Chemokines: Key Players in Innate and Adaptive Immunity

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Healthy individuals initiate an immediate immune response to microbes by using a set of germline-encoded receptors that recognize common molecular patterns found on the surface of pathogens that are distinct from self-antigens. This innate immune response is the first line of defense against microorganisms in vertebrates, and constitutes the only immune response in plants and invertebrates. The innate immune system includes cellular components, as well as a host of soluble products (antimicrobial peptides, complement fragments, cytokines, and chemokines). The adaptive immune response, which provides long-lasting protection, takes days to develop and requires somatic mutations leading to the development of antigen-specific T cell receptors (cell-mediated immunity) and immunoglobulins (humoral immunity). Members of the chemokine superfamily are crucially involved in both innate and adaptive responses. We review the biological actions of the chemokine superfamily, focusing on several functions that are relevant for both immune responses, such as cell recruitment, microbicidal activity, cell activation, polarization of $CD4^+$ T cells, and effects on structural cells. In particular, we will illustrate the central role that chemokines play in host defense, best demonstrated by the tremendous number of chemokine and chemokine receptor homologs found in microbial genomes, which deflect the immune response of the host.

Key words: adaptive/chemokine/inflammation/innate
J Invest Dermatol 125: 615–628, 2005

The superfamily of chemokines consists of more than 40 members and can be divided into four subgroups, based on the number and spacing of the first two conserved cysteine residues in the amino terminus: CXC (α-family), CC (β-family), C (γ-family), and CX3C (δ-family) (where "X" is an amino acid) (Zimmermann *et al*, 2003) (http://cytokine.medic.kumamoto-u.ac.jp/). Recently, a systematic nomenclature system for the chemokine ligands was developed (Table I) (Zlotnik and Yoshie, 2000). Chemokines (from the contraction of the words chemotactic and cytokines) were initially recognized for their effects on cell activation, differentiation, and trafficking (Nickel *et al*, 1999). More recently, their role has been recognized in many biological processes, such as angiogenesis, angiostasis, hematopoiesis, organogenesis, cell proliferation, lymphocyte polarization, apoptosis, tumor metastasis, and host defense (Gerard and Rollins, 2001; Murakami *et al*, 2004).

Functionally, chemokines can be divided into inducible or "inflammatory" chemokines and constitutively expressed or "homeostatic" chemokines (Table I) (Lukacs, 2001). Inflammatory chemokines are critical for attracting a diverse set of effector leukocytes to inflammatory sites and as such they are thought to play a key role in the innate immune response by recruiting neutrophils, monocytes/macrophages, dendritic cells (DC), and natural killer (NK) cells. Inflammatory chemokines typically bind to more than one chemokine receptor, which suggests that there is considerable redundancy in the inflammatory chemokine network. For example, numerous CC chemokines such as CC-chemokine ligand (CCL)5, CCL7, CCL11, CCL13, CCL24, CCL26, and CCL28 bind to CC-chemokine receptor (CCR)3. These molecules are, however, thought to be non-redundant, as they differ in stimuli that induce their release, cells that produce them, their binding affinities as well as biological potencies and efficacies (Zimmermann *et al*, 2003). Furthermore, these secreted chemokines may be modified within their local tissue environment by enzymes such as dipeptidyl peptidase IV (CD26), which cleaves amino-terminal amino acids, resulting in changes in their biological potency or even in the development of a functional antagonist. For example, the truncated version of eotaxin can no longer induce a CCR3 signal and in fact desensitizes the CCR3 receptor (Struyf *et al*, 1999). Many other enzymes frequently found at sites of inflammation or infection, such as neutrophil elastase, cathepsins, chymotrypsin, and matrix metalloproteinases can also modify chemokines at the amino

Abbreviations: APC, antigen-presenting cell; CCL, CC-chemokine ligand; CCR, CC-chemokine receptor; CpG-DNA, unmethylated poly-cytosine or -guanosine dinucleotides; CX3CR, CX3C chemokine receptor; CXCL, CXC chemokine ligand; CXCR, CXC chemokine receptor; DARC, Duffy antigen receptor for chemokines; DC, dendritic cell; ELR, N-terminal ELR (Glu–Leu–Arg) amino acid motif (in CXC chemokines); GPCR, G-protein-coupled seven-transmembrane receptor; LC, Langerhans cell; LPS, lipopolysacharide; MCAF, monocyte chemotactic and activating factor; MIP, macrophage inflammatory protein; MYD88, myeloid differentiation factor 88; NK, natural killer; NOD, nucleotide-binding oligomerization domain; PAMP, pathogen-associated molecular pattern; PGN, peptidoglycan; PRR, pattern-recognition receptor; RBC, red blood cell; TLR, toll-like receptor; vCk, viral homolog of chemokines; vCkBP, virally produced chemokine-binding protein; vCkR, viral homolog of chemokine receptors

Table I. Chemokine ligands and receptors

Name	Human ligand	Receptor agonist or [antagonist]
CXC family		
CXCL1	GROα/MGSA-α	CXCR2
CXCL2	GROβ/MGSA-β	CXCR2
CXCL3	GROγ/MGSA-γ	CXCR2
CXCL4	PF4	
CXCL5	ENA-78	CXCR2
CXCL6	GCP-2	CXCR1, CXCR2
CXCL7	NAP-2	CXCR2
CXCL8	IL-8	CXCR1, CXCR2
CXCL9	MIG	CXCR3 [CCR3]
CXCL10	IP-10	CXCR3 [CCR3]
CXCL11	I-TAC	CXCR3 [CCR3]
CXCL12	**SDF-1α/β**	**CXCR4**
CXCL13	**BLC/BCA-1**	**CXCR5**
CXCL14	BRAK/bolekine	
CXCL15	—	
CXCL16	SR-PSOX	CXCR6
CC family		
CCL1	**I-309**	**CCR8**
CCL2	MCP-1/MCAF	CCR2
CCL3	MIP-1α/LD78α	CCR1/CCR5
CCL4	MIP-1β	CCR5
CCL5	RANTES	CCR1, CCR3, CCR5
CCL6	—	
CCL7	MCP-3	CCR1, CCR2, CCR3 [CCR5]
CCL8	MCP-2	CCR3
CCL9/10	—	
CCL11	Eotaxin	CCR3, CCR5 [CCR2]
CCL12	—	CCR2
CCL13	MCP-4	CCR2, CCR3
CCL14	HCC-1	CCR1
CCL15	HCC-2/Lkn-1/ MIP1δ	CCR1, CCR3
CCL16	HCC-4/LEC	CCR1
CCL17	**TARC**	**CCR4**
CCL18	DC-CK1/PARC/ AMAC-1	[CCR3]
CCL19	**MIP-3β/ELC/ exodus-3**	**CCR7**
CCL20	**MIP-3α/LARC/ exodus-1**	**CCR6**
CCL21	**6Ckine/SLC/ exodus-2**	**CCR7**
CCL22	**MDC/STCP-1**	**CCR4**
CCL23	MPIF-1	CCR1
CCL24	MPIF-2/Eotaxin-2	CCR3
CCL25	**TECK**	**CCR9**
CCL26	Eotaxin-3	CCR3 [CCR1, CCR2, CCR5]
CCL27	**CTACK/ILC**	**CCR10**
CCL28	**MEC**	CCR3, **CCR10**
C family		
XCL1	Lymphotactin/ SCM-1α/ATAC	XCR1
XCL2	SCM-1β	XCR1
CX3C family		
CX3CL1	Fractalkine	CX3CR1

New nomenclature as proposed by Zlotnik and Yoshi (2000) is shown, with older nomenclature indicated in the second column. A *dash* indicates that the homolog has not been identified. Bolded chemokines/ receptors are homeostatic or constitutive chemokines.

