22-q23. The CXC receptor genes (*CXCR1-4*) mostly map to chromosome 2q (Table II).

FUNCTIONAL ACTIVITIES OF CHEMOKINES

Chemotaxis

Chemokines have been implicated in both inflammatory and homeostatic leukocyte migration. Much of our information about the leukocyte specificity and magnitude of the chemotactic effect comes from studies with purified human leukocytes or cell lines studied in the Boyden microchemotaxis chamber. With regard to chemotactic activity, the CXC subfamily can be further subdivided into proteins containing the amino acid motif E-L-R (Glu-Leu-Arg) in the amino terminus (eg, see IL-8 in Fig 1) and those that lack this motif (IFN- γ -inducible protein-10 [IP-10], monokine induced by IFN- γ [MIG], platelet factor-4 [PF-4], and SDF-1). Generally speaking, the E-L-R-containing CXC chemokines are chemoattractants for neutrophils but not monocytes, whereas those lacking this motif do not act on neutrophils but attract mononuclear cells. In contrast, CC chemokines generally attract monocytes and lymphocytes but not neutrophils. Several CC members have been shown to induce eosinophil migration, including eotaxin-1, eotaxin-2, MCP-2, MCP-3, MCP-4, RANTES, MDC, and MIP-1 α ; many of these same chemokines act on basophils as well.²⁷⁻³² The most potent in vitro eosinophil chemoattractants are MCP-3, MCP-4, RANTES, eotaxin-1, and eotaxin-2.^{28,33} Although many of these chemokines have been shown to bind to several chemokine receptors (see Table I), neutralizing antibody experiments have demonstrated that CCR3 is the receptor that mediates the majority of the eosinophil chemotactic effect and is the only receptor for eotaxin-1 and eotaxin-2 identified to date.^{29,33,34} Although eosinophil migration in response to eotaxin-1, eotaxin-2, MCP-4, and RANTES is mediated by CCR3, eosinophil migration in response to MDC appears to involve a receptor other than CCR3 or CCR4.²⁷ The selectivity of the CC subfamily for T cells, monocytes, eosinophils, and basophils has led to numerous studies of their roles in allergic inflammation.

Several members of the C, CC, and CXC chemokine

subfamilies induce T-cell migration. The chemokine receptors expressed on naive T cells have been described and include CXCR4, the receptor for SDF-1, and CCR7, the receptor for EBI-1 ligand chemokine (ELC).³⁵⁻³⁷ This finding, coupled with the fact that SDF-1 is expressed in a variety of tissues, suggests that these receptors may be important in basal lymphocyte trafficking. Because dendritic cells also apparently use CCR7 for migration to lymph nodes, this receptor/ligand pair may aid in colocalizing naive lymphocytes and antigen-presenting cells.^{38,39} Some chemokines display selectivity for activated or memory T cells.⁴⁰⁻⁴³ Polarization of T cells into T_H1 and T_H2 is characterized by differences in chemokine receptor expression. For example, subsets of T_H2 cells have been shown to selectively express CCR3 and CCR4, the latter being a receptor for TARC and MDC.⁴⁴⁻⁴⁶ Andrew et al⁴⁷ have shown that ligands for CCR4 (MDC and TARC) and CCR3 (eotaxin-1 and eotaxin-2) selectively attract T_H2 cells. Furthermore, MDC, TARC, eotaxin-1, and eotaxin-2 are highly expressed in nasal polyp tissues⁴⁸ (Greaves et al, personal communication), possibly explaining the large numbers of T_H2 cells in these tissues. Taken together with the evidence that CCR3 plays a role in basophil and eosinophil recruitment, these observations reinforce the proposal that CCR3 and CCR4 are important chemokine receptors in allergic disease.^{49,50} T_H1 cells, in contrast, express higher levels of CXCR3 than do T_H2 cells, which corresponds with enhanced chemotaxis to the relevant ligands (IP-10, MIG, and IFN-inducible T-cell α chemoattractant [I-TAC]).^{51,52} CCR1 and CCR5 have also been reported to be markers of T_H1 cells.⁵¹⁻⁵³ There is now circumstantial evidence that T_H1 and T_H2 cells may recruit additional polarized T cells by amplifying loops involving selective chemokine induction by IFN- γ and IL-4 (Fig 2).

Transendothelial migration

Early in vitro studies by Huber et al⁵⁴ suggested that IL-8 was necessary for neutrophil transendothelial migration induced by IL-1. Ebisawa et al⁵⁵ demonstrated that RANTES, but not IL-8, induced eosinophil transendothelial migration. This migration could be inhibited by antibodies against the β 2 integrin, CD18. Eosinophil priming with IL-5 resulted in a synergistic increase in transendothelial migration in response to RANTES. Cell trafficking in vivo is thought to involve several steps, including leukocyte rolling, firm adhesion, and transendothelial migration. Rolling is largely mediated by selectins and their carbohydrate counterligands. Rolling leukocytes are able, by virtue of their slower velocity, to sample the environment for chemotactic gradients and adhesion molecules. Firm adhesion is mediated primarily by the interaction of β 1 integrins (very late antigen [VLA-4]) or the β 2 integrins (leukocyte function-associated antigen-1 [LFA-1], Mac-1, and p150,95) on leukocytes with members of the Ig supergene family expressed on the surface of endothelial cells. Several studies have demonstrated that endothelial cells also present chemokines on their luminal surface and thereby

TABLE I. Human chemokines: biologic features

Chemokine	Receptor	Cell sources	Target cells	Biologic effects
CXC (α) family				
BCA-1	CXCR-5	Secondary lymphoid organs	B lymphocyte	Chemotaxis
β -TG	ND	Platelet Monocyte-macrophage	Neutrophil Fibroblast Chondrocyte	Chemotaxis, activation Chemotaxis, proliferation, activation Increased glycosaminoglycan synthesis
CTAP-III	ND	Platelet Monocyte-macrophage	Neutrophil Fibroblast Chondrocyte	Chemotaxis, activation Chemotaxis, proliferation, activation Increased glycosaminoglycan synthesis
ENA-78	CXCR-2	Epithelial cell line A549 Platelet Hepatocyte	Basophil Neutrophil	Modulation of histamine release Chemotaxis
GCP-2	CXCR-1	Osteosarcoma cell line MG63	Neutrophil	Chemotaxis, activation
GRO- α , β , γ	CXCR-2 CXCR-2	Fibroblast Monocyte-macrophage Fibroblast Endothelial cell Synovial cell Hepatocyte Keratinocyte Epithelial cell Neutrophil T lymphocyte	Neutrophil Basophil Fibroblast Melanoma cell Stem cell Endothelial cell T lymphocyte	Chemotaxis, activation Chemotaxis, activation Growth factor Growth factor Suppression of colony formation of immature myeloid precursors (GRO- β , γ) Inh of angiogenesis (GRO- β , γ) Chemotaxis
IL-8	CXCR-1 CXCR-2	Monocyte-macrophage T lymphocytes B lymphocyte/B-CLL Eosinophil Epithelial cell Endothelial cell Fibroblast Hepatocyte Keratinocyte Melanoma cell Mesothelial cell Smooth muscle cell	Neutrophil Basophil T lymphocyte B lymphocyte/B-CLL Endothelial cell Keratinocyte Smooth muscle cell Fibroblast Bone marrow progenitor cell	Chemotaxis, activation Chemotaxis, inh of histamine release Chemotaxis, inh of IL-4 synthesis Chemotaxis, inh of growth and IgE production, prolonged survival of B-CLL Promotion of angiogenesis Chemotaxis, expression of HLA-DR Chemotaxis Chemotaxis Increased survival, suppression of myeloid colony formation
IP-10	CXCR-3	Monocyte-macrophage T lymphocyte Endothelial cell Fibroblast Keratinocyte	Act T lymphocyte Monocyte NK cell CD34 ⁺ bone marrow cell Endothelial cell	Chemotaxis Chemotaxis Chemotaxis, activation Inh of hemopoiesis Inh of angiogenesis
I-TAC	CXCR-3	Astrocyte Epithelial cell Keratinocyte Monocyte Neutrophil	Act T lymphocyte NK cell T _H 1	Chemotaxis Chemotaxis Chemotaxis
MIG	CXCR-3	Monocyte-macrophage NK cell CD34 ⁺ bone marrow cell Endothelial cell	Act T lymphocyte Chemotaxis Inh of hemopoiesis	Chemotaxis
NAP-2	CXCR-1 CXCR-2	Platelet	Inh of angiogenesis Neutrophil Bone marrow precursor Megakaryocyte	Chemotaxis, activation Increased survival Inh of megakaryocytopoiesis
PF-4	ND	Platelet Megakaryocyte T lymphocyte	Neutrophil Monocyte Fibroblast Basophil Endothelial cell Megakaryocyte	Chemotaxis, activation Chemotaxis Chemotaxis Modulation of histamine release Inh of angiogenesis Inh of megakaryocytopoiesis

