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Chemokines, Chemokine Receptors, and Allograft Rejection

Review

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Introduction

The emigration of leukocytes from the peripheral circulation into an allograft is an essential component of organ transplant rejection. Ischemic damage and surgical trauma set the stage for early leukocyte infiltration and activation, leading in turn to the recruitment of additional effector leukocytes and the propagation of damage to the graft. Productive alloreactivity in vivo also requires processes controlled within secondary lymphoid organs (Lakkis et al., 2000). The migration of dendritic cells from the allograft into secondary lymphoid tissue is of paramount importance to the rejection process (Lakkis et al., 2000). The biology of chemokines underlies both leukocyte recruitment and important aspects of the adaptive immune response.

Chemokines were first characterized by their ability to induce migration of leukocytes (Segerer et al., 2000; Zlotnik and Yoshie, 2000). This family of chemotactic cytokines has subsequently been shown to play important roles in the control of leukocyte recruitment, activation, and effector function, as well as hematopoiesis, the modulation of angiogenesis, and aspects of adaptive immunity (Keane and Strieter, 1999; Campbell and Butcher, 2000; Murphy et al., 2000; Rossi and Zlotnik, 2000; Sallusto et al., 2000; Segerer et al., 2000). These diverse biologic actions underlie many of the acute and chronic processes that make up allograft dysfunction.

To date, 44 chemokines and 21 chemokine receptors have been described (Murphy et al., 2000; Rossi and Zlotnik, 2000; Segerer et al., 2000) (Tables 1 and 2). The separation of this chemokine superfamily into four branches (C, CC, CXC, and CX_3C) is based upon the position of the first two cysteine residues in a four-cysteine motif in their primary amino acid sequence and whether or not they are separated by additional amino acids (designated as X). A subgroup of CXC chemokines displays the additional E-L-R-CXC amino acid motif (glutamic acid-leucine-arginine-cysteine-X-cysteine) (ELR^+) (Table 1). These chemokines generally act as neutrophil chemoattractants, while the CXC ELR^- chemokines bind a different set of CXC receptors and are more active on lymphocytes. While most of the chemokines belong to the CC or CXC classes, two additional branches, C and CX_3C chemokines, have also been described (reviewed by Murphy et al., 2000; Rossi and Zlotnik, 2000). Lymphotactin-α/XCL1 and lymphotactin-β/XCL2 are C chemokines that share homology at their carboxyl end with the CC chemokines but lack the first and third cysteines in the four-cysteine motif (Murphy et al., 2000; Rossi and Zlotnik, 2000). Fractalkine/CX_3CL1, the only CX_3C chemokine described, has three intervening amino acids between the first two cysteine residues. It is tethered directly to the cell membrane via a mucin stalk and combines the functions of both a chemokine and an adhesion molecule. Fractalkine/CX_3CL1 can induce cell adhesion and migration as either a membrane-tethered or shed-soluble ligand (Murphy et al., 2000; Rossi and Zlotnik, 2000).

Chemokines can be further classified according to function and regulation of expression (Murphy et al., 2000; Zlotnik and Yoshie, 2000). Some chemokines are important in the control of inflammatory processes, while others are involved in normal trafficking of leukocytes through primary and secondary lymphoid organs (Campbell and Butcher, 2000; Murphy et al., 2000; Rossi and Zlotnik, 2000; Zlotnik and Yoshie, 2000). The "inflammatory" chemokines are regulated by proinflammatory stimuli and help orchestrate innate and adaptive immune responses. The second group contains those chemokines involved in homeostatic activity important in lymphocyte and dendritic cell trafficking during immune surveillance (Table 1). The homeostatic chemokines are generally thought to be "constitutively" expressed (Campbell and Butcher, 2000; Murphy et al., 2000; Zlotnik and Yoshie, 2000).

Chemokine Receptors

The actions of chemokines are mediated through a large family of seven-transmembrane-spanning serpentine Gi/Go protein–coupled receptors that are sensitive to Pertussis toxin (Murphy et al., 2000; Rossi and Zlotnik, 2000; Sallusto et al., 2000) (Figure 1). Each chemokine receptor has a distinct chemokine specificity and a restricted expression for subclasses of leukocytes (Table 2). Nevertheless, the ligand specificities of the receptors can overlap, as some chemokines bind to multiple receptors, and some receptors can bind multiple chemokines (Murphy et al., 2000; Segerer et al., 2000; Zlotnik and Yoshie, 2000). Receptors can be specific and bind only one ligand, or they can share ligands from the same general family. The Duffy antigen, also a chemokine binding protein, can bind members of both the CXC and CC chemokine subfamilies (Table 2) (Murphy et al., 2000; Segerer et al., 2000; Zlotnik and Yoshie, 2000). In general, the proinflammatory chemokine receptors tend to have more promiscuous ligand binding specificities, while the receptors involved in normal leukocyte traffick-

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Table 1. Chemokines: Names and Actions