CXCL, CXC chemokine ligand; GRO, Growth-regulated oncogene; MGSA, melanoma growth stimulatory activity; PF, platelet factor; ENA-78, epithelial neutrophil activating peptide 78; GCP, granulocyte chemotactic protein; NAP, neutrophil-activating peptide; MIG, monokine-induced by IFNγ; IP, IFNγ-inducible protein; I-TAC, IFN-inducible T-cell chemoattractant; SDF-1, stromal cell-derived factor 1; BCA, B-cell attracting chemokine; BRAK, breast- and kidney-expressed chemokine; MCP, monocyte chemoattractant protein; MCAF, monocyte chemotactic and activating factor; MIP, macrophage inflammatory protein; 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 ligand chemokine; LARC, liver- and activation-regulated chemokine; SLC, secondary lymphoid tissue chemokine; MDC, monocyte-derived chemokine; STCP, stimulated T cell chemoattractant protein; MPIF, myeloid progenitor inhibitory factor; TECK, thymus-expressed chemokine; CTACK, cutaneous T cell-activating chemokine; ILC, IL-11 recepter α-locus chemokine; MEC, mucosae-associated epithelial chemokine; SCM, Single C motif; ATAC, activation-induced, chemokine-related molecule (modified from Zimmermann *et al*, 2003).

terminus, altering their receptor–ligand interaction and therefore their function (Rot and von Andrian, 2004). This post-translational modification has been utilized by the helminthic parasite, *Necator americanus*, which releases an enzyme that inactivates eotaxin thereby preventing the recruitment and activation of eosinophils, providing a survival advantage to the parasite (Culley *et al*, 2000).

Homeostatic chemokine receptor/ligand pairs (CXC chemokine receptor (CXCR)4–CXC chemokine ligand (CXCL)12, CXCR5–CXCL13, CCR6–CCL20, CCR7–CCL19, CCR7–CCL21) are important for migration of antigen-presenting cells (APC) and lymphocytes into the lymph node, where antigen education and immune surveillance occur, whereas other homeostatic chemokine receptor/ligand pairs (CCR4–CCL17, CCR4–CCL22, CCR8–CCL1, CCR9–CCL25, CCR10–CCL27, CCR10–CCL28) are important for effector T cells to reach tissues that contain their cognate antigen (reviewed in Rot and von Andrian, 2004). Both actions are critically important for an effective adaptive immune response. Not surprisingly, in studies where several of these chemokine receptors were knocked out, the development of normal lymphoid architecture was affected,

resulting in altered B cell migration (CXCR5 KO), immature DC migration (CCR6 KO), lymphocyte, and activated DC migration (CCR7 KO) (Muller *et al*, 2002). In contrast to inflammatory chemokines, homeostatic chemokines display a more monogamous receptor usage. It is important to recognize, however, that some chemokines clearly do not fit either paradigm. For example, CCL22 is not only expressed in secondary lymphoid tissue but also in inflamed lungs (Godiska *et al*, 1997). Similarly, CCL21 is constitutively expressed on the luminal side of high endothelial venules and as such is important in the homeostatic movement of naïve T and B cells to lymphoid organs. But CCL21 can also be induced on afferent lymphatics by inflammatory stimuli, thereby boosting the numbers of DC that reach the draining lymph node (see Leukocyte recruitment) (MartIn-Fontecha *et al*, 2003).

Chemokines mediate their biological effects by binding to G-protein-coupled seven-transmembrane receptors (GPCR) that can activate an array of signaling pathways. Ten CC (CCR1-10), seven CXC (CXCR1-6 and CXCR3B), one CX3C (CX3C chemokine receptor (CX_3CR)1), and one C (XCR1) receptors have been identified (Table I), in addition to two decoy (non-signaling) chemokine receptors: Duffy antigen receptor for chemokines (DARC) previously known as the erythrocyte antigen Duffy, and D6. Both of these decoy receptors bind primarily inflammatory chemokines of the CXC and CC subfamilies, respectively (Gardner et al, 2004).

Recent studies have demonstrated that these decoy receptors have several functions, including internalization and/or degradation of inflammatory, but not homeostatic, chemokines (the so-called "sink" hypothesis) (Nickel *et al*, 1999). Other than DARC expression on red blood cells (RBC), the expression of these decoy receptors is largely limited to endothelial cells, such as post-capillary venules for DARC and lymphatics for D6. Endothelial-expressed DARC has been shown to transport chemokines from the abluminal to the luminal surface for presentation of the chemokine to circulating leukocytes (Lee *et al*, 2003). This function would suggest that DARC may also have a pro-inflammatory function. D6, expressed on lymphatic endothelial cells, does not mediate such chemokine transcytosis and is thought to function as a clearance mechanism for inflammatory chemokines to prevent their diffusion to draining lymph nodes via afferent lymphatics (Fra *et al*, 2003). The D6-deficient mouse was recently shown to have an enhanced inflammatory response to phorbol esters that was characterized by a notable T cell and mast cell infiltrate and a psoriasiform change to the epithelium (Jamieson *et al*, 2005).

DARC was first recognized as the receptor utilized by *Plasmodium vivax* to infect erythrocytes (Miller, 1975). The majority of Africans and about 70% of African Americans do not express DARC on their RBC. This confers an evolutionary advantage, as DARC-negative erythrocytes are resistant to *Malaria*, which is endemic in most of the African continent. There is evidence that the lack of DARC on RBC leads to a greater susceptibility to tumors (such as prostate cancer) and inflammatory diseases (such as graft rejection and asthma) due to the reduced "sink" of angiogenic and inflammatory chemokines (Lentsch, 2002; Akalin and Neylan, 2003). This anti-inflammatory effect has been shown more directly in an eosinophil shape change assay, which is a surrogate for chemotaxis. In this assay, the magnitude of the shape change observed in response to whole-blood stimulation with the CCR3 ligand, CCL11, was diminished in subjects who were DARC positive (Bryan *et al*, 2002). Although the expression of DARC on RBC is genetically determined, endothelial DARC mRNA and protein can be upregulated in response to inflammatory or infectious stimuli, suggesting that the chemokine-binding properties of Duffy antigen may be biologically relevant during lipopolysacharide (LPS)-induced inflammation (Dawson *et al*, 2000). Similarly, DARC-null mice, lacking the Duffy antigen on RBC and endothelial cells, develop significantly greater inflammation in the lungs and liver following challenge with lipopolysaccharide (Table II) (Dawson *et al*, 2000). The hypothesis that has therefore emerged is that DARC participates in regulating effective chemokine concentrations in tissue but whether the net effect is anti- or pro-inflammatory may depend on the stimulus, the organ, and the relative effects of DARC-expressing RBC *versus* endothelial cells. Less is known about the role of the D6 decoy receptor in health and diseased states.

With regard to chemokine receptor interactions, CC receptors bind mainly CC chemokines, and CXC receptors bind preferentially CXC chemokines. There are several known exceptions, such as the CXC ligands CXCL9, CXCL10, and CXCL11, which bind CCR3 and are functional antagonists (Fulkerson *et al*, 2004). Even within a chemokine subfamily (and its receptors), chemokines can have agonistic actions on one receptor and antagonistic actions on another. An example would be CCL11, which can signal through CCR3 and CCR5, but acts as an antagonist when binding to CCR2 (Ogilvie *et al*, 2001).

