

Chemokines and Chemokine Receptors as Therapeutic Targets in Lupus Nephritis

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Summary: Recruitment of leukocytes is a characteristic feature of tissue injury in systemic lupus erythematosus, including lupus nephritis. Locally secreted chemokines and their receptors are important mediators of leukocyte recruitment to the specific sites of immune complex injury, and contribute to renal inflammatory disease in the initiation and progression phase. Therefore, chemokines and chemokine receptors represent potential therapeutic targets in lupus nephritis. In this review we summarize available experimental and human data supporting their functional role in lupus nephritis. Moreover, interventional studies with chemokine and chemokine receptor antagonists that show the therapeutic potential of chemokine antagonists in experimental models of lupus nephritis and potentially in human renal disease are discussed.

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The local infiltration of inflammatory cells at the sites of immune complex-mediated injury represents the hallmark of renal disease in systemic lupus erythematosus (SLE). Among the mediators of inflammation, the chemokines and chemokine receptors play a major role. The chemokines are a subgroup of cytokines specifically mediating cell migration and chemotaxis. Although chemokines have a relatively low level of sequence identity, their 3-dimensional structure is highly homologous in that they all have the same monomeric fold. This fold results from a 4-cysteine motif that forms 2 characteristic disulfide bridges. Depending on the relative position of the first 2 cysteines, chemokines are divided into CC, CXC, C, and CX3C subfamilies, as listed in Table 1.¹ Members of the chemokine family markedly differ in their

function and specific expression patterns. For example, a group of chemokines (homeostatic chemokines) are involved in physiologic homing of leukocytes to lymphoid tissues and in lymphocyte and dendritic cell trafficking during immune surveillance.²⁻⁴ Other chemokines (inflammatory chemokines) mediate the recruitment of leukocytes to sites of tissue injury.^{5,6} Chemokines act via a family of 7-transmembrane-spanning G protein-coupled receptors that are classified according to the class of their respective chemokine ligands (eg, CR, CCR, CXCR, and CX₃CR receptors) (Table 1). Inflammatory chemokine receptors tend to ligate more than 1 chemokine, leading to a certain degree of redundancy in the system.

After injury, parenchymal tissues produce inflammatory mediators that enhance endothelial expression of adhesion molecules such as selectins and integrins, and the presentation of chemokines by heparan sulfate proteoglycans at the luminal endothelial cell surface adjacent to the tissue lesion. Selectins mediate the rolling process of leukocytes along the endothelial surface by interaction with their respective carbohydrate ligands.⁷⁻⁹ During this rolling phase the leukocytes are brought into contact with chemokines retained on the endothelial surface. Chemokine binding to their respective, leuko-

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Table 1. Chemokine and Chemokine Receptor Classification

Chemokine Ligand	Systematic/Common Name	Chemokine Receptor
CC chemokines		
CCL1	I-309, TCA-3	CCR8
CCL2	MCP-1	CCR2
CCL3	MIP-1 α	CCR1, 5
CCL4	MIP-1 β	CCR5
CCL5	RANTES	CCR1,3,5
CCL7	MCP-3	CCR1,2
CCL8	MCP-2	CCR1,2,3,5
CCL9	MIP-1 γ	CCR1
CCL11	Eotaxin	CCR3
CCL13	MCP-4	CCR1,2,3
CCL14	HCC-1, HCC-3	CCR1,3,5
CCL15	HCC-2	CCR1,3
CCL16	HCC-4	CCR1,8
CCL17	TARC	CCR4
CCL18	DC-CK1	CCR3 ?
CCL19	ELC	CCR7
CCL20	MIP-3 α , LARC	CCR6
CCL21	SLC, 6Ckine	CCR7
CCL22	MDC	CCR4
CCL23	MIP-3, MPIF-1	CCR1
CCL24	Eotaxin-2	CCR3
CCL25	TECK	CCR9
CCL26	Eotaxin-3	CCR3
CCL27	CTAK, ESkine	CCR10
CXC chemokines		
CXCL1	Gro- α , KC	CXCR2
CXCL2	Gro- β , CINC-2 α , MIP-2	CXCR2
CXCL3	Gro- γ , CINC-2 β , MIP-2	CXCR2
CXCL4	PF4	CXCR3-B
CXCL5	ENA-78	CXCR2
CXCL6	GCP-2	CXCR1,2
CXCL7	NAP-2	CXCR2
CXCL8	IL-8	CXCR1,2
CXCL9	MIG	CXCR3
CXCL10	IP-10	CXCR3
CXCL11	I-TAC	CXCR3
CXCL12	SDF-1	CXCR4
CXCL13	BCA-1	CXCR5
CXCL14	Bolekine, BRAK	
CXCL15	Lungkine	
CXCL16	SR-PSOX	CXCR6
XC chemokines		
XCL1	Lymphotactin	XCR1
XCL2	SCM-1 β	XCR1
CX ₃ C chemokines		
CX ₃ CL1	Fractalkine	CX ₃ CR1