CC	-ELR-	H/I	Other Nomenclature
CCL1	NA	I	I-309, TCA3, P500
CCL2	NA	I	MCP-1, MCAF, (mouse; JE)
CCL3	NA	I	LD78α, LD78β, MIP-1α
CCL4	NA	I	Act-2, G-26, HC21, H400, MIP-1β, LAG-1, SISγ, MAD-5
CCL5	NA	I	RANTES
CCL7	NA	I	MCP-3
CCL8	NA	I	MCP-2, HC14
CCL11	NA	I	eotaxin
CCL12	NA	I	(mouse) MCP-5
CCL13	NA	I	MCP-4, NCC-1, CKβ10
CCL14	NA	I	HCC-1, HCC-3, NCC-2, CKβ1, MCIF
CCL15	NA	I	HCC-2, NCC-3, MIP-5, Lkn-1, MIP-1δ
CCL16	NA	I	NCC-4, LEC, HCC-4, LMC, LCC-1, CKβ12
CCL17	NA	H	TARC
CCL18	NA	H?	DC-CK1, PARC, MIP-4, CKβ7, DCCK1
CCL19	NA	H	ELC, MIP-3β, exodus-3, CKβ11
CCL20	NA	H	MIP-3α, LARC, exodus-1, ST38, CKβ4
CCL21	NA	H	SLC, 6Ckine, exodus-2, TCA4, CKβ9
CCL22	NA	H	MDC, STCP-1, DC/B-CK
CCL23	NA	I	MIP-3, MPIF-1, CKβ8
CCL24	NA	I	MPIF-2, CKβ6, eotaxin-2
CCL25	NA	H	TECK, Ckβ15
CCL26	NA	I	eotaxin-3, IMAC, MIP-4α, TSC-1
CCL27	NA	H	ALP, skinkine, ILC, ESkine, PESKY, CTAK
CCL28	NA	H	MEC, CCK1
CXC	**-ELR-**	**H/I**	**Other Nomenclature**
CXCL1	ELR^+	I	GROα, MGSA-α, NAP-3, (mouse/rat; KC, MIP-2, CINC-2β)
CXCL2	ELR^+	I	GROβ, MIP-2α, MGSA-β, CINC-2α
CXCL3	ELR^+	I	GROγ, MIP-2β, CINC-2β
CXCL4	ELR^-	I	PF4
CXCL5	ELR^+	I	ENA-78
CXCL6	ELR^+	I	GCP-2
CXCL7	ELR^+	I	CTAPIII, NAP-2, LA-PF4, MDGF, LDGF, β-TG
CXCL8	ELR^+	I	IL-8, NAP-1
CXCL9	ELR^-	I	mig
CXCL10	ELR^-	I	IP-10
CXCL11	ELR^-	I	I-TAC
CXCL12	ELR^-	H	SDF-1α, SDF-1β, PBSF
CXCL13	ELR^-	H	BLC, BCA-1
CXCL14	ELR^-	I	BRAK, bolekine, MIP-2γ, BMAC, KS1
CXCL15	ELR^+	H	lungkine
CXCL16	ELR?	?	SR-Psox
C	**-ELR-**	**H/I**	**Other Nomenclature**
XCL1	NA	I	Lymphotactin, SCM-1α, ATAC
XCL2	NA	I	SCM-1β
CX_3C	**-ELR-**	**H/I**	**Other Nomenclature**
CX_3CL1	NA	I	Fractalkine, neurotactin

Chemokines (common and official names), the presence of ELR-CXC motifs, and homeostatic (H) versus inflammatory (I) designation, based on chemokine expression and receptor specifity. NA, not applicable. For definitions of the various acronyms, see Murphy et al. (2000) (see also http://cytokine.medic.kumamoto-u.ac.jp/ for detailed up-to-date information on the various chemokines).

ing have relatively few ligands. The nomenclature used to describe the individual chemokine receptors is based upon the class of chemokine ligands that interacts with the receptor (i.e., C, CC, CXC, and CX_3C receptors) (Murphy et al., 2000; Rossi and Zlotnik, 2000).

Chemokine receptors generally undergo internalization and phosphorylation following ligand binding. The binding of a chemokine ligand to its receptor activates Gα protein subunits. The subsequent Gi or Gq signal transduction cascade leads to the activation of phospholipase Cα1 and α2 and the generation of inositol (1,4,5)-trisphosphate and diacylglycerol (Murphy et al., 2000; Segerer et al., 2000). This brings about a transient rise in intracellular calcium and subsequent activation of protein kinase C. A series of protein kinases are then involved in downstream signal transduction cascades, including serine/threonine kinases (e.g., members of the MAP kinase cascade) and tyrosine protein kinases (Murphy et al., 2000; Segerer et al., 2000).

Chemokine regulation of leukocyte migration appears to occur in a complex milieu of chemotactic signals where several receptors may be triggered simultaneously or successively. Within this complex environment of receptor cross-talk and desensitization, the migrating

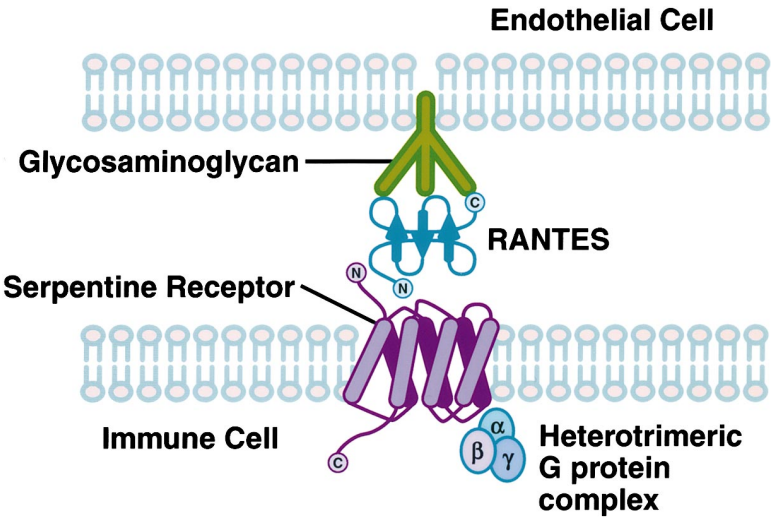

Figure 1. Multimolecular Complex in Chemokine Action

A multimolecular complex is generally involved in chemokine action. For example, the chemokine RANTES/CCL5 can be presented by a glycosaminoglycan moiety on the endothelial cell surface to a seven-transmembrane-spannning G protein–associated serpentine receptor expressed on the surface of immune cells.

leukocyte must distinguish a hierarchy of signals within the tissue to reach the site of inflammation (Foxman et al., 1997; Hancock et al., 2000a; Segerer et al., 2000).