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TABLE I. CONT

Chemokine	Receptor	Cell sources	Target cells	Biologic effects
PBP	ND	Platelet lymphocyte	Bone marrow precursor	Increased survival
SDF-1	CXCR-4	Numerous tissues	T lymphocyte	Precursor of CTAP-III and NAP-2
			B lymphocyte	Chemotaxis, inh of lymphocyte-trophic HIV cell entry, adh to endothelial ICAM-1
			CD34 ⁺ hematopoietic progenitor cell	Proliferation, chemotaxis
CC (β) family				
AMAC	ND	Activated macrophage	Naive T cell	Chemotaxis
Ckβ8	ND	Aortic endothelium	Monocyte	Chemotaxis
		Monocyte	Resting T lymphocyte	Chemotaxis
		Liver	Myeloid progenitor	Inh of proliferation
		Gastrointestinal tract		
6-C-kine	ND	Lymphoid organs	Thymocyte	Chemotaxis
			Act T lymphocyte	Chemotaxis, adh to endothelial ICAM-1
			Bone marrow progenitor	Inh of hematopoiesis
DC-CK1	ND	Dendritic cell	Naive T lymphocyte (CD45RA ⁺)	Chemotaxis
Eotaxin	CCR-3	Epithelial cell	Eosinophil	Chemotaxis
		Endothelial cell	Basophil	Chemotaxis, activation
		Eosinophil	TH2	Chemotaxis
		Fibroblast		
		Macrophage		
		Monocytic cell line U937		
Eotaxin-2	CCR-3	Act monocyte	Eosinophil	Chemotaxis
		Basophil	Chemotaxis, activation	
		Resting T lymphocyte	Chemotaxis	
		Myeloid progenitor	Inh of proliferation	
HCC-1	ND	Several normal tissues	Monocyte	Ca ⁺⁺ mobilization, enzyme release
		CD34 ⁺ myeloid progenitor	Enhanced proliferation	
HCC-2	CCR-1	Liver	Monocyte	Chemotaxis
CCR-3	Intestine	T lymphocyte	Chemotaxis	
	Lung	Eosinophil	Chemotaxis	
I-309	CCR-8	Monocyte	Monocyte	Chemotaxis
		Act T lymphocyte	T lymphocyte	Antiapoptotic
		Mast cell		Inh of infection by several HIV strains
MCP-1	CCR-2	Monocyte	Monocyte	Chemotaxis
	CCR-10	Eosinophil	Basophil	Activation
		Epithelial cell	T lymphocyte	Chemotaxis, inh of cytokine production (TH1 cell), differentiation of CD4 ⁺ from TH0 to TH1 phenotype
		Endothelial cell		
		Fibroblast		
		Mast cell	NK cell	Chemotaxis, activation
		Mesothelial cell	Dendritic cell	Chemotaxis
		Smooth muscle cell		
MCP-2	CCR-1	Osteosarcoma cell	Monocyte	Chemotaxis
	CCR-2	Fibroblast	T lymphocyte	Chemotaxis, inh of CCR-5-mediated HIV entry
			Eosinophil	Chemotaxis, activation
			Basophil	Chemotaxis, activation
		NK cell	Chemotaxis, activation	
		Dendritic cell	Chemotaxis	
MCP-3	CCR-1	Monocyte	Monocyte	Chemotaxis
	CCR-2	Osteosarcoma cell	T lymphocyte	Chemotaxis
	CCR-3	Epithelial cell	Eosinophil	Chemotaxis
	CCR-10		Basophil	Chemotaxis, activation
			NK cell	Chemotaxis, activation
			Dendritic cell	Chemotaxis
MCP-4	CCR-2	Epithelial cell	Eosinophil	Chemotaxis, activation
	CCR-3	Endothelial cell	Monocyte	Chemotaxis

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TABLE I. CONT

Chemokine	Receptor	Cell sources	Target cells	Biologic effects
MDC	CCR-4	Monocytic cell line U937	T lymphocyte	Chemotaxis
			Basophil	Chemotaxis, activation
		Macrophage	Monocyte-derived dendritic cell	Chemotaxis
		Monocyte-derived dendritic cell	NK cell	Chemotaxis
		Thymus	Monocyte	Chemotaxis
MIP-1 α	CCR-1 CCR-5	Lung	Eosinophil	Chemotaxis, shape change
		Spleen	TH2	Chemotaxis
		Mononuclear cell	T lymphocyte	Chemotaxis, differentiation of CD4+ lymphocyte from TH0 to TH1 phenotype
		Endothelial cell	Monocyte-macrophage	Chemotaxis, inh of CCR-5-mediated HIV cell entry
		Mast cell line HMC-1		Increased IgE/IgG4 production
MIP-1 β	CCR-5 CCR-8	Smooth muscle cell		Chemotaxis, activation
			B lymphocyte	Activation
			NK cell	Chemotaxis
			Basophil	Chemotaxis
			Dendritic cell	Modulation of proliferation
MIP-3 α	CCR-6		Hematopoietic progenitor cell	
		Mononuclear cell	T lymphocyte	Chemotaxis, inh of CCR-5-mediated HIV cell entry
		Endothelial cell	NK cell	Chemotaxis, activation
		Mast cell line HMC-1	Dendritic cell	Chemotaxis
			Megakaryocyte	Inh of colony formation
MIP-3 β	CCR-7		B lymphocyte	Increased IgE/IgG4 production
		Lymphocyte	Mononuclear cell	Chemotaxis
		Monocyte	T lymphocyte	Chemotaxis
		Liver	Dendritic cell	Chemotaxis
		Myeloid progenitor	Inh of proliferation	
MIP-5	CCR-1	Lymphoid tissue	Act T lymphocyte	Chemotaxis, adh to endothelial ICAM-1
		Act B lymphocyte	Chemotaxis	
		Liver	Monocyte	Chemotaxis
		Small intestine	Chemotaxis	
		Lung leukocyte	Chemotaxis	
PARC	ND	Lung	T lymphocyte	Chemotaxis
		Lymphoid tissue		
		Monocytic cell line		
		T lymphocyte	Eosinophil	Chemotaxis
		Monocyte	T lymphocyte	Chemotaxis
RANTES	CCR-1 CCR-3 CCR-5	Platelet	Monocyte	Chemotaxis, inh of CCR-5-mediated HIV cell fusion
		Eosinophil		
		Epithelial cell	Basophil	Chemotaxis
		Endothelial cell	NK cell	Chemotaxis, activation
		Dendritic cell	B lymphocyte	Increased IgE/IgG4 production
SLC	ND	Mast cell line HMC-1	Dendritic cell	Chemotaxis
		Smooth muscle cell		
		Lymphoid tissue	T lymphocyte	Chemotaxis
		Activated macrophage	Act T lymphocyte	Chemotaxis
		Lymphoid tissue		
TARC	CCR-4 CCR-8	Lymphoid tissue	T lymphocyte	Chemotaxis
		Mononuclear cell	TH2	Chemotaxis
		Thymic dendritic cell	Activated macrophage	Chemotaxis
		Small intestine	Dendritic cell	Chemotaxis
		Liver	Thymocyte	Chemotaxis
TECK	ND			
C (γ) family	Lymphotactin XCR1	CD8+ T lymphocyte	Lymphocyte	Chemotaxis
		$\gamma\delta$ Epithelial T lymphocyte	Act NK cell	Chemotaxis, activation
		Thymocyte		
		NK cell		
		Mast cell line HMC-1		