In summary, chemokines are important, multifunctional mediators of inflammation and immunity. The complexity of their biological role is suggested by many of their features, from the large number of members in the superfamily, to their complex ligand–receptor interactions, the multiple conformations of the receptor (homo- and hetero-dimerization), as well as naturally-occurring splice variants, polymorphisms, and post-translational modifications (observed with locally released proteases); which all play a role in finely tuning their local biological actions (Mellado *et al*, 2001; Comerford and Nibbs, 2005).

Chemokine Functions

Leukocyte recruitment The mechanisms responsible for selective recruitment of leukocytes into tissues (under homeostatic or inflammatory conditions) are thought to involve cytokines that activate the expression of the endothelial adhesion molecules E- and P-selectin, ICAM-1 and VCAM-1, as well as leukocyte-specific chemoattractants such as chemokines (Bochner, 2000). The earliest step in leukocyte recruitment involves the rolling of leukocytes on the endothelial surface. This process is primarily mediated by the selectin family with the exception of the T cell, which like the eosinophil, may also use VLA-4/VCAM-1 for rolling (Sriramarao *et al*, 1994; Alon *et al*, 1995). Rolling is followed by firm adhesion, which is mediated by leukocyte β1 and β2

Table II. Mouse models demonstrate that the chemokine family is important in host defense

Knockout	Clinical and immunological consequence Reference[a]	Microbe
Receptors		
CCR1		
	Reduced inflammation, increased mortality Domachowske, J Immunol, 2000	Paramyxovirus
	Increased susceptibility to infection Khan, J Immunol, 2001	*Toxoplasma gondii*
	Increased susceptibility to infection Gao, J Exp Med, 1997	*Aspergillus fumigatus*
	Smaller lesions containing fewer parasites **Rodriguez-Sosa, Immunol Cell Biol, 2003**	***Leishmania major***
	No effect on corneal PMN or opacities **Hall, J Immunol, 2001**	***Onchocerciasis***
CCR2		
	Defective macrophage recruitment and host defense Kurihara, J Exp Med, 1997	*Listeria monocytogenes*
	Decreased macrophage and CD8 + T cell recruitment Huffnagle, Immunopharmacology, 2000	*Cryptococcus neoformans*
	Prolonged pulmonary infection Up to 800-fold greater dissemination to spleen and brain Reduced macrophage recruitment Traynor, J Immunol, 2000	*C. neoformans*
	Failure to control infection Block in infection-induced relocalization of splenic DC Sato, J Exp Med, 2000	*Leishmania major*
	Significantly decreased survival Macrophages exhibit recruitment defects to lungs 100-fold higher bacterial load in lungs Peters, PNAS, 2001	*Mycobacterium tuberculosis*
CCR4		
	Decreased mortality in endotoxic shock **Chvatchko, J Exp Med, 2000**	**LPS**
CCR5		
	Impaired macrophage function ANCE Reduced efficiency in bacterial clear Zhou, J Immunol, 1998	*Listeria monocytogenes*
	No protection against infection or death Elvin Nature, 2004	*Yersinia*
	Decreased survival Defect in leukocyte migration to brain Huffnagle, J Immunol, 1999	*C. neoformans*
	Lower parasite burden in liver **Sato, J Immunol, 1999**	**Leishmania donovani**

Table II. Continued

Knockout	Clinical and immunological consequence Reference[a]	Microbe
	Reduced macrophage infiltration **Glass, Virology, 2001**	**Mouse hepatitis virus**
	Antiviral T-cell response appears to be augmented **Nansen, Immunobiology, 2002**	**Lymphocytic choriomeningitis virus**
	Decreased susceptibility to Cryptosporidiosis **Campbell, J Parasitol, 2002**	**Cryptosporidium parvum**
	Decreased susceptibility to cerebral malaria **Belnoue, Blood, 2003**	**Plasmodium berghei**
IL8Rh/*CXCR2*		
	Dysfunctional neutrophil migration Godaly, J Immunol, 2000	*Escherichia coli*
	Subepithelial neutrophil entrapment and renal scarring Hang, J Infect Dis, 2000	*E. coli*
	Enhanced susceptibility to pyelonephritis Frendeus, J Exp Med, 2000 Frendeus, J Infect Dis, 2001	*E. coli*
	Impaired neutrophil recruitment Del Rio, J Immunol, 2001	*Toxoplasma gondii*
	Impaired neutrophil extravasation Increased bacterial burden Kielian, J Immunol, 2001	*S. aureus*
	Reduction in neutrophil recruitment Goncalves, Scand J Immunol, 2002	*Mycobacterium avium*
	Enhanced susceptibility to herpetic stromal keratitis Banerjee, J Immunol, 2004	HSV-1
	Decrease in Lyme arthritis severity *Brown, J Immunol, 2003*	***B. burgdorferi***
	Decreased mucus production and airway hyperreactivity *Miller, J Immunol, 2003*	***Respiratory syncytial virus***
CXCR5		
	Accelerated transfer of intraperitoneally administered prions into the spinal cord Prinz, Nature, 2003	Prions
DARC		
	Increased inflammatory infiltrates in lung and liver Dawson TC, Blood, 2000	LPS
Ligands		
CCL2		
	Reduced NKT cell recruitment Kawakami, J Immunol, 2001	*Cryptococcus neoformans*
	Enhanced susceptibility to gingivitis Chae, Infect Immun, 2002	*Streptococcus mutans*, *Streptococcus intermedius*, *Peptostreptococcus micros*, *Porphyromonas gingivalis*, *Prevotella intermedius*, *Fusobacterium nucleatum*

Table II. Continued

Knockout	Clinical and immunological consequence Reference[a]	Microbe
	Failure to expel infection deSchoolmester, J Immunol, 2003	*Trichuris muris*
CCL3		
	Reduced antiviral host defense Domachowske, J Immunol, 2000	Pneumonia virus
	Inhibited inflammatory and protective liver responses Salazar-Mather, J Exp Med, 1998	Murine cytomegalovirus
	Decreased resistance to infection Reduced NK cell accumulation Salazar-Mather, J Clin Invest, 2000	Murine cytomegalovirus
	Impaired survival Lindell, Infect Immun, 2001	*Klebsiella pneumonia*
	Decreased survival Olszewskin, J Immunol, 2000	*C. neoformans*
	Impaired prevention of eosinophilic pneumonia Olszewski, Infect Immun, 2001	*C. neoformans*
	Reduced protective innate immunity against sepsis Cecal ligation and puncture Takahashi, J Leuk Biol, 2002	*C. neoformans*
	Delayed viral clearance Trifilo, J Virol, 2003	Mouse hepatitis virus
	Lower parasite burden in liver **Sato, J Immunol, 1999**	***Leishmani donovani***
CCL11		
	Suppressed endotoxemia-associated peritoneal neutrophils Cheng, Exp Mol Pathol, 2002	LPS
CXCL15		
	Impaired pulmonary host defense Chen, J Immunol, 2001	*Klebsiella pneumonia*

Knockout mice (in bold) experienced *improved* survival advantage compared with wild-type mice.
[a]All references in this table can be found in the supplemental material available online for this article.

integrins binding to endothelial adhesion molecules (ICAM-1 and VCAM-1) (Bochner, 2000).

Chemokines exert their effect at two points in the extravasation process. First, they transiently activate integrins on the leukocyte surface, which results in enhanced avidity of the cell for the endothelial adhesion molecules (ICAM-1 and/or VCAM-1) (Constantin *et al*, 2000). In so doing, they facilitate the transition of leukocytes from fast to slow rolling and ultimately, to firm adhesion. Chemokines are presented to the rolling leukocyte bound to glycosaminoglycans present on the apical surface of endothelial cells. Second after transendothelial migration, the chemokine gradients found within the tissues determine where the leukocytes will localize, in conjunction with integrin-based adhesion signals that the leukocytes experience to extracellular matrix proteins.