As a further complication of this already baroque biology, at least some chemokine receptors form homo- or heterodimers, and it appears that posttranslationally modified receptor variants exist (Rodriguez-Frade et al., 1999; Murphy et al., 2000; Nelson et al., 2001). It has been suggested that heterodimers of receptors could add considerable flexibility to the overall functional significance of receptor–ligand interactions by allowing a different function for heterodimers relative to that for

Table 2. Chemokine Receptors, Chemokines Reported to Bind, and Cell Types Expressing the Receptor

Chemokine Receptor	Chemokine	Receptor-Expressing Cell Type
CCR1	CCL3, CCL5, CCL7, CCL8, CCL13, CCL14, CCL15, CCL23	monocyte, dendritic cell (immature), T cell, neutrophil, eosinophil, mesangial cell, platelet
CCR2	CCL2, CCL7, CCL8, CCL13	monocyte, dendritic cell (immature), basophil, T cell, natural killer cell, endothelial cell, fibroblast
CCR3	CCL5, CCL7, CCL8, CCL11, CCL13, CCL14, CCL15, CCL24, CCL26	eosinophil, basophil, Th2 T cell, dendritic cell, platelets
CCR4	CCL17, CCL22	dendritic cell (immature), basophil, Th2 T cell, platelets
CCR5	CCL3, CCL4, CCL5, CCL8, CCL11, CCL13, CCL14	Th1 T cell, dendritic cell (immature, mature), monocyte, natural killer cell, thymocyte
CCR6	CCL20	dendritic cell (immature), T cells, B cells
CCR7	CCL19, CCL21	dendritic cell (mature), T cell, B cell
CCR8	CCL1, CCL16	monocyte, B cell, T cell, thymocyte
CCR9	CCL25	T cell, thymocyte
CCR10	CCL27, CCL28	T cell, melanocytes, dermal endothelium, dermal fibroblast, Langerhans cells
CCR11	CCL19, CCL21, CCL25	astrocyte
CXCR1	CXCL5, CXCL6, CXCL8	neutrophil, monocyte, astrocytes, endothelium
CXCR2	CXCL1, CXCL2, CXCL3, CXCL5, CXCL7, CXCL8	neutrophil, monocyte, eosinophil, endothelium
CXCR3	CXCL9, CXCL10, CXCL11	Th1 T cell, B cell, mesangial cell, smooth muscle cell
CXCR4	CXCL12	T cell, dendritic cell (immature, mature), monocyte, B cell, neutrophil, platelet, astrocyte
CXCR5	CXCL13	T cell, B cell, astrocyte
CXCR6	CXCL16	
XCR1	XCL1, XCL2	T cell
CX_3CR1	CX_3CL1	natural killer cell, T cell, astrocyte
Duffy	CXCL8, CCL1, CCL5, CXCL1, CXCL7	red blood cells, endothelium
D6	CCL2, CCL4, CCL5, CCL8, CCL13, CCL14, CCL15	B cell

Chemokines, chemokine receptors, their ligand specificity, and tissue distribution (Murphy et al., 2000; Nelson et al., 2001) (see http://crf.medic.kumamoto-u.ac.jp for recent updates on chemokine/receptor information).

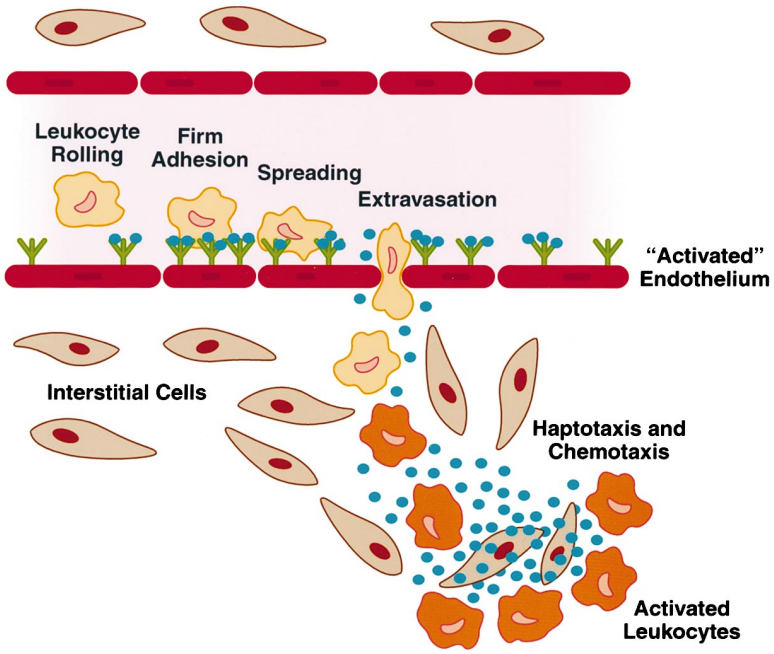

Figure 2. Multistep Process in Generation of Inflammatory Infiltrate in Transplant Rejection

A series of chemokine gradients are thought to be required for the recruitment of inflammatory cells from the bloodstream into sites of inflammation.

homodimers or through altered sensitivity to chemokine stimulation. Posttranslational modification of receptors could also yield a cell type- or activation-specific form of chemokine receptor (Murphy et al., 2000; Nelson et al., 2001).