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TABLE I. CONT

Chemokine	Receptor	Cell sources	Target cells	Biologic effects
SCM-1	ND	Mononuclear cell Jurkat T cell line Spleen	Lymphocyte	Chemotaxis, activation
CX3C family				
Fractalkine	CX3CR1	Endothelial cell	Monocyte	Chemotaxis, adh to endothelium-bound fractalkine
			T lymphocyte	Chemotaxis, adh to endothelium-bound fractalkine

BCA-1, B cell-chemokine; *ND*, not determined; *CTAP*, connective tissue-activating peptide; *inh*, inhibition; *B-CLL*, B cell chronic lymphocytic leukemia; *Act*, activated; *NK*, natural killer; *PBP*, platelet basic protein; *adh*, adhesion; *AMAC*, alternative macrophage activation-associated CC chemokine; *Ckβ8*, chemokine b8; *6-C-kine*, 6-cysteine chemokine (exodus-2); *DC-CK1*, dendritic cell chemokine; *HCC*, hemofiltrate CC chemokine; *PARC*, pulmonary and activation-regulated chemokine; *SLC*, secondary lymphoid tissue chemokine; *STCP*, stimulated T-cell chemotactic protein; *TECK*, thymus-expressed chemokine.

TABLE II. Nomenclature and chromosomal locations for human chemokines and their receptors

Gene symbol	Chemokine	Synonym	Location
CC chemokines			
<i>SCYA1</i>	I-309 (human)	TCA-3 (mouse)	17q11.2
<i>SCYA2</i>	MCP-1	MCAF, human JE	17q11.2
<i>SCYA3</i>	MIP-1α	LD-78a	17q21.1-21.3
<i>SCYA3L</i>	LD-78b		17q21
<i>SCYA4</i>	MIP-1β	Act-2, AT744.1	17q21-q23
<i>SCYA5</i>	RANTES		17q11.2
<i>SCYA6</i> (mouse only)	C-10	MRP-1	
<i>SCYA7</i>	MCP-3		17q11.2
<i>SCYA8</i>	MCP-2		17q11.2
<i>SCYA9</i> = <i>SCYA10</i> (mouse only)	MRP-2	CCF-18, MIP-1γ	
<i>SCYA11</i>	Eotaxin		17q21.1-21.2
<i>SCYA12</i>	MCP-5		Not mapped
<i>SCYA13</i>	MCP-4	NCC-1	17q11.2
<i>SCYA14</i>	NCC-2	HCC-1	17q11.2
<i>SCYA15</i>	NCC-3	HCC-2, MIP-5, MIP-1δ Leukotactin (LKN1)	17q11.2
<i>SCYA16</i>	NCC-4	LEC	17q11.2
<i>SCYA17</i>	TARC		16q13
<i>SCYA18</i>	PARC	DCCK-1, AMAC-1, MIP-4	17q11.2
<i>SCYA19</i>	ELC-1	MIP-3β, exodus-3	9p13
<i>SCYA20</i>	LARC	MIP-3α, exodus-1	2q27-q33
<i>SCYA21</i>	SLC	6-C-kine, exodus-2	9p13
<i>SCYA22</i>	MDC		16q13
<i>SCYA23</i> (mouse only)			
<i>SCYA24</i>	Eotaxin-2	MPIF-2	7q11.23
<i>SCYA25</i>	TECK		19p13.2
CXC, C, and CX3C chemokines			
<i>SCYB1</i>	GRO-α oncogene, MGSA	GRO-1	4q12-q13
<i>SCYB2</i>	GRO-β, MIP-2A	GRO-2	4q12-q13
<i>SCYB3</i>	GRO-γ, MIP-2B	GRO-3	4q12-q13
<i>SCYB4</i>	Platelet factor 4	PF4	4q12-q13
<i>SCYB4V1</i>	PF4-like	PF4L	4q12-q13
<i>SCYB5*</i>	ENA-78		4q13-q21
<i>SCYB6*</i>	GCP2		4q12-q21
<i>SCYB7</i>	TGB/THBGB1, NAP-2, CTAP-III, PBP	PPBP	4q12-q13
<i>SCYB8</i>		IL-8	4q12-q13
<i>SCYB9</i>		MIG	4q21
<i>SCYB10</i>	IP-10	INP10	4q21
<i>SCYB11</i>	I-TAC	SCYB9B	4q21
<i>SCYB12†</i>		SDF	10q11.1
<i>SCYC1</i>	LPTN, ATAC, SCM-1a	Lymphotactin (LTN)	1q21-q25

Continued page 730

TABLE II. CONT

Gene symbol	Chemokine	Synonym	Location
SCYC2 SCYD1	CX3Ckine	SCM-1b Fractalkine, neurotactin (NTT, NTN)	Not mapped 16q13
Gene symbol	Receptor	Location	
Receptors for CC chemokines			
CMKBR1	CCR-1	3p21.3	
CMKBR2‡	CCR-2	3p21.3	
CMKBR3	CCR-3	3p21.3	
CMKBR4	CCR-4	3p24	
CMKBR5	CCR-5	3p21	
CMKBR6	CCR-6	ND in humans	
CMKBR7	CCR-7/EBI-1	17q12-q21.1	
CMKBR8	CCR-8	3p22	
CMKBR9 = CMKBR10	CCR9 = CCR10 = hD6		
Gene symbol	Receptor	Synonym	Location
Receptors for CXC, C, and CX3C chemokines			
CXCR1	CXC-R1	IL8RA/IL8R1	2q35
CXCR2	CXC-R2	IL8RB/IL8R2	2q35
CXCR3	CXC-R3	GPR-9	8p12-p11.2
CXCR4§	CXC-R4	NPY3R, LESTR, D2S201E, FUSIN	2q21
XCR1	XCR1	GPR5	
CX3CR1	CX3CR1	CMKBRL1/V28	

MCAF, Monocyte chemotactic and activating factor; *Act-2*, activation protein-2 (MIP-1 β); *MRP*, MIP-related protein; *LEC*, liver-expressed chemokine; *PARC*, pulmonary and activation-regulated chemokine; *DCCK*, dendritic cell chemokine; *AMAC*, alternative macrophage activation-associated CC chemokine; *SLC*, secondary lymphoid tissue chemokine; *6-C-kine*, 6-cysteine chemokine (exodus-2); *MPIF*, myeloid progenitor inhibitory factor; *TECK*, thymus-expressed chemokine; *CTAP*, connective tissue-activating protein; *PBP*, platelet basic protein; *INP10*, IP-10; *ND*, not determined.

*As of Feb 6, 1999, only *SCYB5* and *SCYB6* are approved CXC chemokine gene symbols by the HUGO gene nomenclature committee.

†*SCYB12A* and *SCYB12B* are isoforms (alternative splicing).

‡CCR-2A and CCR-2B isoforms are the result of alternative splicing.