Interestingly, it has recently been appreciated that several chemokines (CXCL12 and CCL26) mediate chemorepulsion through CXCR4 and CCR2, respectively (Poznansky *et al*, 2002; Ogilvie *et al*, 2003). This is thought to be relevant for leukocyte departure from tissue compartments such as the bone marrow and thymus.

The chemotactic actions of chemokines are thought to be critical for the recruitment and activation of leukocytes important in the innate immune response such as neutrophils, monocytes, DC, and NK cells as well as those involved in the adaptive immune response (naïve and memory CD4 and CD8 cells, and immature DC).

Innate immunity A key element in the initiation of the innate immune response is the detection of components com-

monly found on the invading pathogen that are not normally found on host cells. These components are constitutive and conserved products of microbial metabolism and include among others, LPS, lipoproteins, peptidoglycan (PGN), and unmethylated DNA containing a CpG motif (CpG-DNA). These pathogen-associated molecular patterns (or PAMP) are recognized by receptors of the innate immune system, which are referred to as pattern-recognition receptors (PRR), such as nucleotide-binding site leucine-rich repeat proteins (NOD1 and NOD2) and Toll-like receptors (TLR) (see accompanying Modlin review). The major inflammatory cytokines released by epithelial cells or DCs as a direct consequence of PRR signaling include IL-1, TNFα, Interferons, IL-4, IL-5, IL-6, IL-13, IL-17, and chemokines (Kopp and Medzhitov, 2003). Many of these molecules can have autocrine and paracrine effects, amplifying chemokine release by adjacent cells. When the innate response results in the production of a polarized (Th1 *vs* Th2) cytokine profile, a unique profile of chemokines is released and referred to as Th1 chemokines or Th2 chemokines (see below). Interestingly, the most potent stimuli for chemokine production by structural cells or APC are those that signal through myeloid differentiation factor 88 (MyD88) signaling (such as TLR, IL-1, and TNFα) (Kopp and Medzhitov, 2003), although even antimicrobial peptides, like the α defensins (human neutrophil peptide 1–3) have been shown to augment the expression and/or production of the neutrophil chemoattractants, CXCL8 and CXCL5, from bronchial epithelial cells (Yang *et al*, 2004).

The innate response is best characterized by the recruitment of leukocytes such as phagocytic granulocytes, monocyte/macrophages that are capable of engulfing or combating the pathogen with a variety of humoral mediators such as antimicrobial substances, activated components of the complement cascade, and cytokines. In general, neutrophils expressing CXCR1 and CXCR2 arrive early in response to specific CXC ligands containing the Glu–Leu–Arg amino acid motif in their NH_2 terminus, also called ELR^{+pos} CXC chemokines, such as CXCL8, CXCL5, and CXCL1 (Rot and von Andrian, 2004) (Figure 1). Mice lacking CXCR2 have a defect in neutrophil-mediated killing and are highly susceptible to infection with *Staphylococcus aureus*, *Mycobacterium avium*, and *Toxoplasma gondii* (Table II) (Del Rio *et al*, 2001; Kielian *et al*, 2001; Goncalves and Appelberg, 2002). The CC chemokines, CCL3 and CCL4, are not chemotactic for neutrophils *in vitro*, but promote local influx of neutrophils *in vivo*, which is CCR1 mediated (Lee *et al*, 2000). CCR1 appears to be involved in neutrophil-mediated host defense, as the CCR1 knockout mouse is more susceptible to infection with *Aspergillus fumigatus*, a ubiquitous fungus that causes invasive and highly lethal infections in humans and mice mainly when neutrophil number or function are impaired (Table II) (Gao *et al*, 1997). In contrast, CCR1 was not responsible for the neutrophil recruitment observed in the cornea in the helminth-mediated keratitis called Onchocerciasis or river blindness (Hall *et al*, 2001). Collectively, these findings suggest that the role CCR1 plays in neutrophil recruitment and function is organ specific. Monocytes and other mononuclear cells expressing CCR1, CCR2, and CCR5 arrive later primarily in response to CCL2, CCL3, and CCL5 (Rot and von Andrian, 2004).

NK cells are involved in the early protection against viral infection. These cells do not undergo genetic recombination events to increase their affinity for particular ligands, and are thus considered part of the innate immune system. NK cells are found predominantly in peripheral blood and spleen. Resting $CD56^{dim}$ NK cells do not express the chemokine receptor CCR7, which is important for cell homing to secondary lymphoid organs (Maghazachi, 2003). Consequently, NK cells are thought to migrate to peripheral non-lymphoid tissues. The expression of chemokine receptors by human NK cells is a subject of controversy. Campbell *et al* (2001) found high-level expression of CXCR1, CXCR4 and CX3CR1 and low-level expression of CXCR2 and CXCR3). In contrast, Inngjerdingen *et al* (2001), reported that purified, resting human NK cells expressed CXCR4 but not CXCR1, CXCR2, CXCR3, or CX3CR1. Functional studies have demonstrated that resting human NK cells migrate in response to ligands for CXCR3 (CXCL9-11), CXCR4 (CXCL12), XCR1 (XCL1), and CX3CR1 (CX3CL1), strongly suggesting that these receptors are in fact expressed (Inngjerdingen *et al*, 2001). Following *in vitro* activation, human NK cells upregulate CCR2, CCR4, CCR7, and CCR8 and exhibit increased chemotactic responses to known ligands for these receptors (Inngjerdingen *et al*, 2001). Macrophage-inducible protein (CCL3)-1α recruits NK cells toward the liver of cytomegalovirus-infected mice, resulting in increased inflammation and decreased susceptibility to infection with this virus (Salazar-Mather *et al*, 1998). In summary, chemokines not only recruit NK cells to inflammatory sites *in vivo*, but they potentiate NK cell-mediated killing (Taub *et al*, 1995).

Adaptive immunity The orchestration of the adaptive immune response is mediated by both homeostatic and inflammatory chemokines (Figure 1). The binding of the homeostatic chemokines CCL19 and CCL21 to CCR7 on naïve T cells, B cells, mature DC/LC (Langerhans cells) and $CD56^{bright}$ NK cells induce their migration to the T cell zone of secondary lymphoid organs (Ono *et al*, 2002). The B cells ultimately localize to the follicles of the lymph node in response to the homeostatic CXCR5 ligand, CXCL13. Therefore, the homeostatic chemokines are important for the development and maintenance of lymph node architecture (Muller *et al*, 2002). Inflammatory chemokines, on the other hand, are responsible for the recruitment of immature DC to sites of inflammation, and include ligands for CXCR1, CCR1, CCR2, CCR5, and CCR6 (Caux *et al*, 2000).