Chemokines in Transplantation

Transplantation research is important not only for its clinical relevance but also as a tool to identify and dissect more general aspects of immune regulation. One of the benefits of animal models of transplantation is that, by adjusting the MHC disparity between the graft and host, one can regulate the severity of the rejection process. In addition, both acute and chronic inflammatory events can be studied, and the disease process is initiated at the time of transplantation, thus allowing precise kinetic monitoring of events.

Chemokines and Leukocyte Recruitment into Allografts

Following transplantation, ischemic injury enhanced by reperfusion of the allograft is associated with microvascular stress and trafficking of leukocytes into microvascular spaces (Ambrosio and Tritto, 1999). The multistep process of effector leukocyte recruitment into the allograft involves a series of interactions between molecules expressed on the leukocyte and the endothelial surface (Butcher and Picker, 1996; Campbell and Butcher, 2000; Segerer et al., 2000) (Figure 2).

Stressed cells (for example, within kidney grafts) produce increased oxygen and nitric oxide radicals (Calo et al., 2000). This in turn leads to the production and release of inflammatory mediators such as platelet-activating factor and tumor necrosis factor and enhances the expression of adhesion molecules that mediate leukocyte rolling and firm adhesion to the endothelium (Butcher and Picker, 1996; Calo et al., 2000; Segerer et al., 2000). During selectin-mediated rolling, the leukocyte integrins and endothelial immunoglobulins remain in an unactivated state (Butcher and Picker, 1996; Campbell and Butcher, 2000). Specific chemokines either generated by activated endothelial cells or released following platelet activation bind to the surface of "activated" endothelium. Endothelium is an early discriminator of leukocyte infiltration, as different endothelial cells show altered ability to present individual chemokines (Grone et al., 1999; von Hundelshausen et al., 2001). Chemokines presented by the endothelium are thus ideally situated to direct and sort leukocytes through the allograft parenchyma by triggering the activation of the leukocyte-expressed integrins, resulting in shear-resistant, firm adhesion of the leukocyte to the endothelial surface (Butcher and Picker, 1996; Weber et al., 1999; Segerer et al., 2000; von Hundelshausen et al., 2001; Weber et al., 2001). Additional chemokines appear to then influence subsequent events associated with leukocyte emigration, including spreading, diapedesis, and extravasation (Weber et al., 1999) (Figure 2).

The release of microparticles from eukaryotic cells is a well-recognized phenomenon (Mack et al., 2000). It was recently shown that microparticles containing CCR5 can transfer the receptor from peripheral blood mononuclear cells to endothelial cells during transendothelial migration. Thus, intercellular transfer of chemokine receptors by microparticles might have broad implications for intercellular communication (Mack et al., 2000).

A specialized role of apparently redundant receptors in early stages of leukocyte trafficking was recently demonstrated (Weber et al., 2001). Monocytes and Th1-like T cells can express both CCR1 and CCR5, which in turn share ligand specificities, including RANTES/CCL5. The role of these receptors in the selective recruitment of monocytes and Th1 T cells was tested in an in vitro system where firm arrest under flow conditions was triggered by RANTES/CCL5 immobilized to endothelium.

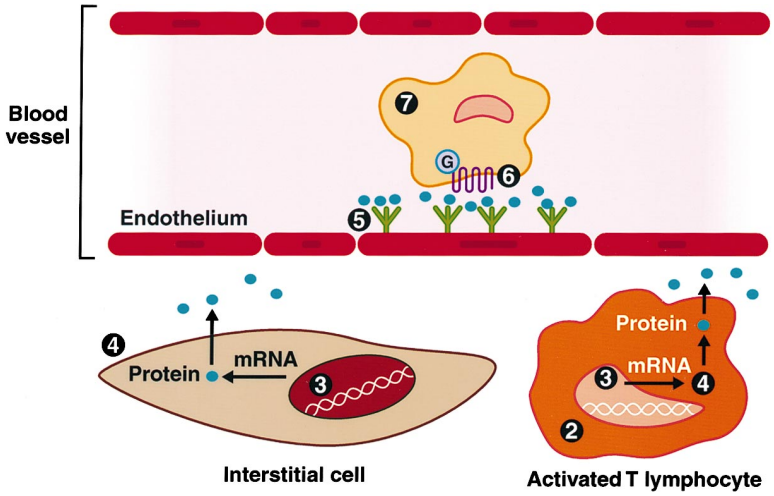

Figure 3. Potential Sites for Therapeutic Intervention in Chemokine Action

(1) Proinflammatory cytokine induction in stromal cells (TNF or IL-1 inhibitors; not shown), (2) early T cell activation (classical immunosuppressives like cyclosporin, tacrilomus, or anti-CD3), (3) gene expression (corticosteroids, novel transactivators), (4) translation and secretion (select cytostatics), (5) binding to endothelium (heparin), (6) serpentine receptors (CCR1 or CXCR3 receptor blockade), and/or (7) G proteins and downstream signal (select kinase inhibitors).

Using selective receptor antagonists, it was found that RANTES-induced arrest of these cells was predominantly mediated by CCR1, while CCR5 mainly contributed to leukocyte spreading in shear flow. Nevertheless, both CCR1 and CCR5 could support transendothelial chemotaxis toward RANTES/CCL5. Thus, not all chemokine receptors expressed by effector leukocytes are necessarily involved in direct recruitment from the peripheral circulation. Understanding the functional role of individual chemokine receptors in leukocyte adhesion, emigration, and effector cell activation will be beneficial in the development and application of specific chemokine receptor antagonists for transplant rejection (Figure 3).