§As of Feb 6, 1999, only *CXCR4* is an approved CXC chemokine receptor gene symbol by the Human Gene Organisation gene nomenclature committee.

establish a local chemokine gradient (Fig 3). It is not clear what presentation molecules are responsible for this, although specific receptors and surface proteoglycans are being considered.⁵⁶⁻⁵⁸ The migratory response to such a gradient is referred to as haptotaxis. Some chemokines have been shown to transiently activate integrin adhesiveness and thereby increase their avidity to their cognate ligand.^{30,59} For example, RANTES induces a transient activation of VLA-4 on eosinophils, increasing their adhesiveness to vascular cell adhesion molecule (VCAM)-1, whereas MCP-3 stimulation results in a prolonged conformational change in Mac-1, leading to increased intercellular adhesion molecule (ICAM)-1 adhesion.⁶⁰ Chemokines can also affect adhesion by regulating the surface expression of integrins.^{61,62} For example, MIP-1 α increases the surface expression of CD11a (LFA-1) and CD11c (p150,95) on human monocytes, resulting in enhanced endothelial adhesion.⁶¹ Although CC chemokines have been shown to enhance leukocyte adhesion in several in vitro systems, they have also been shown to reduce eosinophil adhesion.^{60,63} Chemokine-induced integrin deactivation may also be important in the movement of leukocytes into tissues.^{63,64} Last, it has been proposed that chemokines or their receptors may themselves function as adhesion molecules. This has

been suggested for fractalkine and its recently identified receptor, CX3CR1, which are both membrane bound.¹ Additionally, CCR1 has been shown to be posttranslationally modified to express the carbohydrate adhesion ligand sialyl Lewis X.¹

Hematopoiesis and angiogenesis

Recent studies suggest that the biologic effects of chemokines extend far beyond just leukocyte activation and recruitment. Chemokines have been shown to have effects on hematopoiesis, angiogenesis, tumor immunity, fibrosis, host defense, cell survival/apoptosis, and embryonic development. Several chemokines have been implicated as both negative and positive regulators of proliferation and differentiation of hematopoietic stem cells and progenitors. For example, MIP-1 α suppresses the proliferation of immature erythroid and myeloid progenitors but promotes the proliferation of lineage committed cells.^{65,66} Other chemokines shown to inhibit the proliferation of immature stem/progenitor cells in vitro or in vivo include PF4, β -thromboglobulin (β -TG), neutrophil-activating peptide (NAP)-2, and IL-8.^{67,68} Mice lacking the receptor for the latter 3 chemokines, CXCR2, demonstrated a large expansion of myeloid progenitors compared with wild-type mice.⁶⁹ Mice engineered to be

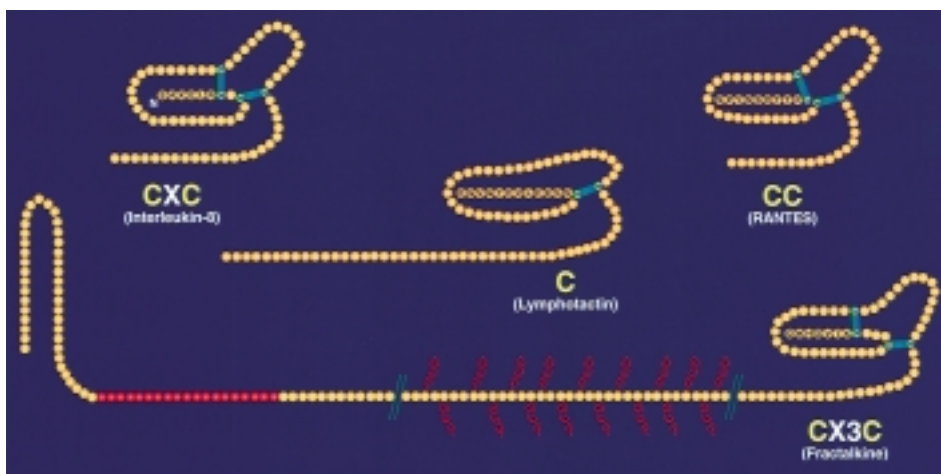


FIG 1. Representative illustrations of chemokine subfamilies. Cysteines and disulfide bonds from which nomenclature is determined are highlighted in blue. Stalk of fractalkine (CX3C) is not drawn to scale, as indicated by parallel green lines. Transmembrane portion of fractalkine is indicated in red, and carbohydrate groups in mucin-rich stalk are indicated by magenta forms. Sequence of amino termini for structures is shown.

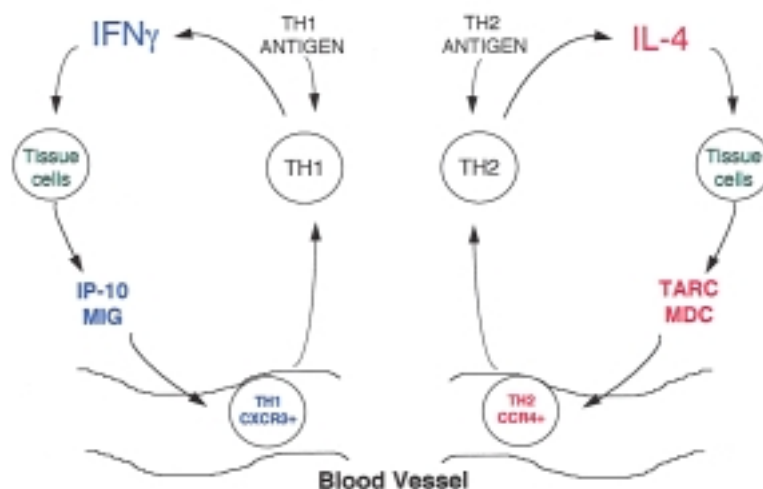


FIG 2. Hypothetic model for selective amplification of recruitment of T_H1 and T_H2 cells. T_H1 cells may recruit more T_H1 cells to tissue site by release of $IFN-\gamma$, resulting in production of IP-10 and MIG by tissue cells (*left*). These chemokines induce migration of CXCR3-expressing T_H1 cells. An analogous mechanism is displayed for selective recruitment of TH2 cells involving IL-4 induction of MDC and TARC, which recruit CCR4 expressing T_H2 cells (*right*). See text for references.

deficient in SDF-1 or its receptor, CXCR4, exhibit defective myelopoiesis and B-cell development as well as several deficiencies in organogenesis.⁷⁰⁻⁷³

More germane to allergic disease is the CC chemokine eotaxin, which acts synergistically with stem cell factor to enhance the development of mature mast cells and promotes the growth of Mac-1⁺ myeloid progenitor cells.^{74,75} These effects are thought to be mediated through CCR3, which is expressed at embryonic sites of hematopoiesis, including the yolk sac, fetal liver, and fetal blood.⁷⁴ Furthermore, systemic administration of eotaxin induces a rapid and profound blood eosinophilia in rodents.^{76,77} The addition of IL-5 resulted in a marked synergism with eotaxin; eotaxin-induced blood

eosinophilia was significantly greater in wild-type mice versus IL-5 ^{-/-} mice.⁷⁶

Chemokines with reported angiogenic properties include several ELR-containing CXC chemokines (IL-8, epithelial-derived neutrophil-activating peptide-78 [ENA-78], GRO- α , GRO- β , GRO- γ , granulocyte chemotactic protein [GCP]-2 and NAP-2, see Table I), which are thought to be relevant for wound healing, tumor growth and metastasis, and some angioproliferative diseases such as rheumatoid arthritis.^{78,79} Their role in allergic disease is currently unknown. Interestingly, the CXC chemokines lacking the ELR motif (PF-4, IP-10, and MIG) have been shown to inhibit angiogenesis and tumor cell growth,^{80,81} and it has been hypothesized that it is the balance between