Abbreviations: GCP-2, granulocyte chemotactic protein-2; Gro, growth-regulated oncogene; KC, keratinocyte-derived chemokine; MIP, macrophage inflammatory protein; CINC, cytokine-induced neutrophil chemoattractant; ENA78, epithelial cell-derived neutrophil-activating peptide 78; NAP-2, neutrophil-activating peptide-2; PF4, platelet factor 4; MIG, monokine induced by IFN- γ ; IP-10, interferon-inducible protein-10; I-TAC, interferon-inducible T-cell α chemoattractant; SDF-1, stromal cell-derived factor-1; BCA-1, B-cell-attracting chemokine-1; BRAK, breast and kidney-expressed chemokine; SR-PSOX, scavenger receptor for phosphatidylserine and oxidized lipoprotein; RANTES, regulated on activation, normal T-cell expressed and secreted; MPIF, myeloid progenitor inhibitory factor; HCC, hemofiltrate CC chemokine; TARC, thymus and activation-regulated chemokine; MDC, macrophage-derived chemokine; DC-CK1, dendritic cell chemokine 1; LARC, liver and activation-regulated chemokine; ELC, EB11 ligand chemokine; SLC, secondary lymphoid tissue chemokine; TCA-3, T-cell activation protein-3; TECK, thymus-expressed chemokine; CTACK, cutaneous T-cell-attracting chemokine; ESkine, embryonic stem cell-derived chemokine; MEC, mucosae-associated epithelial chemokine; SCM-1 β , single cysteine motif-1 β chemokine.