Chemokines have additional biological activities that are also relevant to diverse processes that contribute to allograft rejection. The ELR^+ CXC chemokines IL-8/CXCL8, ENA-78/CXCL5, and GRO-α/CXCL1 act as angiogenic agents, while the ELR^- chemokines PF4/CXCL4, IP-10/CXCL10, Mig/CXCL9, and SDF-1/CXCL12 are angiostatic factors (Keane and Strieter, 1999; Hancock et al., 2000a; Murphy et al., 2000). Thus, for example, during chronic rejection, the expression of chemokines could influence the microvasculature within the allograft.

In addition, leukocytes extravasate by partially digesting and loosening the basement membrane. Chemokines induce T cell and monocyte secretion of the matrix metalloproteinases required for migration through basement membranes (Segerer et al., 2000). This phenomenon could promote the degradation of matrix and the perturbation of tissue homeostasis and thus enhance tissue damage in acute and chronic allograft rejection.

The functional activity of chemokines can also be modified through posttranslational proteolytic processing (Van Damme et al., 1999). Dipeptidyl peptidase IV enzymes, such as CD26, can cleave chemokines that share a conserved NH_2-X-Pro (where X is any amino acid) sequence at their amino terminus. The enzymes cleave after the Pro residue, resulting in a two amino acid deletion. This modification can alter the receptor specificity of the chemokine. The chemokine substrates for these enzymes include MCP-2/CCL2, IP-10/CXCL10, RANTES/CCL5, and eotaxin/CCL11 (Van Damme et al., 1999). An additional example of protease regulation of chemokine function is the cleavage of MCP-3/CCL7 by matrix metalloproteinase gelatinase A (MMP2) (McQuibban et al., 2000). The cleavage product binds to CCR1, CCR2, and CCR3 and acts as a potent antagonist that suppresses inflammation. This suggests that matrix metalloproteinases are both effectors and regulators of the inflammatory response (McQuibban et al., 2000) and that proteolytic processing may provide an additional level of control for differential cell recruitment and for the regulation of target cell specificity. Thus, the interpretation of in situ hybridization or immunohistochemical data may be difficult, as the presence of a given chemokine does not necessarily prove that it is acting to specifically recruit leukocytes. Through proteolytic processing, for example, a chemokine could be acting as a selective antagonist.

Dendritic Cells, Chemokines, Chemokine Receptors, and T Cell Responses

Dendritic cells appear to play dual roles in the immune response to allografts, both as promoters of rejection and mediators of tolerance (Sallusto et al., 2000). Dendritic cells derived from the allograft can act as potent inducers of an alloresponse. In response to signals produced during inflammation, dendritic cells undergo maturation leading to migration to secondary lymphoid organs such as spleen, lymph nodes, and mucosal lymphoid tissues where they present their complement of MHC-associated antigens. Secondary lymphoid tissues provide the environment for dendritic cells to selectively activate naive T and B lymphocytes. The importance of this process in allograft rejection was recently demonstrated by Lakkis et al., who showed that cardiac allografts could be accepted indefinitely in recipient mice that lack secondary lymphoid tissue, suggesting that the alloimmune response to a vascularized organ transplant requires trafficking of dendritic cells to secondary nodes (Lakkis et al., 2000).

Immature dendritic cells express inflammatory chemokine receptors CCR1, CCR2, CCR5, and CXCR1. This group of receptors helps account for the ability of these cells to migrate into inflamed tissues (Murphy et al., 2000; Sallusto et al., 2000; Sozzani et al., 2000). During maturation, these receptors decrease in expression, and

the chemokine receptors that help direct the mature dendritic cell to the lymphoid environments, CCR4, CXCR4, and CCR7, increase in expression (Murphy et al., 2000; Sallusto et al., 2000; Sozzani et al., 2000). In this regard, CCR7 appears to play a pivotal role in migration of dendritic cells from inflamed tissue to secondary lymphoid tissue (Forster et al., 1999). In contrast, CCR6 appears to be a mucosa-specific regulator of humoral immunity and lymphocyte homeostasis (Cook et al., 2000).

Within the secondary lymphoid organs, chemokines may also act to position antigen-loaded dendritic cells and antigen-specific T cells for efficient cell–cell communication. In a reciprocal manner to dendritic cells, activation of naive T cells results in decreased expression of chemokine receptors such as CXCR4 and CCR7 and increased expression of inflammatory receptors, including CCR5, CCR3, CXCR3, and CCR8 (Murphy et al., 2000; Sallusto et al., 2000; Sozzani et al., 2000). This switch in receptor expression facilitates the migration of the activated T cells to sites of inflammation. Subsequent selective tissue homing to specific anatomic sites has also been correlated with the expression of unique chemokine receptor patterns (Campbell and Butcher, 2000).

Chemokine receptor expression is also associated with the events surrounding the outcome of an adaptive immune response (Murphy et al., 2000; Sallusto et al., 2000; Sozzani et al., 2000). Th1-like and Th2-like responses represent the extremes of a spectrum for a potential immune response. The Th1 subtype produces strong cellular immune responses that are stimulated by pathogens that invade and inhabit cells and result in an activation of cytotoxic T lymphocytes and delayed-type hypersensitivity. Allograft rejection is mediated in part by Th1-like processes. The Th2-like subtypes evoke strong antibody responses. In addition, the Th2-derived cytokines can suppress the proinflammatory reactions induced by the Th1 cytokines (Murphy et al., 2000; Sallusto et al., 2000; Sozzani et al., 2000). Thus, the Th1/Th2 balance can either elicit immunity or reinforce tolerance.