We recently demonstrated that epithelial cells express mRNA for all TLR, and that several known TLR ligands activate epithelial cells to express chemokines, cytokines, and host defense molecules, including acute-phase proteins and complement proteins (Sha *et al*, 2004). Among the induced genes were CCL20 (the CCR6 ligand) and GM-CSF, which would be expected to recruit and activate immature DC and LC that are important initiators of an adaptive immune response. It is interesting to note that keratinocyte expression of CCL20 is induced by IL-1 and TNFα and not surprisingly, CCL20 has been detected in the epidermis of skin biopsies from subjects with psoriasis, contact dermatitis, and mycosis fungoides, suggesting that these diseases develop in response to mi-

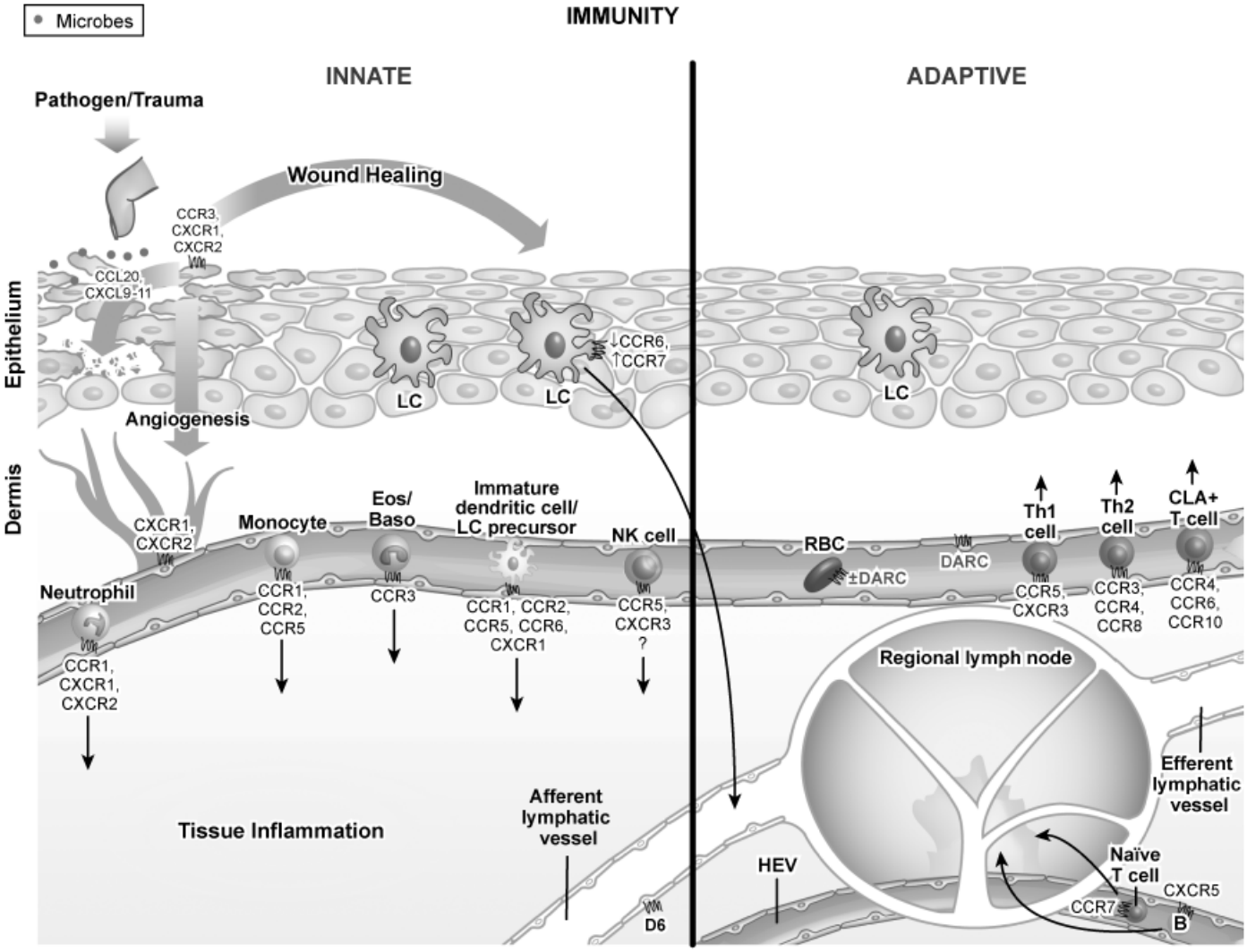

Figure 1
The Role of Chemokines in the Innate and Adaptive Immune Responses in the Skin. Chemokines orchestrate effects that impact both innate and adaptive immune responses. Microbial invasion or injury (*upper left*) initiates innate immune pathways at least in part through TLR signaling. This results in the release of inflammatory cytokines and chemokines from both structural cells (epithelial and fibroblasts) and APC. These mediators are responsible for the activation of the endothelium (e.g., upregulation of adhesion molecules) and the recruitment and activation of leukocytes critical for innate immune responses (neutrophils, eosinophils, basophils, NK cells, monocytes, and immature DC/LC precursors). Several chemokines produced by keratinocytes are also thought to act as antimicrobial peptides (CCL20, CXCL9-11), directly killing microbes. Keratinocytes express several chemokine receptors (CCR3, CXCR1, and CXCR2) which likely play a role in the wound repair response (epithelial proliferation, and chemotaxis) and release chemokines important for angiogenesis following an innate insult. When resident, immature LC/DC are exposed to a danger signal (e.g., pathogen or injury) they mature resulting in a reduction in CCR6 and enhanced CCR7 expression. This releases the LC/DC from effects of the keratinocyte-derived CCR6 ligand (CCL20), and enables them to respond to the CCR7 ligand (CCL21), released by lymphatic vessels and promotes their migration to the draining LN. Maintenance of the normal LN architecture under homeostatic or inflammatory conditions is largely due to the directional effects of chemokines released by stromal cells of the lymph node. The CCR7 ligands (CCL19, CCL21) are responsible for the recruitment of naïve T cells to the T-zone and the CXCR5 ligand (CXCL13) is responsible for recruitment of B cells and T helper cells to the B cell follicle. The polarization of T helper cells into Th1 *versus* Th2 cells may be in part determined by the relative effects of CCR5 or CCR2 ligands, respectively. The trafficking of these memory cells back to the tissue sites is also under chemokine control with Th1 cells responding to CXCR3 ligands (CCL9-11) and CCR5 ligands (CCL3-5) and Th2 cells responding primarily to the CCR4 and CCR8 ligands (CCL1, CCL17 and CCL22). The specific recruitment of skin homing memory T cells, identified by the surface marker, CLA is under the influence of the CCR10 ligand (CCL27) in *both* Th1- and Th2-mediated skin diseases. In the case of atopic dermatitis (Th2 polarized), CCR4 ligands (CCL17 and CCL22) seem to be critical and in the case of psoriasis (Th1 polarized), the CCR6 ligand (CCL20) seems to be pivotal. There are several known mechanisms responsible for containing the inflammatory response initiated by inducible chemokines and include among other things; decoy receptors (DARC on RBC and endothelial cells, D6 on lymphatic endothelium) and tissue enzymes which can cleave and in some cases inactivate chemokines.

crobial invasion or injury (Dieu-Nosjean *et al*, 2000; Schmuth *et al*, 2002).