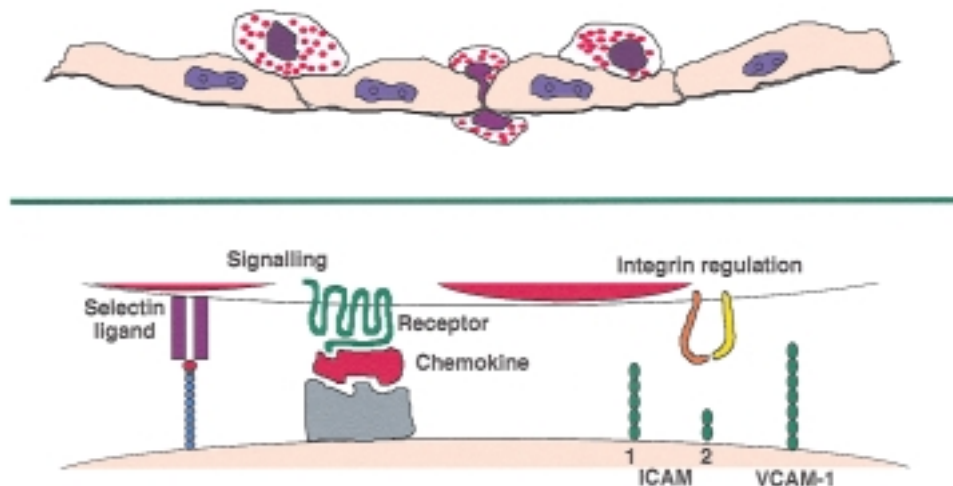


FIG 3. Chemokines trigger firm leukocyte adhesion and transendothelial migration. Activation of chemotactic 7TM receptor in context of selectin binding may trigger both inflammatory gene expression and coordinated integrin adhesion/deadhesion (see text).

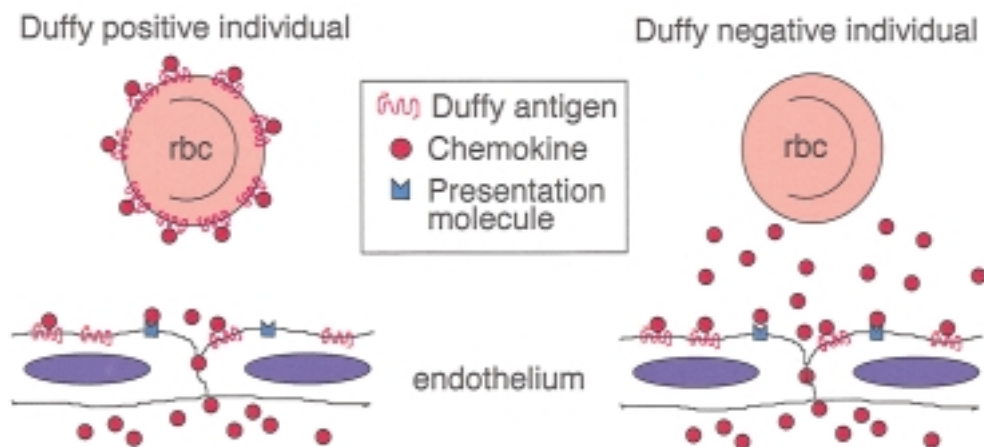


FIG 4. Theoretical regulation of chemokine-mediated inflammation by Duffy antigen/receptor for chemokines on erythrocytes (rbc). Absence of Duffy (right) may lead to higher local concentrations of chemokines associated with endothelial presentation molecules or free in plasma (see text).

these 2 CXC subgroups that determines whether tissues undergo vascular proliferation or regression.⁸²

EXPRESSION OF CHEMOKINES

Cellular sources

Numerous cell types have been found to be sources of chemokines, as summarized in Table I. Although chemokines are highly inducible by proinflammatory and immunomodulatory stimuli,⁸³ constitutive expression of chemokine mRNAs has also been documented in several human tissues, with variable patterns of expression among the different molecules. Among the chemokines associated with allergic disease, eotaxin mRNA is most abundant in the small intestine,⁸⁴ whereas MCP-4 is markedly expressed in lung and heart tissue.²⁸ MDC is primarily found in thymus, lung, and spleen, whereas TARC has been observed in lymphoid tissue. The func-

tion of the basal chemokine expression in uninflamed tissues is largely unknown; recently, constitutive eotaxin expression has been postulated to have a role in regulating the tissue levels of eosinophils in healthy states on the basis of a lack of intestinal villus eosinophils in eotaxin-deficient animals.⁸⁵ Many of the cell sources of chemokines in allergic disease are the same cell types that have been implicated in the pathogenesis of allergic disorders, including monocytes, lymphocytes, eosinophils, basophils, or resident tissue cells such as mast cells, dendritic cells, macrophages, fibroblasts, epithelial cells, and endothelial cells.⁸⁶ In particular, epithelial-derived chemokine production is abundant in inflamed airways and is widely believed to be critical in the establishment of a chronic mucosal inflammatory infiltrate because eosinophils and other infiltrating cells are often found in close contact with the epithelial lining.⁸⁷⁻⁸⁹ In situ

hybridization and immunohistochemical studies of RANTES, MCP-1, MCP-4, and eotaxin show that epithelium is among the predominant staining cell type in biopsy specimens of inflamed upper and lower airways of humans and mice^{28,29,88,90-95} (see below). Finally, several in vitro studies have shown that airway epithelial cells produce substantial quantities of RANTES and other CC, as well as CXC chemokines.^{28,84,96-99}

Regulation of chemokine expression

Cytokines and mediators. Among cytokines with broad proinflammatory activity, TNF- α and IL-1 have been shown in vitro to be potent inducers of both CC and CXC chemokine expression from a variety of cell sources.¹⁰⁰ IFN- γ greatly potentiates the production of RANTES^{91,96,101} and MCP-4^{28,97} and less markedly of eotaxin⁹⁸ induced in epithelial cells by TNF- α . The molecular mechanisms of the synergistic effect of IFN- γ with TNF- α are not completely known. Studies on transcriptional regulation of RANTES have shown that this synergy is at least partially dependent on cooperation between the transcription factor STAT-1 α with nuclear factor- κ B (NF- κ B).¹⁰² This is notable in light of the recent finding that STAT-1 is constitutively elevated in epithelial cells of asthmatic compared with healthy individuals.¹⁰³ Two of the cytokines derived from T_H2 cells, IL-4 and IL-13, induce expression of eotaxin. Transplantation of IL-4-secreting tumor cells in the skin of immunodeficient mice led to a marked eosinophil infiltrate and to a strong local up-regulation of eotaxin mRNA, suggesting that eotaxin may have mediated the eosinophil-recruiting activity of IL-4.¹⁰⁴ Indeed, in vitro studies have shown that IL-4 induces eotaxin expression in human fibroblasts¹⁰⁵ as well as in epithelial cells, where it also up-regulates the expression of MCP-4 but not RANTES.¹⁰⁶ IL-13 induces the production of eotaxin in human dermal fibroblasts¹⁰⁷ and in murine airway epithelium.¹⁰⁸ Thus IL-4 and IL-13 derived from T_H2 cells may lead to selective recruitment of eosinophils by induction of VCAM-1 and release of eotaxin.¹⁰⁹ Induction of eotaxin by IL-4 is dramatically potentiated by TNF- α , apparently because of interactions between STAT-6 and NF- κ B response elements, which overlap in the eotaxin promoter.^{110,111} Infection of epithelial cells with respiratory viruses such as respiratory syncytial viruses, influenza virus, rhinovirus (RV)-14 or RV-16 induces the expression of RANTES and other chemokines, an event that could mediate exacerbations of asthma during upper airway viral respiratory infections.¹¹²⁻¹¹⁴

Glucocorticoids. The treatment of chronic allergic diseases with glucocorticoids results in a profound inhibition of the infiltration of inflammatory cells within the affected tissue.¹¹⁵ A key molecular mechanism of this action is the suppression of the activation of proinflammatory genes.¹¹⁶ Glucocorticoids have been shown in vitro to inhibit the expression of many chemokines suspected to be involved in allergic disease, including RANTES,^{96,117,118} eotaxin,^{98,106} MCP-1,¹¹⁹⁻¹²² MCP-4,²⁸ and MIP-1 α .¹²³ Glucocorticoids inhibit chemokine

production at low concentrations⁹⁶ and in a glucocorticoid-specific manner.^{96,119,120} Suppression of chemokine expression is thought to be an important mechanism by which glucocorticoids prevent the accumulation of inflammatory cells in the airways.