Differential expression of chemokine receptors has been linked to the development of a Th1- or Th2-type immune response. While the patterns are not absolute, Th1-like cells appear to preferentially express the chemokine receptors CXCR3 and CCR5, while Th2 cells generally display CCR4 and CCR8, with a subpopulation that also expresses CCR3 (Murphy et al., 2000; Sallusto et al., 2000; Sozzani et al., 2000). Thus, the chemokine receptors expressed by different populations of T cells may be linked to tissue infiltration of Th1 versus Th2 cells and/or the functional response of receptor-expressing cells, thus determining the ultimate outcome of an immune response (Hancock et al., 2000a; Sallusto et al., 2000; Segerer et al., 2000; Sozzani et al., 2000; Nelson et al., 2001).

Chemokine and Chemokine Receptor Expression by Nonlymphoid Tissues

The biology of chemokines has become more complicated with the demonstration that nonhematopoietic tissues also express functional chemokine receptors. For example, human mesangial cells express functional CXCR3, CCR1, and CCR7 (reviewed in Nelson et al., 2001). In addition, endothelial cells, epithelial cells, microglial cells, and neurons can express chemokine receptors (Keane and Strieter, 1999; Murphy et al., 2000; Rossi and Zlotnik, 2000). Although the overall function of chemokine receptor expression by nonhematopoietic cells remains incompletely understood, it may play a role in angiogenesis, atherosclerosis, and wound healing. It is of interest that chemokines and their receptors are expressed differentially during renal ontogeny (CXCR3, IP-10/CXCL10, CXCR4, SDF-1/CXCL12, Fractalkine/CX_3CL1, CX3CR1, CCR7, and SLC/CCL21) (reviewed by Nelson et al., 2001). As genes that are expressed differentially during ontogeny often play a role in tissue regeneration, these embryonal chemokine/chemokine receptor patterns may reflect the role of these factors in renal injury and repair, a potentially important issue when considering chronic blockade of chemokine receptors as a means of therapeutic intervention (see below).

Regulation of Chemokine Expression

Inflammatory chemokines are generally found in two clusters on human chromosomes 4 (CXC) and 17 (CC) (Murphy et al., 2000; Segerer et al., 2000). Due to genomic clustering of these genes, it has been suggested that there may be coordinate regulation at both the individual and regional levels analogous to that seen for other gene family clusters (Murphy et al., 2000; Segerer et al., 2000; Nelson et al., 2001). It is now thought that, rather than acting as single molecules, inflammatory chemokines may be functionally coregulated in groups that may in turn activate common chemokine receptors (Murphy et al., 2000; Segerer et al., 2000). This apparent redundancy reflects a complexity that could provide a high degree of flexibility in vivo and may explain why chemokine and chemokine receptor knockouts often do not display pronounced phenotypes (Segerer et al., 2000; Nelson et al., 2001).

Inflammatory chemokines are regulated at multiple levels, involving transcriptional, posttranscriptional, translational, and posttranslational events (Segerer et al., 2000). IL-1β, IFN-γ, or TNF-α alone or in combination induce many of the proinflammatory chemokines (Segerer et al., 2000). CXC chemokines, Mig/CXCL9 and IP-10/CXCL10, were initially identified on the basis of their selective induction by IFN-γ (Murphy et al., 2000). RANTES/CCL5 is of particular interest as an example of this specialized regulation. Although it, like many other CC chemokines, is expressed as an immediate early gene in fibroblasts, epithelial cells, and endothelial cells within minutes of stimulation by proinflammatory cytokines like IL-1β and TNF-α, it is expressed "late," 3–5 days after activation, in T lymphocytes (Song et al., 2000). This late expression kinetic appears to help amplify the immune response in both time and space. Inflammatory cells first enter the site of inflammation in response to the early chemokines, but only T cells with receptors for specific antigen(s) will be activated, proliferate, and release additional RANTES days later. This unusual expression makes RANTES a novel target for immunotherapy (Song et al., 2000) (Figure 3).

RANTES expression in T lymphocytes is regulated transcriptionally by RANTES Factor of Late-Activated T

Lymphocytes (RFLAT)-1 (Kruppel-like Factor 13, KLF-13) and other transactivators (Song et al., 1999). RFLAT-1 itself is translationally regulated (Song et al., 2000). Its 5′ untranslated region regulates translation of RFLAT-1 in response to factors that affect eIF4e. Thus, the original concept of "late T cell activation" and a developmental switch in expression of transcription factors (Ortiz et al., 1997) may be replaced by a new concept of a translational–transcriptional rheostat that regulates chemokine expression in a dynamic way in a terminally differentiated T lymphocyte (Song et al., 2000). For example, growth factors and/or cellular stress may increase eIF4E, which in turn leads to an increase in RFLAT-1 expression. RFLAT-1 binds the RANTES promoter, modulating expression in a more responsive time frame than previously recognized.

For "inflammatory" chemokines, signaling pathways can be different for each stimulus and transcription factor, may vary from cell type to cell type, and can be further complicated by "cross-talk" between various pathways. This complex control system may permit fine tuning and integration of the multiple signals required for a tissue-specific response. Such signal transduction pathways are potential targets for therapeutic intervention (Figure 3).

Kidney Transplantation

Rat models of renal transplantation have allowed an analysis of the expression and functional role of select chemokines and chemokine receptors in acute and chronic allograft rejection (reviewed by Segerer et al., 2000; Nelson et al., 2001). RANTES/CCL5, MCP-1/CCL2, and lymphotactin/XCL1 expression increases during rejection. RANTES/CCL5 mRNA expression is localized to infiltrating leukocytes, tubular epithelial cells, and endothelial cells by in situ hybridization. Lymphotactin/XCL1 is expressed in infiltrating leukocytes. In studies of transplantation of Fisher kidneys into Lewis rats, treatment with a RANTES/CCL5-receptor antagonist, Met-RANTES, significantly reduced recruitment of inflammatory cells and vascular and tubular damage in acute renal allograft rejection (Grone et al., 1999). In a high-responder model (Brown Norway kidney into Lewis rat), the combination of Met-RANTES and low-dose cyclosporin A markedly reduced damage to vessels and tubules and interstitial rejection (Grone et al., 1999).