In addition to its effects on immature DC, CCL20 has also been shown to induce the selective migration of bone marrow-derived LC precursors and freshly isolated LC as well as memory T cells to sites of inflammation. (Dieu-Nosjean *et al*, 2000; Homey *et al*, 2000). Interestingly, the CCR6-deficient mouse had reduced CD4+ cell recruitment in a contact hypersensitivity and DTH model but had no effect on epidermal or dermal LC numbers, suggesting that there may be other pathways to recruit LC to the skin under physiological conditions (Varona *et al*, 2001). Chemokine

ligands and receptors are also critical for the trafficking of epidermal LC to regional lymph nodes after a "danger signal" or inflammatory stimuli. After such a signal, LC undergo a maturation process that leads to enhanced CCR7 and the loss of CCR6 expression (Jakob *et al*, 2001). The loss of CCR6 is thought to release LC from the local CCL20-rich environment, and enables them to respond to CCR7 ligands such as CCL19 and CCL21, both of which are constitutively expressed in T cell areas of regional lymph nodes (Jakob *et al*, 2001). Studies in CCR7-deficient mice have clearly demonstrated that this receptor is indispensable for activation-induced but is also important for steady-state migration of LC into afferent lymphatics (Ohl *et al*, 2004). Migration under steady-state conditions is thought to be important in the maintenance of peripheral tolerance. CCL21 is also constitutively expressed on lymphatic vessels and blocking antibody studies confirmed the involvement of CCL21 in the entry of activated LC into lymphatic vessels (Saeki *et al*, 1999). In plt/plt mice, which carry a spontaneous mutation that abolishes CCL19 expression and restricts CCL21 expression to the lymphatic endothelium, LC and also naïve T cells fail to enter the T cell zone of secondary lymphoid organs (Gunn *et al*, 1999). Thus, migration of mature DC and LC may be regulated at the level of entry into into lymphatic vessels via upregulation of CCR7 and CCL19/CCL21. In summary, a switch in chemokine receptor expression promotes LC trafficking from sites of epidermal antigen uptake to lymphoid organs. The restricted set of chemokine receptors expressed by trafficking LC stands in striking contrast to the broader chemokine receptor panel of DC that appears under inflammatory conditions.

Several studies have demonstrated that human Th1 and Th2 cells differentially express chemokine receptors. The C-C chemokines, CCL1, CCL11, CCL17, and CCL22, selectively recruit Th2 lymphocytes. This chemotaxis is mediated primarily by expression of CCR4 and CCR8 on Th2 cells with a potential role for CCR3 in highly polarized cells (Ono *et al*, 2003). CCR5 and CXCR3 are preferentially expressed on Th1 cells producing IFN-γ, and as a consequence, they migrate in response to CCL3, CCL4, and CCL5 or the ELR^{-neg} CXC chemokines, respectively (Luther and Cyster 2001). Interestingly, memory T cells that home preferentially to the skin also express CCR4, regardless of their polarized phenotype (Th1 or Th2) (Andrew *et al*, 2001).

These skin-homing cells express a unique receptor called cutaneous lymphocyte-associated antigen (CLA) (Beck and Leung, 2000). This notion emerged from immunohistochemical evidence showing that the majority of the T lymphocytes infiltrating the skin in a wide variety of inflammatory and neoplastic conditions expressed CLA, whereas very few CLA^+ T cells are found at extracutaneous inflammatory sites (Beck and Leung, 2000). High expression of CCR4 has been found on skin-homing lymphocytes, and high levels of its ligands (CCL17 and CCL22) have been detected in the skin of a mouse model of atopic dermatitis (AD) (NC/Nga) and in skin biopsies and serum of AD subjects (Vestergaard *et al*, 1999; Galli *et al*, 2000; Kakinuma *et al*, 2001; Fujisawa *et al*, 2002). Interestingly, serum CCL17 levels are much higher in subjects with AD compared with psoriasis or healthy controls, suggesting that another chemokine receptor besides CCR4 may be utilized for memory T cell recruitment in a Th1-polarized disease (Kakinuma *et al*, 2001; Vestergaard *et al*, 2003). Both CCL20/CCR6 and CCL27/CCR10 have also been implicated in the skin homing of memory T lymphocytes (Morales *et al*, 1999; Homey *et al*, 2000). The marked upregulation of CCL20 and its receptor, CCR6, in psoriasis and the enhanced chemotactic response of psoriatic lymphocytes compared with normal controls to CCL20 suggest that CCL20/CCR6 may be more relevant for the Th1 lymphocyte recruitment observed in this disease (Homey *et al*, 2000). The C-C chemokine, CCL27, constitutively expressed by human keratinocytes, selectively induces the migration of CLA^+ T cells *in vitro* by binding to CCR10, and has been postulated to be involved in basal memory T cell trafficking to the skin (Morales *et al*, 1999). CCL27, however, can be upregulated by inflammatory signals (IL-1 and TNF-α) and not surprisingly, there is enhanced expression in inflammatory skin diseases (allergic contact dermatitis, AD, and psoriasis) (Homey *et al*, 2002). Therefore, it appears that CCL27/CCR10 are important for T cell homing to the skin regardless of whether the memory T cell is Th1 or Th2 polarized.

The importance of various chemokine ligands/receptor pairs in the host response to a wide variety of pathogens has been demonstrated in deficient mouse strains and has been recently reviewed (see Table II) (Le *et al*, 2002; Power, 2003). Similarly, several groups have demonstrated improved host survival as a result of overexpression of chemokines in murine infection models (Le *et al*, 2002). Although most of the literature supports the notion that chemokine/chemokine receptors are important in host defense, there are a few examples where chemokine/chemokine receptor-deficient mice have improved survival following pathogen exposure, suggesting that an overly brisk innate response may not always be advantageous (Table II, sections in bold).

It has been suggested that an exaggerated innate response may result in chronic inflammatory or autoimmune diseases (Kobayashi and Flavell, 2004). Several diseases that may develop as the consequence of an overly robust chemokine-mediated, inflammatory response include acute respiratory distress syndrome, glomerulonephritis, ischemia–reperfusion injury of the heart, viral mediated cardiomyopathies, and possibly even herpes simplex virus-associated erythema multiforme (Kobayashi and Flavell, 2004). Conversely, there are disease states where the innate response appears to be compromised, as has been observed in atopic dermatitis, where the epithelial production of antimicrobial peptides and the neutrophil chemoattractant, CXCL8, is inappropriately low compared with subjects with psoriasis (Ong *et al*, 2002; Nomura *et al*, 2003).

Microbicidal activity A critical feature of an effective innate immune response is the release of substances that have direct antimicrobial actions. A wide variety of host substances (e.g., hydrogen peroxide, RNase 7, nitric oxide, lactoferrin, lysozyme, dermcidin, psoriasin) have been shown to have antimicrobial activity in mammals but the antimicrobial peptides (defensins (α and β) and cathelicidins) are the best characterized (Schittek *et al*, 2001; Ganz, 2003; Glaser *et al*, 2005). These effector proteins are produced by many of the same stimuli (IL-1, TNFα, TLR ligands)

and cells (epithelial cells and phagocytic leukocytes) that are responsible for chemokine release. Their direct antimicrobial activity is mediated by the insertion of the peptide into the pathogens' cell wall, resulting in the formation of multiple pores and ultimately cell permeabilization or lysis (Ganz, 2003).

Defensins and chemokines have overlapping functions as they both have chemotactic activity. For example, many defensins are leukocyte chemoattractants at nanomolar concentrations, whereas tissue levels can reach the millimolar range (Yang *et al*, 2004). The α-defensins are chemotactic for monocytes, naïve $CD4^+$ T cells, $CD8^+$ T cells, and neutrophils and exert their effects by binding to a not yet identified, seven-transmembrane GPCR. The β-defensins recruit immature DC and memory $CD4^+$ T cells by interaction with CCR6, whereas the human cathelicidin, LL-37, chemoattracts neutrophils by binding to the seven-transmembrane GPCR, fMetLeuPhe (FMLP) receptor-1 (Oppenheim *et al*, 2003).