Glucocorticoids can down-regulate gene expression at either the transcriptional or posttranscriptional level.¹¹⁶ DNA-dependent transcriptional regulation occurs when the ligand-activated glucocorticoid receptor binds directly to either positive or negative DNA glucocorticoid response elements (GREs) within the promoter region of the targeted gene.^{124,125} Alternatively, glucocorticoids can exert transrepression in a DNA-independent way, through protein-protein interactions with other transcription factors, such as NF- κ B, activator protein-1 (AP-1), cyclic AMP-responsive element binding protein (CREB), octamer-binding protein-1, NF-IL-6, and others.¹²⁶⁻¹²⁸ Such complex formation prevents the interaction of transcription factors with their promoter binding sites, interfering with their ability to activate inflammatory gene expression. Both DNA-dependent and DNA-independent pathways have been demonstrated for the transcriptional inhibition of chemokine expression by glucocorticoids. Ablation of the GRE in the IL-8 promoter led to the loss of glucocorticoid sensitivity of a reporter gene in a human fibrosarcoma cell line.¹²⁹ A GRE is also present in the promoter region of eotaxin, but its function has not yet been determined.^{130,131} In a pivotal study Mukaida et al¹³² demonstrated that the interaction of ligand-activated glucocorticoid receptor (GR) with the transcription factor NF- κ B was mediating the repression of IL-8 promoter-driven reporter gene activity. Both NF- κ B and AP-1 are known to be involved in the up-regulation of several CC chemokines, including MCP-1,¹³³ RANTES,¹³⁴ and eotaxin.^{130,131} Therefore it can be hypothesized that expression of several chemokines induced by proinflammatory cytokines is inhibited by glucocorticoids in part by interference with AP-1- and NF- κ B-activating pathways.

Glucocorticoids can also inhibit gene expression by accelerating the decay of mRNA species, possibly through interactions—still not fully characterized—with AUREs. These are sequences present in the 3'-UTR of mRNAs involved in the regulation of mRNA half-life.¹³⁵ AUREs are present in the 3'-UTR of eotaxin,⁸⁴ MCP-1,¹³⁶ MIP-1 α ,¹³⁷ and IL-8,¹³⁸ whereas RANTES¹³⁹ and MCP-4⁹⁷ mRNA 3'-UTRs do not contain AUREs. In the epithelial cell line BEAS-2B, the topical glucocorticoid budesonide markedly accelerated the decay of eotaxin and MCP-4 mRNA while having no effect on the RANTES mRNA half-life.^{96,106,140} Interestingly, budesonide increased the rate of decay of MCP-4 mRNA in epithelial cells, despite the lack of an AURE in the 3'-UTR of the MCP-4 transcript. Therefore it can be expected that other regulatory elements present in the 3'-UTR, or in intronic sequences of nuclear, unspliced forms of chemokine RNA, may also contribute to glucocorticoid-induced chemokine mRNA destabilization.

IN VIVO STUDIES OF CHEMOKINES

Challenge studies

Skin. Cutaneous challenge of human subjects with RANTES induced eosinophil recruitment that was both dose- and time-dependent with no effect on neutrophils.¹⁴¹ Furthermore, a remarkable difference in the rate of eosinophil recruitment between nonallergic and allergic subjects was noted. No tissue eosinophilia was observed in challenged skin of nonallergic subjects at 30 minutes and 6 hours after challenge, whereas significant eosinophilia was observed in allergic subjects by 30 minutes, reaching near-maximum levels by 6 hours. The peak responses at 24 hours were similar in both groups, and the 2 groups had comparable circulating eosinophil numbers. One possible explanation for this result is that greater eosinophil priming in the allergic subjects may hasten the response to RANTES. Similar conclusions were derived from a cutaneous RANTES challenge study performed in dogs, which demonstrated tissue eosinophilia only after previous exposure to parasites.¹⁴² Studies of rheumatoid joints, sarcoidal granulomas, inflamed endometrium, and normal thymus have all demonstrated the presence of RANTES in the absence of a significant infiltration of eosinophils.¹⁴³⁻¹⁴⁵ These findings would fit with a model in which eosinophil priming is important or critical for chemokine-induced migration. Possible underlying mechanisms include enhanced eosinophil expression of the relevant chemokine receptors, increased ligand affinity, or enhanced receptor signaling in primed eosinophils. Eosinophils from allergic subjects have, on average, 2-fold higher surface expression of CCR3 compared with those from nonallergic individuals.^{50,146}

Challenge of human subjects with RANTES also induced the recruitment of memory (CD45RO⁺) T cells but not monocytes (CD68⁺), in agreement with in vitro studies demonstrating that RANTES induces migration of these cell types. In a recently completed MIP-1 α human challenge study, significant recruitment of monocytes was observed, with only modest recruitment of eosinophils and T lymphocytes, also in agreement with in vitro chemotaxis assays.¹⁴⁷ The maximum tissue eosinophilia seen in response to MIP-1 α (1000 pmol) was 5-fold less than that seen after RANTES (800 pmol) challenge. MIP-1 α also induced significant tissue neutrophilia, as observed by Alam et al¹⁴⁸ in a mouse footpad challenge model. Cutaneous challenge with RANTES or MIP-1 α had no effect on mast cell histamine release because none of the subjects had swelling or itch after injection. Both of these chemokines induced dermal endothelial activation as measured by enhanced E-selectin expression. This may be an indirect or downstream effect of chemokine injection because in vitro stimulation of endothelial cell lines failed to induce any endothelial adhesion molecules (Beck et al, unpublished observations).

In vivo challenge studies with chemokines relevant to allergic disease in animals have demonstrated some important species differences. For example, several groups have demonstrated that the cutaneous injection of recombinant

human RANTES has little to no inflammatory effect in BALB/C mice (Beck et al, unpublished observations), guinea pigs,¹⁴⁹ or in *Ascaris*-sensitized cynomolgus macaques.¹⁵⁰ Interestingly, even murine RANTES had no eosinophil-recruiting activity in a mouse model in which both mouse eotaxin-1 and mouse MIP-1 β induced significant cutaneous eosinophilia.¹⁵¹ Injection of human CC chemokines into the skin of *Ascaris*-sensitized cynomolgus macaques resulted in significant tissue eosinophilia 24 hours after challenge, with a rank order of potency of MCP-4 $\geq$ eotaxin-1 > MCP-3 > MIP-1 α .¹⁵⁰ Teixeira et al,¹⁵¹ with use of a mouse model, also found eosinophil migration in response to mEotaxin-1, which was greater than that seen with mMIP-1 α but found no tissue eosinophilia in response to murine (m) MCP-1, mMCP-5 or mMIP-1 β .

Airways. Challenge of guinea pigs with an aerosol of eotaxin led to a selective increase of eosinophils in the airways.¹⁵² Intranasal eotaxin led to the influx of large numbers of eosinophils in the lungs of IL-5 transgenic mice.¹⁵³ In humans, intranasal RANTES challenge led to significant increases in the number of eosinophils, metachromatic cells, and lymphocytes but not neutrophils.¹⁵⁴ Pre-exposure to allergen amplified this response significantly.