In humans, we first showed that RANTES/CCL5 protein could be identified in 17 of 20 biopsies from human kidneys undergoing acute cellular rejection (Pattison et al., 1994). RANTES/CCL5 mRNA was expressed by infiltrating mononuclear cells and renal tubular epithelial cells. Interestingly, RANTES/CCL5 protein was also prominently localized to the endothelial surface of peritubular capillaries, although the endothelium was largely negative for RANTES mRNA based on in situ hybridization. This finding suggested that the protein was deposited on the endothelium by activated platelets, and, so positioned, it could enhance recruitment of monocytes and T cells into the graft (Pattison et al., 1994; von Hundelshausen et al., 2001). Increased expression of chemokines, ENA-78/CXCL5, IL-8/CXCL8, MIP-1α/CCL3, MIP-1β/CCL4, MCP-1CCL2, IP-10/CXCL10, lymphotactin/XCL1, and corresponding chemokine receptors, CCR5, CCR2, CXCR4, and Duffy, have subsequently been demonstrated in biopsy samples from human renal allografts undergoing rejection (reviewed by Segerer et al., 2000; Nelson et al., 2001).

Infiltration by CCR5-expressing leukocytes occurs during both acute and chronic phases of human renal allograft rejection (reviewed by Segerer et al., 2000; Nelson et al., 2001). The distribution of the CCR5-positive infiltrate in areas of endothelialitis, tubulitis, and interstitial infiltrates during cellular rejection mirrors the general expression of RANTES/CCL5 in these tissues (Segerer et al., 2000; Nelson et al., 2001). The functional importance of CCR5-positive leukocytes in human renal allograft survival was dramatically demonstrated in a large cohort of patients genetically lacking CCR5. Approximately 1% of individuals of northern European heritage are homozygous for CCR5Δ32, a null allele of CCR5 (Murphy et al., 2000; Rossi and Zlotnik, 2000). Due to a 32 base pair deletion within the coding region of the gene, these individuals lack functional receptor. These individuals do not show an obvious phenotype in general but appear to be highly resistant to productive HIV infection (Murphy et al., 2000; Rossi and Zlotnik, 2000; Segerer et al., 2000). Fischereder and Luckow studied the prevalence of the CCR5Δ32 genotype in over 1200 renal transplant recipients. Twenty-one patients within this group were identified as homozygous for the null allele. Of 22 renal transplants performed in these 21 patients, only one showed a loss of function during followup. Based upon these observations, it appears that the absence of CCR5 results in a significantly prolonged allograft half-life, as compared to the heterozygous or wild-type allele (60 versus 17 years) (Fischereder et al., 2001). These are the strongest data to date supporting an important role of chemokines, especially CCR5-positive leukocytes, in human renal transplant nephropathy (Segerer et al., 2000; Nelson et al., 2001). It is intriguing that, with the explosion of interest in the development of novel CCR5 antagonists for treatment of HIV infection, strategies designed to block viral fusion may have the added benefit of moderating transplant rejection.

The monitoring of chemokines in urine may provide a dynamic picture of the inflammatory state of the kidney. At present, data have been reported for the urinary excretion of MCP-1/CCL2 and IL-8/CXCL8 during renal allograft rejection (reviewed by Nelson et al., 2001). While in the past similar efforts have not been informative, the urinary excretion of chemokines may reflect the intrarenal inflammatory cell infiltrate and in this regard may be of prognostic value as a measure of continued intrarenal inflammation.

Heart Transplantation

Targeted disruption of chemokine receptor genes in mice has implicated chemokines in cardiac transplant rejection (Gao et al., 2000; Hancock et al., 2000a, 2000b). Mice lacking CCR1 ($-/-$) have prolonged cardiac allograft survival. Allografts across a class II mismatch were permanently accepted by *CCR1*$^{-/-}$ recipients, and class I- and class II–mismatched cardiac allografts were rejected more slowly than controls. Levels of cyclosporin A with marginal effects in *CCR1*$^{+/+}$ mice resulted in permanent allograft acceptance in *CCR1*$^{-/-}$ recipients, with

no sign of chronic rejection 50–200 days after transplantation (Gao et al., 2000). The efficacy of CCR1 receptor blockade was tested in a rat heterotopic heart transplant model using a specific small molecule antagonist, BX 471. Analogous to the results with *CCR1*$^{-/-}$ mice, treatment of rats with BX 471 and a subtherapeutic dose of cyclosporin A was more effective in prolonging graft survival than in animals treated with either cyclosporin or BX 471 alone, thus showing a species-independent effect of a CCR1 blockade (Horuk et al., 2001).

During acute cardiac allograft rejection, ligands for CXCR3 (IP-10/CXCL10, Mig/CXCL9, and I-TAC/CXCL11) are increased in expression. This is accompanied by infiltration of CXCR3-expressing activated T cells. CXCR3 appears to play a key role in T cell activation, recruitment, and allograft destruction. Mice treated with anti-CXCR3 monoclonal antibody show prolonged allograft survival. Mice deficient for CXCR3 (−/−) are highly resistant to acute allograft rejection and, when treated with a transient, subtherapeutic dose of cyclosporin A, maintained their allografts permanently, without evidence of chronic rejection (Hancock et al., 2000a, 2000b).