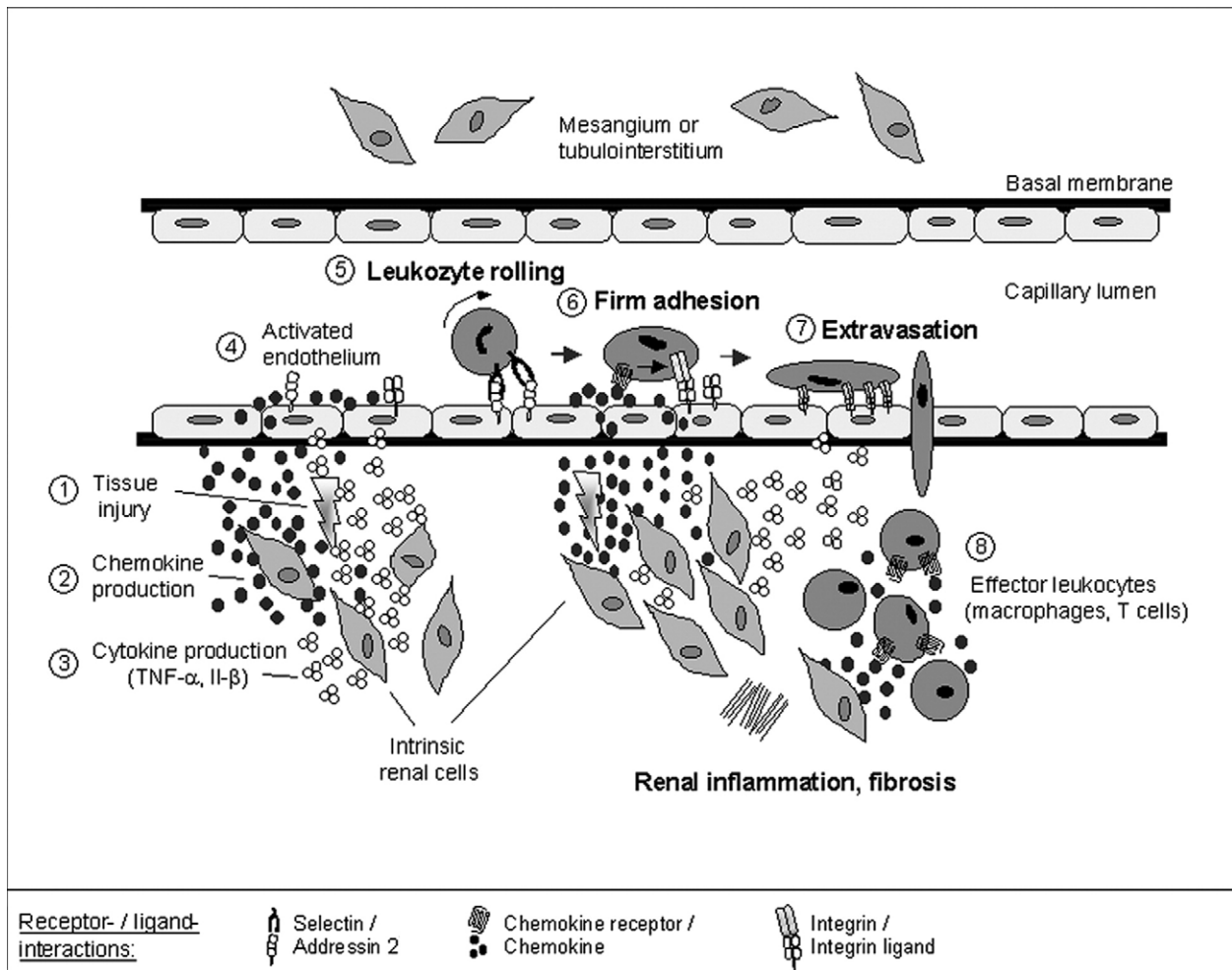


Figure 1. Role of chemokines and chemokine receptors in renal leukocyte infiltration. After tissue injury (1) intrinsic renal cells secrete inflammatory chemokines (2) and cytokines (3). This activates endothelial cells of adjacent glomerular or tubulointerstitial capillaries, which upregulate expression and luminal presentation of adhesion molecules and chemokines (4). The multistep cascade of leukocyte–endothelial cell interactions is initiated by leukocyte rolling along the endothelial surface, a process mediated through transient and reversible binding of selectins to selectin ligands, the addressins (5). Rolling leukocytes are brought into contact with chemokines that are retained on the endothelial surface. Chemokine binding to respective leukocyte-expressed receptors rapidly activates leukocyte integrins, resulting in shear-resistant, firm adhesion of the leukocyte to the endothelium through irreversible interaction with integrin ligands, the endothelial adhesion molecules of the immunoglobulin superfamily (6). This is the prerequisite for subsequent spreading, diapedesis, and migration of the leukocyte into the inflamed tissue (7). The latter processes are supported by interstitial chemokine gradients. Moreover, locally secreted chemokines may induce proliferation and activate effector functions of recruited leukocytes (8). As activated leukocytes become a major source of chemokine production, a positive feedback loop is formed, ultimately leading to progressive renal inflammation and fibrosis.