Both defensins and chemokines have antimicrobial activity. For example, truncated forms of the CXC chemokine, CXCL7, isolated from the α-granules of platelets, named thrombocidins, have antimicrobial activity against Gram-positive and -negative bacteria (Yang *et al*, 2004). The IFNγ-inducible ELR^{-neg} CXC chemokines (CXCL9-11) were found to have defensin-like activity against *Escherichia coli* and *Listeria monocytogenes* with minimal inhibitory concentrations (MIC) equal to, or 2–3-fold higher, than that necessary for the α defensin, human neutrophil peptide-1 (Cole *et al*, 2001). The antimicrobial activity was thought to be due to the high isoelectric point ($pI > 10.6$) and the specific distribution of positively charged residues. Based on a few shared structural motifs, and an abundance of cationic residues shared by β-defensins and CCL20, it has recently been shown that CCL20 also has antibacterial activity mainly against gram-negative bacteria at low μg per mL concentrations (Hoover *et al*, 2002). Recently, the CCR3 and CCR10 ligand, CCL28, expressed at high concentrations on mucosal surfaces of exocrine glands such as the parotid, salivary glands, and within milk and saliva, and was found to have antimicrobial activity against *Candida albicans*, Gram-negative, and -positive bacteria (Hieshima *et al*, 2003). Yang *et al* (2003) recently screened 30 human chemokines and demonstrated that about two-thirds of these have antibacterial activity.

Taken together, it appears that many chemokines demonstrate defensin-like antimicrobial activity. It remains to be established whether these chemokines achieve the *in vivo* concentrations necessary to exert their antimicrobial actions. This is indeed a likely scenario, as chemokine measurements from biological fluids significantly underestimate the levels achieved within tissue compartments, since chemokines are immobilized by glycosaminoglycans present on cell surfaces.

Cell activation Shortly after chemokines were recognized for their effects on cell motility, it was appreciated that they also had effects on cell activation. Their effects are numerous and best characterized on granulocytes. They include generation of oxygen radicals, lipid mediators, cytokines, chemokines, upregulation, and conformational changes of adhesion molecules, etc (Thelen, 2001). Leukocyte degranulation, which results in the release of myeloperoxidase, elastase, and lactoferrin from PMN, cationic proteins from eosinophils, histamine and chemokines from mast cells, and the respiratory burst from the macrophages, is important for a rapid and effective innate immune response. Additionally, chemokines also regulate NK cell proliferation and cytotoxicity. For example, CCL2-5, CCL7, CCL8, CXCL10, and CX3CL1 promote cytotoxic granule release, and the two known CCR7 ligands, CCL19 and CCL21, can costimulate IL-2-induced proliferation of $CD56^{dim}$ NK cells (Taub *et al*, 1995).

Chemokines are also thought to have effects on immature DC (those expressing inflammatory chemokine receptors) that may directly or indirectly alter their trafficking patterns and ultimately their functional state. This could have consequences for T helper cell polarization or even immune priming or tolerance, and has recently been reviewed (Kapsenberg *et al*, 2003).

Polarization of CD4+ T cells As noted above, chemokines are well recognized for their activation of myeloid cells and only more recently, has this been extended to their effects on T helper cell differentiation (Kapsenberg *et al*, 2003). Optimal clearance of the various pathogens encountered by the human body requires the selective activation of particular cellular and/or humoral immune responses. The balance of Th1 or Th2 cytokines determines the types of effector responses. The traditional paradigm is that the APC provides T cells not only with the antigen and costimulatory signals but also with a polarizing signal. Data recently reviewed suggest that the CCR5 ligands function similarly to IL-12, IL-23, and IL-27 in promoting the development of a Th1 phenotype, whereas CCR2 ligands have homology to IL-4 or IL-10 with regard to their capacity to polarize T helper cells toward Th2 differentiation (Kapsenberg *et al*, 2003). Interestingly, individuals with a CCR5 allele variant (CCR5Δ32), which leads to a non-functional receptor, have been shown to have reduced risk for the development of asthma, a prototypic Th2 disease (Hall, 1999).

Chemokine receptor expression has also become a marker of cell maturation, phenotype, and homing capacity for T lymphocyte and DC (Muller *et al*, 2002). As noted in the section on Cell recruitment, the interaction of chemokines with specific receptors on Th1 (CCR5, CXCR3) and Th2 (CCR4, CCR8, and CCR3) cells is thought to be critical to direct trafficking of these cells to tissue-specific areas where their biological actions would be most effective. In atopic dermatitis, a Th2-initiated disease, the percentage of $CCR5^+$ cells among circulating $CD4^+CD45RO^+$ T cells was found to be significantly reduced, and this correlated positively with the skin severity score and IFN-γ production (Okazaki *et al*, 2002). Conversely, in psoriasis, a Th1 condition, almost all of the skin-infiltrating $CD3^+$ cells expressed CCR5 (Uchida *et al*, 2002).

Effects on structural cells A coordinated and successful innate response to pathogens or injurious stimuli results in containment of the pathogen and tissue repair (Figure 1). Angiogenesis, or the growth of new blood vessels from preexisting vessels or capillaries, is a physiologic response that

arises in the context of an innate immune response, as it provides the tissue sites with a greater supply of relevant leukocytes and mediators necessary to neutralize the pathogen. Only recently have studies demonstrated that chemokines also communicate with structural cells (e.g., epithelial cells, endothelial cells, fibroblasts, and smooth muscle cells), and that this is the basis for their angiogenic, angiostatic, and wound repair actions.

Structural cells are thought to play a vital role in the regulation of leukocyte trafficking into organs such as the skin (Stellato and Beck, 2000). Our laboratory and others have demonstrated that these structural cells express a number of chemokine receptors, suggesting that chemokines may have autoregulatory or juxtaregulatory functions (Petering *et al*, 2001; Stellato *et al*, 2001; Loveless *et al*, 2003). Chemokine receptors have been identified on structural cells in normal as well as in inflammatory tissues: CCR3 immunoreactivity has been detected on airway epithelium from asthmatics, and patients with idiopathic hypereosinophilia (Stellato *et al*, 2003), and on keratinocytes from skin biopsies of AD subjects (Petering *et al*, 2001). *In vitro* studies have demonstrated that these receptors are functional, based on agonist-induced intracellular Ca^{2+} flux, or phosphorylation of downstream signaling proteins (Stellato *et al*, 2001; Eddleston *et al*, 2002; Viedt *et al*, 2002). The expression of these receptors can be regulated *in vitro* by inflammatory mediators such as TNFα, IL-1β, or LPS (Stellato *et al*, 2001; Eddleston *et al*, 2002; Lundien *et al*, 2002), indicating that the function they convey is likely to be altered by an innate immune response. Recent research demonstrates that the biological actions of chemokine receptors on structural cells include cell proliferation, chemotaxis, and the expression of proinflammatory and profibrotic genes.

The first function attributed to chemokine receptors expressed on structural cells was uncovered by Strieter *et al* (1992), who demonstrated that a member of the CXC chemokine subfamily CXCL8 was angiogenic. Subsequently, it was shown that ELR^{+pos} CXC chemokines, such as CXCL1, CXCL5, and CXCL8, promote angiogenesis, as well as neutrophil migration (see Leukocyte recruitment). It is interesting to note that angiogenesis is another biological action shared by antimicrobial peptides (LL-37) and chemokines (Elsbach, 2003). The interferon γ-induced, ELR^{-neg} chemokines, CXCL9, CXCL10, and CXCL11, which are CXCR3 agonists, instead display angiostatic properties. These chemokines were able to inhibit angiogenesis in nude mice, suggesting that these chemokines mediate their effects independent of their actions on T cell recruitment (Gurtsevitch *et al*, 1988).