The role of fractalkine/CX_3CL1 and its receptor, CX_3CR1, was recently studied in murine models of heart allograft rejection (MHC-mismatched H-2^d hearts into H-2^b mice) (Robinson et al., 2000). Factalkine/CX_3CL1 expression was upregulated early on vascular tissues and endothelium in rejecting allografts. At later stages of rejection, increased expression was found around vessels and on cardiac myocytes. In in vitro flow assays across murine endothelium, treatment with either anti-fractalkine- or anti-CX3CR1-blocking antibody significantly inhibited peripheral blood mononuclear cell binding to activated endothelium, suggesting that a large proportion of leukocyte binding to murine endothelium occurs via the fractalkine/CX_3CL1 and CX_3CR1 adhesion receptors (Robinson et al., 2000). Finally, the treatment of transplanted animals with blocking anti-CX_3CR1 antibody significantly prolonged allograft survival from 7 ± 1 to 49 ± 30 days, suggesting a critical role for fractalkine/CX_3CL1 in the pathogenesis of acute rejection (Robinson et al., 2000).

Chronic heart allograft dysfunction in humans is characterized by graft atherosclerosis following transplantation. RANTES/CCL5 is expressed by infiltrating neointimal lymphocytes and macrophages in arterioles and venules adjacent to the wall of the coronary artery and in myofibroblasts within the neointima (von Hundelshausen et al., 2001). In this regard, circulating platelets and the chemokine RANTES may contribute to interaction and activation of monocytes and endothelium, thereby playing an important role in the pathogenesis of inflammatory and atherosclerotic disease (von Hundelshausen et al., 2001). It was recently demonstrated that deposition of RANTES by platelets could trigger shear-resistant monocyte arrest on inflamed or atherosclerotic endothelium (von Hundelshausen et al., 2001). Therefore, delivery of RANTES by platelets may contribute to the rapid atherosclerosis seen in heart transplantation.

Skin, Lung, and Liver Transplantation

CXC and CC chemokine expression has been studied in skin allograft rejection in mouse (Koga et al., 1999; Watarai et al., 2000). IP-10/CXCL10 and Mig/CXCL9 were expressed in allografts 3 days before rejection was complete, suggesting a role for these chemokines in recruiting primed T cells into the allograft. Treatment of recipients with rabbit antiserum to Mig/CXCL9 but not to IP-10/CXCL10 delayed rejection of allografts 3–4 days, suggesting that Mig/CXCL9 mediates optimal recruitment of T cell allografts during rejection (Koga et al., 1999; Watarai et al., 2000).

An increase in RANTES/CCL5 in bronchoalveolar lavage fluid was observed in patients undergoing lung transplant rejection compared with healthy lung transplant recipients (Belperio et al., 2000). An increase in RANTES expression is also found in rat lung allografts and correlates with recruitment of mononuclear cells expressing CCR1 and CCR5 (Belperio et al., 2000). In vivo neutralization of RANTES/CCL5, using anti-RANTES antibody, decreased mononuclear cell infiltration and reduced acute lung allograft rejection (Belperio et al., 2000). Patients with bronchiolitis obliterans, a manifestation of chronic lung transplant rejection, have increased IL-8/CXCL8 in bronchoalveolar lavage fluid (Elssner et al., 2000; Hancock et al., 2000a). Bronchial epithelial cells are thought to be the source of IL-8/CXCL8, which in turn may mediate airway inflammation and fibroproliferation in the pathogenesis of this most important long-term complication after lung transplantation.

Enhanced expression of the chemokine CINC mRNA and prominent accumulation of neutrophils are characteristic features of the immune response during acute rejection of liver transplants (Hancock et al., 2000a). Immunostaining in rat hepatic allografts during acute rejection revealed that CINC is expressed in the portal areas by infiltrating mononuclear cells and neutrophils (Yamaguchi et al., 1997). Serum CINC concentration increased significantly at a constant rate over time following transplantation (Yamaguchi et al., 1997). Hepatic allografts treated with FK506 or isografts showed much lower levels of CINC mRNA and less neutrophil infiltration compared with untreated allografts (Yamaguchi et al., 1997).

Chemokines may also play an important role in tissue regeneration after liver injury. After acetaminophen-induced hepatotoxicity in mice, liver regeneration was facilitated by the exogenous addition of ELR-containing CXC chemokines, MIP-2/CXCL2, ENA-78/CXCL5, or IL-8/CXCL8. Intravenous administration of ELR-CXC chemokines after liver damage significantly reduced histological and biochemical markers of hepatic injury (Hogaboam et al., 1999). These observations demonstrate that ELR-CXC chemokines may be novel hepatic regenerative factors that may have prolonged therapeutic effects in damaged liver.

Conclusions

Chemokines are pivotal mediators in allograft rejection by virtue of their activity as regulators of leukocyte movement, adhesion, and effector function. Because the regulation of effector cell infiltration is complex, it is difficult to dissect the relative role of each chemokine in the inflammatory processes leading to allograft rejection, especially since many chemokines and chemokine

receptors are seemingly redundant. Nevertheless, it is clear from knockout and inhibitor experiments that specific chemokine and/or chemokine receptor blockades may influence various aspects of the rejection process. Therefore, therapeutic interventions aimed at chemokines and/or their receptors may prove useful in treatment of transplant rejection and/or induction of immunologic tolerance. Therapeutic effects will likely differ, depending upon the stage of rejection and the other therapeutics administered. The marked enhancement of long-term kidney transplant survival in patients homozygous for a CCR5 null allele is very encouraging for future therapies directed at CCR5-positive mononuclear cells. Results with CCR1, CXCR3, and CX_3CR1 blockades are also remarkable. Clearly, antagonists for chemokines and their receptors have the potential to become important therapeutics in treatment of acute and chronic allograft rejection.

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