cyte-expressed receptor activate integrins.¹⁰ Integrin heterodimers are present in a low-affinity, low-avidity state on the leukocyte surface but are induced to rapidly bind endothelial cell-expressed ligands, the intercellular adhesion molecules of the immunoglobulin superfamily. On chemokine receptor activation this increased integrin affinity and avidity is

achieved through conformational changes, association of integrins with the cytoskeleton, and integrin clustering.¹¹ The firm binding of leukocytes to the endothelial integrin ligands results in an irreversible arrest, which is the prerequisite for subsequent transendothelial migration and diapedesis of leukocytes into the subendothelial tissue. Thus, chemokine signal-

ing converts the low-affinity, selectin-mediated, leukocyte-endothelial cell interaction into the high-affinity, integrin-mediated arrest that leads to extravasation of the leukocytes (Fig. 1). Interestingly, selectin-mediated rolling is not an essential step in leukocyte adhesion in all situations. CX₃CL1/fractalkine, a transmembrane mucin-chemokine chimeric molecule induced on the surface of activated endothelial cells, mediates rapid capture (without rolling) and integrin-independent firm adhesion of free-flowing monocytes, T cells, and natural killer cells through interaction with its receptor CX₃CR1.¹²

The chemokines may be produced and/or released by activated endothelial cells, activated platelets, and injured subendothelial parenchymal cells.¹³ Chemokines generated in the subendothelial tissue cross the endothelium via transcytosis after binding to glycosaminoglycans or the Duffy antigen-related chemokine receptor and are selectively presented on the apical microvilli.¹⁴ The activated endothelium also upregulates structures such as heparan sulfate proteoglycans, which retain and present these chemokines to rolling leukocytes.¹⁴ The concerted interaction of adhesion molecules, endothelial heparan sulfate proteoglycans, and the chemokine system provides a versatile system that allows for a high degree of variability and thereby specificity for leukocyte subclass adhesion, depending on, for example, the specific microvascular bed.

The kidney is characterized by different compartments with serial microvascular networks. The expression of proteoglycans, integrins, selectins, and chemokines is likely to differ between high shear-stress and high-permeability glomerular capillaries and the low shear-stress peritubular microcirculation. Moreover, the glomerular capillaries are unique in that they are fenestrated and not separated by a basement membrane from the adjacent mesangium. Furthermore, leukocyte subsets express different types of chemokine receptors in a restricted manner.⁵ The system is fine-tuned further by the fact that T cells and monocytes/macrophages will change the type of chemokine receptor expressed on their cell surface depending on their state of activation, maturation,

tissue location, and the local cytokine milieu.¹⁵⁻¹⁷ Thus, expression and endothelial presentation of a chemokine in a given tissue, and the presence of its receptor on a circulating leukocyte, contribute to the selectivity of the local leukocyte recruitment. Because multiple chemokine receptors are involved in adhesion and chemotaxis of the different leukocyte subsets, it would be desirable to identify those that have nonredundant functions for recruitment of the immune cell subsets of interest. In the context of chronic kidney disease the leukocyte subsets of interest include monocytes/macrophages and T cells.^{18,19}

In this context we discuss the following questions. First, which renal cells express which chemokines during the initiation and progression of lupus nephritis, and what are the specific stimuli for chemokine expression? Second, what is the functional role of chemokines and chemokine receptors in the recruitment of leukocytes in lupus nephritis? Third, is chemokine or chemokine receptor antagonism a feasible concept for the treatment of lupus nephritis? Finally, how do the data from animal models translate to human beings?

WHICH RENAL CELLS EXPRESS CHEMOKINES DURING THE INITIATION AND PROGRESSION OF LUPUS NEPHRITIS, AND WHAT ARE THE SPECIFIC STIMULI FOR CHEMOKINE EXPRESSION?