Assessment of chemokine expression in vivo

Skin. Many of the CC chemokines are thought to play a role in the recruitment and activation of eosinophils and lymphocytes at cutaneous sites of allergic inflammation in humans. Increased mRNA for RANTES, MCP-3, MCP-4, eotaxin-1, and eotaxin-2 have been demonstrated by in situ hybridization in human cutaneous late-phase reactions.^{155,156} In these studies eotaxin-1 and MCP-3 mRNA⁺ cells were maximal at 6 hours, when tissue eosinophils are at their peak, suggesting that these chemokines precede and may mediate the early tissue eosinophilia. At a later time point (24 hours) the tissue eosinophilia correlated with eotaxin-2 and MCP-4 mRNA⁺ cells.¹⁵⁶ Interestingly, RANTES expression closely mirrored the tissue infiltration of CD3⁺ lymphocytes and CD68⁺ monocytes.¹⁵⁵ With use of the skin blister chamber model, Zweiman et al¹⁵⁷ demonstrated elevated levels of IL-8, MCP-1, and RANTES in skin chamber fluids (from 2 to 5 hours). IL-8 expression and neutrophil recruitment were observed in this model, regardless of whether antigen was introduced into the blister chamber. This presumably represents a response to the injury associated with generation of the blister. IL-8 levels correlated with lactoferrin levels (a marker of neutrophils) and RANTES correlated with the number of eosinophils.

Airways. Within the last few years, numerous studies have documented expression of mRNA or protein for several CC chemokines in chronic diseases of the airways characterized by tissue eosinophilia. In nasal polyposis intense epithelial and endothelial staining for RANTES^{88,158,159} and epithelial and leukocyte staining for eotaxin²⁹ and MCP-4⁹⁷ has been documented by immunohistochemistry or by in situ hybridization. RANTES,^{88,159} eotaxin,⁹⁵ eotaxin-2,⁴⁸ and MCP-4⁹⁷

were found to be constitutively expressed in normal sinus mucosa, but their expression was significantly increased in polyps compared with noninflamed sinus mucosa.^{29,48,88,95,97,159,160} RANTES, eotaxin, and MCP-4 levels were similarly elevated in polyps from both nonatopic and atopic subjects.^{95,97,158-160} However, eotaxin²⁹ but not RANTES immunoreactivity⁸⁸ strongly correlated with the eosinophil infiltration observed within the mucosa of the polyp tissue. Nasal biopsy specimens of subjects with allergic rhinitis undergoing allergen challenge display a significant up-regulation of eotaxin mRNA and protein.⁹⁵

The presence of RANTES,^{91,92,161-165} eotaxin,^{93,166,167} MCP-1,^{90,163,165} MCP-3,^{162,164} MCP-4,²⁸ and MIP-1 α ^{163,165} has been demonstrated at the mRNA or protein level in bronchial biopsy specimens or bronchoalveolar lavage (BAL) of asthmatic patients. RANTES, MCP-3, MCP-1, and MIP-1 α have been found to be significantly elevated in asthmatic in comparison with nonasthmatic subjects.^{162,163} The increase in RANTES and MCP-3 mRNA in asthmatic patients is detectable regardless of the presence of atopic status.¹⁶⁴ Asthmatic patients have also been found to have a higher concentration of eotaxin than healthy control subjects in the BAL⁹³ and increased expression of eotaxin mRNA and protein in the bronchial mucosa.^{93,166} With at least one exception,¹⁶⁸ correlation of chemokine levels with infiltrating inflammatory cells has been observed in the airways. RANTES and MCP-3 mRNA expression correlated with eosinophil infiltration within the airway mucosa of unchallenged asthmatic patients¹⁶² and after allergen challenge of patients with mild asthma.⁹² RANTES and MCP-1 protein levels found in the BAL of patients with mild asthma were found to correlate with lymphocytes.¹⁶³ Eotaxin mRNA-positive cells in the bronchial mucosa of asthmatic subjects correlated with activated eosinophils identified by immunohistochemistry as EG2⁺ cells, and eotaxin expression was inversely correlated with a marker of bronchial hyperresponsiveness, the histamine provocative concentration causing a 20% fall in FEV₁.¹⁶⁶ The eosinophil chemotactic activity of BAL fluid tested in vitro can be blocked to a varying degree with antibodies against eotaxin, RANTES, MCP-3, or MCP-4.^{92,93,161}

Studies in murine models of allergic inflammation have firmly established up-regulation of chemokines on lung challenge and yielded information on the role of chemokines in the orchestration of inflammatory cell recruitment in allergic inflammation. Gonzalo et al⁹⁴ demonstrated that the differential accumulation of specific leukocyte types correlates with a distinct pattern of chemokine expression: challenge of ovalbumin-sensitized mice led to the sequential expression in the lungs of MCP-1, eotaxin, and RANTES, which correlated with the accumulation of monocytes-macrophages, eosinophils, and T lymphocytes, respectively. In a similar model MacLean et al¹⁶⁹ found that the increased eotaxin production induced in the lungs by ovalbumin challenge is blocked by anti-CD3 antibody treatment of the animals, suggesting that eotaxin might be the molecule mediating eosinophil recruitment

driven by antigen-specific T-cell activation. Individual blockade of eotaxin, MCP-5, MIP-1 α , RANTES, and MCP-1 in ovalbumin-induced airway allergic inflammation in mice indicated profound differences in the contribution of chemokines to cellular recruitment and bronchial hyperreactivity.^{170,171} Little is known about chemokine expression by T_H1 versus T_H2 cells either directly or indirectly by activation of other cells. In a recent study Chen-sue et al¹⁷² examined the production of RANTES after challenge with antigens inducing a type 1 (ie, T_H1) response (purified protein derivative) or a type 2 response (*Schistosoma mansoni* egg antigen). Greater elevations of RANTES were seen in the type 1 challenge, and anti-RANTES modestly reduced the type 1 response yet enhanced the type 2 response (increasing lesion size as well as the appearance of IL-4, IL-5, etc). The authors concluded that RANTES may down-regulate type 2 inflammatory responses. In related studies, Li et al¹⁷³ found that adoptive transfer of antigen-specific T_H2 cells led to lung eosinophilia associated with eotaxin after challenge, whereas T_H1 cell transfer was associated with neutrophils and little eotaxin. The cellular source of the eotaxin was not determined. Disruption of the eotaxin gene has been shown by Rothenberg et al¹⁷⁴ to suppress the early eosinophil recruitment to the lungs after antigen challenge but not the late eosinophil influx, although in a different eotaxin knock-out system the eosinophil influx in the bronchoalveolar lavage induced by ovalbumin challenge was not modified in comparison with that of wild-type mice.¹⁷⁵ In the mouse, eotaxin may have a greater role in antigen-induced eosinophilia in the gastrointestinal tract than in the lung (Hogan and Rothenberg, personal communication).

Treatment for 1 week with intranasal beclomethasone dipropionate (BDP) or fluticasone significantly inhibited the recovery of IL-8, RANTES, and MIP-1 α in nasal secretions of 10 allergic patients after experimental allergen challenge.^{176,177} In a double-blind, placebo-controlled study of 4 months of BDP treatment of patients of mild asthmatics, evaluation by immunohistochemistry of bronchial biopsy specimens showed a significant decrease of RANTES staining within the epithelium in the BDP-treated group but not in the placebo group.⁹¹ The local suppression of epithelial-derived chemokines by topical glucocorticoids may be an important component of the clinical efficacy of topical glucocorticoids, by preventing chemokine-driven infiltration of lymphocytes, eosinophils, and mast cells within the airway mucosa.