Another biological process in which chemokine receptors appear to play an active role is wound repair. Disruption of the epithelial barrier triggers a multi-step process, in which the cells at the edge of the wound need to migrate, proliferate, and differentiate in an effort to restore the integrity of the skin or mucosal surface. Along these lines, CCR2 and CCR3 expressed on airway epithelial cells have been reported to mediate CCL2- and CCL24-induced proliferation and chemotaxis of the airway epithelial cells (Lundien *et al*, 2002; Stellato *et al*, 2003). Both effects appear to be specific, as blocking antibodies or small-molecule inhibitors abrogated them. Similarly, keratinocytes have been shown to proliferate in response to CXCR2 and CCR3 ligands (Metzner *et al*, 1999; Petering *et al*, 2001).

Gene expression induced by chemokine stimulation of structural cells provides further proof that these receptors are important in inflammation and wound repair. For example, gene array studies of epithelial cells stimulated with a CCR3 ligand have noted upregulation of numerous chemokines, proinflammatory cytokines, and growth factors indicating that chemokines can amplify proinflammatory pathways while providing key signals important for tissue repair/remodeling (Haley *et al*, 2000; Loveless *et al*, 2003; Stellato *et al*, 2003).

In conclusion, chemokine receptors on structural cells clearly mediate effects of the chemokine network that go beyond the regulation of leukocyte trafficking, and expand the role of these molecules to the involvement of processes equally relevant for chronic inflammatory diseases and the innate immune response, such as fibrosis, tissue remodeling, and angiogenesis.

Viruses: harnessing chemokines/chemokine receptors to subvert host defenses As part of evolution, many infectious agents have exploited the chemokine system to improve their survival in the host and enhance their dissemination. Viruses, more than any other microbial class, have taken lessons from host defense strategies to develop protein homologs of chemokines (vCk), homologs of chemokine receptors (vCkR) and unique viral products able to bind chemokines (vCkBP) that ultimately lead to a survival advantage for the virus (Liston and McColl, 2003).

Viral-encoded chemokine agonist and antagonists are expressed by large DNA viruses, specifically herpes and poxviruses (Liston and McColl, 2003). For example, the Kaposi's sarcoma-associated herpesvirus (HHV8) encodes a chemokine homolog, vMIP-II/K4, that acts as an agonist to CCR3-, CCR8- and CXCR2-expressing cells (Crump *et al*, 2001). This is likely done to promote HHV8 dissemination. But this same chemokine homolog, vMIP-II/K4, acts as an antagonist for cells expressing CCR1, CCR2, CCR5, CXCR4, XCR1, and CCR10 (Crump *et al*, 2001). Similarly, the HIV-1 transactivator protein (Tat) appears to mimic β-chemokines, by recruiting monocyte/macrophages toward HIV-producing cells, facilitating their infection (Albini *et al*, 1998). Cross-desensitization experiments suggest that Tat may share receptors with CCL2, CCL7, and CCL5. Additionally, Tat may also modulate the host response, by decreasing the release of anti-HIV chemokines such as CCL3 and CCL4 from uninfected T cells (Zagury *et al*, 1998).

Expression by the vCkR can serve several purposes. First, it allows the virally infected cell to migrate or proliferate in response to chemokines to which they would otherwise be unresponsive to. This can have obvious advantages, as it can help spread the virus to other regions of the body and lead to greater viral load. Second, the vCkR can act as a decoy receptor preventing the activity of the endogenous chemokine, thus interfering with a robust innate or adaptive immune response. Lastly, virally encoded chemokine receptors may have constitutive signaling activity. This may result in general cell activation or more specifically, could promote angiogenesis and oncogenesis (Liston and McColl, 2003).

Viral binding proteins, CkBP, are not homologs of host chemokine receptors. They are unique viral products with no host homology (Liston and McColl, 2003). For example, the poxvirus vCkBP-II binds CC chemokines with high affinity and thereby prevents interaction with their receptors, preventing recruitment of leukocytes important for the innate or adaptive immune response (Alcami *et al*, 1998). The herpesvirus protein, vCkBP, has even broader actions, binding to members of all chemokine subfamilies (C, CC, CXC, and CX3C) (Liu *et al*, 2000). In summary, virus-encoded chemokines are either antagonists that block leukocyte recruitment to sites of infection, or agonists that could enhance the recruitment of immune cells supporting viral replication.

It is also recognized that chemokine receptors can facilitate viral entry into permissive cells (Le *et al*, 2003). Probably the most well-known examples are the demonstration that HIV utilizes several chemokine receptors as co-receptors (along with CD4) for viral entry and infection (Berger *et al*, 1999) and the demonstration that *Plasmodium vivax* and *knowlesi* invade RBC by binding to the chemokine receptor, DARC (Miller *et al*, 1975). The chemokine receptor used varies based on the HIV isolate in general; CCR5 appears to be more important in primary HIV infection, whereas CXCR4 acts as a co-receptor during disease progression (Berger *et al*, 1999). This is supported by the finding that individuals who are either homozygous or heterozygous for the 32-bp deletion in the coding region of CCR5 are resistant to HIV infection or progress more slowly to AIDS, respectively (Berger *et al*, 1999). The fact that patients with this detection were otherwise healthy increased the enthusiasm for the pharmaceutical development of a CCR5 antagonist as an anti-HIV therapy, which is undergoing human trials (Scozzafava *et al*, 2002).

Conclusion

The discovery of the chemokine family was a revelation for investigators studying leukocyte recruitment. Finally, the unique footprint of leukocytes recruited in each inflammatory disease could be explained. It became increasingly clear that chemokines probably orchestrate the trafficking of almost all cells found in the body, but also that they possess many other key biological effects. Few of these effects are more important than those directed at containing pathogens and responding to injury as outlined in this review. Nevertheless, it has been suggested that an exaggerated innate response may result in chronic inflammatory or autoimmune diseases. The pluripotency of chemokine actions suggests that they are central players in a variety of inflammatory diseases, as well as in host defense, wound repair, and tumorigenesis. To date, pharmacological efforts have primarily focused on developing chemokine and chemokine receptor antagonists as anti-inflammatory drugs or as means to prevent HIV entry (Scozzafava *et al*, 2002). The first human study to test the utility of a chemokine receptor antagonist in a chronic inflammatory disease tested the effect of a CCR1 antagonist in adults with rheumatoid arthritis (Haringman *et al*, 2003). In this 14-d study, they observed a significant reduction in CCR1-bearing leukocytes (CD4+, CD8+, and macrophages [CD68+]) in the synovial tissues and a modest clinical improvement (Haringman *et al*, 2003).

It remains to be seen whether the clinical consequences of such drugs may also bring broader, unexpected effects, such as an increased risk for infections. The idea that inflammatory chemokines are, to some extent, redundant would predict that the use of a single chemokine or chemokine receptor antagonist might have little effect on immunity. On the other hand, we may find that a more prolonged use of these agents will compromise the immune response to a focused group of microbes, possibly only when they invade a specific mucosal surface. Will chemokine receptor antagonists affect a patient's ability to mount a Th1 or Th2 response? Will some anti-chemokine strategies prevent post-infection organ damage that too are the result of an overly brisk innate or adaptive immune response? The answers to at least some of these questions are forthcoming as chemokine antagonists are developed for treatment of inflammatory diseases, and as recombinant chemokines are utilized as vaccine adjuvants and anticancer therapies.

This work has been supported in part by NIH Grant AI 045839 (L. A. B.), NIH Grant AI 050530 (L. A. B.), the American Skin Association (C. E.), and NIH Grant AI 044242 (C. S.). We acknowledge the administrative assistance of Ms Bonnie Hebden.

Supplementary Material

The following material is available online for this article.
Supplementary references.

DOI: 10.1111/j.0022-202X.2005.23841.x

Manuscript received July 1, 2004; revised April 21, 2005; accepted for publication April 25, 2005

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