Inflammatory Stimuli Induce Chemokine Expression in Intrinsic Renal Cells

In the kidney, all types of glomerular (endothelial cells, podocytes, mesangial cells), tubular, and interstitial cells can produce inflammatory chemokines on stimulation.¹³ Proinflammatory stimuli include immune complexes, complement activation, reactive oxygen species, proinflammatory cytokines (eg, tumor necrosis factor [TNF]- α , interferon [IFN]- γ , and interleukin [IL]-1), angiotensin II, and pathogen-associated molecules such as lipopolysaccharide.¹³ From early animal studies it became clear that expression of inflammatory chemokines (eg, CC-chemokine ligand [CCL]2/monocyte chemoattractant protein [MCP]-1 and CCL5/regulated on acti-

vation, normal T-cell expressed and secreted [RANTES]) generally was restricted to the injured compartment of the kidney. In acute glomerulonephritis chemokines are produced exclusively within glomeruli,²⁰ whereas in primary tubulointerstitial diseases such as obstructive nephropathy, chemokine expression is confined to tubular epithelial cells and interstitial infiltrates.²¹ When progression of a glomerular disease leads to secondary tubulointerstitial damage, chemokines, albeit of different types, can be produced in both compartments.²² Moreover, the spatial expression of chemokines correlates with local accumulation of chemokine receptor-positive leukocytes at sites of renal damage.²⁰⁻²²

Renal Chemokine Expression Mediates Local Inflammation

Chemokines are involved both in the initiation and progression of renal disease.¹⁸ The expression of chemokines usually precedes the infiltration of chemokine receptor-positive leukocytes and the clinical manifestation of renal injury such as proteinuria.²⁰⁻²² On the other hand, termination of the renal chemokine production correlated with the resolution of the inflammatory process in models of reversible renal injury.²⁰ However, when local chemokine expression is augmented and prolonged by additional inflammatory stimuli that may be independent of the initial injury, the ongoing recruitment of inflammatory cells leads to accelerated progression of the pre-existing renal disease toward severe renal damage with loss of organ function.^{23,24}

The functional roles of chemokines in the pathogenesis of renal inflammation such as glomerulonephritis have been identified in various rodent models by blocking chemokine activity with neutralizing antibodies, chemokine receptor antagonists, and targeted disruption of chemokine and chemokine receptor genes.^{19,25} However, neutralizing chemokine or chemokine receptor activity also exacerbated renal inflammation under certain conditions. CCR1- and CCR2-deficient mice subjected to nephrotoxic serum nephritis, a model of immune complex glomerulonephritis, developed enhanced disease,^{26,27} and blocking CCL5/RANTES in a

model of acute glomerulonephritis aggravated glomerular damage and proteinuria despite reducing renal leukocyte infiltrates.²⁸ These data show that chemokines and their cognate receptors not only play a role in renal leukocyte recruitment and activation, but also are involved in orchestrating effector mechanisms of the innate and adaptive immune response that mediate renal injury.

Expression of Chemokines and Chemokine Receptors in Experimental Lupus Nephritis

The study of SLE and lupus nephritis has been aided by the availability of several mouse strains that develop a spontaneous autoimmune disease displaying many features in common with SLE. These models include the MRL/MpJ-*fas*^{lpr} (MRL/lpr) strain and the New Zealand Black (NZB) mice crossed with the New Zealand White (NZW) strain (NZB/W-F1 mouse).²⁹ Lupus-prone MRL/lpr mice carry the *lpr* mutation in the apoptosis-related Fas gene, which results in an aberrant nonfunctional transcript. As a consequence of this mutation, autoreactive lymphocytes escape thymic selection, leading to their proliferation and activation. Autoimmune disease in MRL/lpr mice is characterized by high levels of circulating autoantibodies, immune complex deposition in the microvasculature of various organs such as skin and kidney, local complement activation, leukocyte infiltration, local tissue damage, and early death as a result of renal dysfunction. In the kidneys, circulating immune complexes are deposited in the glomerular microvasculature, leading to a progressive mesangioproliferative glomerulonephritis with secondary interstitial inflammation and fibrosis.²²