POTENTIAL USE OF CHEMOKINE ANTAGONISTS IN ALLERGIC DISEASE

Pharmaceutical companies have vigorously pursued chemokine antagonists with the hope that a variety of disorders, including allergic diseases, can be alleviated. Nowhere have these efforts been greater than in the area of AIDS and HIV, where interest in the potential therapeutic or prophylactic value of antagonists of chemokine

receptor HIV entry cofactors, especially CCR5 and CXCR4, is high. For allergic diseases, CCR3 and its ligands have attracted significant interest as targets because of their role in eosinophil recruitment and possibly the recruitment of mast cells and T_H2 cells. Teixeira et al,¹⁵¹ with use of ovalbumin-sensitized mice, demonstrated that the eosinophil recruitment seen in response to cutaneous ova challenge could be reduced by nearly half with the coadministration of a neutralizing antibody to eotaxin-1, whereas anti-MIP-1 α antibody had no effect. Similar results were obtained in a lung challenge model by Gonzalo et al^{194,171} and Conroy et al¹⁷⁸ with antieotaxin. Significant inhibition of eosinophil influx after antigen challenge was also obtained in mice by pretreatment with anti-MCP-3/fibroblast-induced chemokine (FIC) antibody.¹⁷⁹ In contrast, Lukacs et al¹⁷⁰ found that anti-MIP-1 α reduced airway eosinophilia by more than 50%. They also found that anti-MCP-1 blocked the increase in airway reactivity.¹⁷⁰ The chemokine metRANTES is a potent and effective antagonist of CCR3 that can block antigen-induced eosinophilia in mice.¹⁵¹ An antibody to CCR3 has been found by Sabroe et al¹⁸⁰ to inhibit the appearance of eosinophils in eotaxin-challenged guinea pigs. Several companies have developed low-molecular-weight antagonists for CCR3, and clinical trials are likely to be forthcoming soon. As information evolves regarding selective chemokine receptor expression on T_H2 cells, additional targets may become obvious (eg, CCR4, see above).

ETHNIC VARIATIONS IN CHEMOKINE BIOLOGY: IMPLICATIONS FOR ALLERGIC DISEASES

The prevalence of allergic airway disease as well as asthma severity and mortality are particularly high among African Americans, even after adjustment for demographic and socioeconomic factors.¹⁸¹⁻¹⁸⁴ Atopic dermatitis also appears to be more prevalent among phenotypically black populations,¹⁸⁵ and higher total IgE levels have been observed in individuals of African descent compared with Caucasians.^{186,187} These findings strongly suggest that distinct genetic factors render individuals of African descent more susceptible to atopic disorders than Caucasians are. Among potential candidate genes for asthma and asthma-associated traits in African populations are CC chemokines and chemokine receptors. In a genome-wide screen for asthma susceptibility loci in 3 different ethnic groups, evidence for linkage of asthma to chromosome 17q11.2, the location in which the CC chemokine gene cluster maps, was exclusively observed in African American families. No evidence for linkage of asthma or asthma-related traits to chromosome 17 has been observed in 3 independent genome-wide searches for asthma genes in white families.¹⁸⁸⁻¹⁹⁰

Recently, regulatory regions of the RANTES and eotaxin genes were screened for mutations that may affect transcriptional activity or transcript stability. A polymorphism in the proximal RANTES promoter that

results in the formation of a new binding site for the GATA binding transcription factor family was identified.¹⁹¹ GATA 1-3 are expressed primarily in cells of hematopoietic origin and are centrally involved in hematopoietic cell differentiation.¹⁹² Gel shift experiments showed binding of GATA 1-3 to the novel GATA binding site in the mutant RANTES promoter. Also, studies of reporter constructs indicate a 5-fold greater constitutive transcriptional activity of the mutant promoter when transfected into hematopoietic cell lines (the Jurkat T-cell line or the HMC-1 mast cell line, both GATA expressing) but not in an epithelial cell line (BEAS-2B-GATA nonexpressing).¹⁹¹ Taken together, these studies demonstrate a functionally relevant polymorphism in the promoter for RANTES. Five populations of different ethnic origin were subsequently screened for wild-type and mutant alleles. Significant ethnic differences in allele and genotype frequencies were observed. The mutation was most frequent in individuals of African ancestry (allele frequency >0.4 in African Americans and Afro-Caribbeans compared with >0.2 in American whites).

Although no association of the mutation with asthma was detected, the mutant allele was observed significantly more frequently in German children with atopic dermatitis than in unaffected control subjects.¹⁹¹ All German genotyped children were participants of the German Multicenter Allergy Study.¹⁹³ Whether associations exist with atopic dermatitis in African-descended populations has not yet been tested because this phenotype has not been assessed in the available populations. Clearly, more studies will be required to evaluate the importance of this polymorphism in the induction or maintenance of allergic inflammation.

Recent studies focusing on the 3'-UTR of eotaxin indicate a remarkable degree of polymorphism, with distinct profiles of alleles between African-descended and white individuals.¹⁹¹ Preliminary studies suggest associations of some of these alleles with asthma in African-descended populations, whereas a lesser degree of polymorphism and no allelic association with asthma or asthma-related phenotypes were observed in whites. Because the 3'-UTR contains sequences that may dramatically alter the half-life of eotaxin mRNA (see above), functional studies of selected polymorphisms are currently in progress.

Several other differences in CC chemokine receptor biology have been described in individuals of African versus white descent. A 32-bp deletion in the CCR5 receptor, which is a common receptor for RANTES, MIP-1 α , and MIP-1 β and is a coentry factor for macrophage-tropic HIV strains,^{194,195} is found in >10% of whites, whereas this mutation is completely absent in African populations.¹⁹⁶ Second, in contrast to white individuals, the DARC is not expressed on red blood cells in the majority of African people.¹⁹⁷ This confers an evolutionary advantage because Duffy-negative erythrocytes are resistant to infection with the malarial parasite *Plasmodium vivax*,¹⁹⁸ which is endemic in most of Africa. As discussed above, Duffy has been shown to bind with high affinity to

chemokines of both the CXC and CC classes and is expressed on erythrocytes, endothelial cells of postcapillary venules, and Purkinje cells of the cerebellum.¹⁹⁹ Because of Caucasian admixture, more than 30% of African Americans do express Duffy on red blood cells (and are classified as Duffy positive).²⁰⁰ The physiologic role of this receptor on erythrocytes has not been clearly defined. No signal transduction in response to chemokines on cells transfected with cloned Duffy could be observed by Neote et al,²⁰¹ suggesting that Duffy may function as a clearance receptor for chemokines in blood and tissues. This hypothesis is intriguing with regard to evidence for linkage of asthma to the CC chemokine cluster occurring exclusively in populations of African descent.¹⁸⁸ Higher or longer exposure to chemokines within blood vessels because of the absence of Duffy on erythrocytes might contribute to asthma pathogenesis (Fig 4). The importance of Duffy is evidenced by the conservation of this gene among species.¹⁰

The molecular basis for the Duffy-negative phenotype, *Fy(a-b-)*, has been identified by Tournamille et al.²⁰² A point mutation in the Duffy promoter (T-46C) selectively abolishes erythrocyte gene expression by disrupting a GATA-binding motif. This finding enabled the development of a PCR-based technique for retrospective determination of Duffy-positive and Duffy-negative alleles in large populations of African descent with varying degrees of Caucasian admixture.¹⁹¹ Preliminary results of subsequent studies indicate that an interaction of genes on chromosome 1q22 (*FY*-Duffy locus) and on chromosome 17q11.2 (CC chemokine gene cluster) may determine susceptibility to asthma and allergic rhinitis in individuals of African ancestry (Nickel et al, unpublished observations). To test the hypothesis that Duffy can modify chemokine-mediated inflammation, cutaneous challenges with chemokines in Duffy-positive and Duffy-negative individuals are underway.

No evidence for linkage of asthma or asthma-associated phenotypes to chromosome 3p21-p22 (CC chemokine receptor gene cluster) or chromosome 7q11.2 (eotaxin-2) has been reported in 3 genome-wide searches.¹⁸⁸⁻¹⁹⁰ Inconclusive results from genome-wide searches for other asthma susceptibility genes demonstrate the difficulties of genetic analysis of complex genetic traits with varying penetrance and phenotypic pleiotropy. However, screening of candidate genes with obvious biologic importance in the pathogenesis of asthma or the response to asthma medication has shown important associations of genetic variants with distinct asthma-related phenotypes^{203,204} and will remain a most valuable approach in genetic studies of asthma and atopy.

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