In MRL/lpr mice we found a limited number of chemokines and chemokine receptors being upregulated during progressive lupus nephritis. Out of 9 chemokines tested, renal expression of CCL2/MCP-1, CCL4/MIP-1 β , CCL5/RANTES, and CXCL10/IP-10 was induced, with CCL2/MCP-1 and CCL5/RANTES being the most abundant.²² Immunohistochemical and in situ hybridization analyses localized expression of CCL2/MCP-1 and CCL5/RANTES to glomeruli, tubular epithelial cells, and interstitial mononu-

clear cell infiltrates.²² A similar localization of CCL2/MCP-1 protein in MRL/lpr kidneys has been described in other reports.^{30,31} An increased CCL2/MCP-1 expression in intrinsic glomerular cells, tubular epithelium, and infiltrating mononuclear cells also was reported in the NZB/W-F1 mouse.³² In the MRL/lpr model renal chemokine receptor expression was restricted to an upregulation of CCR1, CCR2, and CCR5, but not CCR3 or CCR4.²² Because the expressed receptors bind CCL2/MCP-1, CCL4/MIP-1 β , and CCL5/RANTES, the presence of the earlier-described chemokine receptors on infiltrating leukocytes is consistent with the pattern of chemokines induced. Chemokine receptor expression was restricted to infiltrating mononuclear leukocytes and could not be detected in intrinsic renal cells.^{22,33} Macrophages prominently expressed CCR1, but also CCR2 and CCR5. In contrast, renal T lymphocytes, especially CD8-positive T cells, were CCR5 positive, with relatively low levels of CCR1 and CCR2 expression.^{22,33,34} Glomerular, interstitial, and perivascular infiltrates of chemokine receptor-positive leukocytes generally colocalized with sites of chemokine expression and renal injury.²²

A pathogenetic role for CXCR3 signaling in lupus is supported by a recent study that performed a genome-wide messenger RNA (mRNA) expression analysis in the spleens and kidneys of MRL/lpr mice throughout the disease course. The CXCR3 ligands CXCL9/MIG and CXCL10/IP-10 were induced early in the spleens of MRL/lpr mice, with CXCL10/IP-10 expression being increased also in nephritic kidneys.³⁵ Interestingly, both chemokine genes map into known lupus susceptibility loci in MRL/lpr mice.³⁵ CXCL9/MIG and CXCL10/IP-10 are known to mediate Th1 responses, which are a characteristic feature of lupus. CXCL10/IP-10 also may have noninflammatory functions in mediating glomerular injury. Human mesangial cells proliferate in response to CXCL10/IP-10 and express a CXCR3-related receptor,^{36,37} indicating a role for this chemokine in mediating mesangioproliferative glomerular lesions. Other upregulated chemokines found in MRL/lpr spleens were CCL4/MIP-1 β and CCL5/RANTES, and the corresponding receptor

CCR5. In addition, nephritic kidneys revealed increased levels of the CCR1 ligand CCL9/MIP-1 γ and an increased CCR2 expression.³⁵

Inoue et al³⁸ reported an increased CX₃CL1/fractalkine expression in MRL/lpr mice during lupus nephritis. Significant expression of CX₃CL1/fractalkine was seen in the glomeruli and, to a lesser extent, in the interstitial microvasculature. CX₃CL1/fractalkine was localized predominantly in the glomerular endothelial cells, with occasional expression also in the mesangial cells.³⁸

When the temporal expression of chemokines and chemokine receptors was characterized during MRL/lpr lupus nephritis, chemokine expression was proximal within the cascade of events leading to kidney injury.²² A progressive increase in renal chemokine expression was detected as early as week 8. At this time point, levels of circulating and glomerular immune complexes were increased, but no proteinuria, leukocyte infiltrates, or histopathologic signs of renal damage could be observed. Moreover, expression of chemokine receptors and inflammatory cytokines occurred later in the disease process, subsequent to the onset of chemokine expression, and in parallel with the occurrence of renal leukocyte infiltrates and injury.²² This sequence would be consistent with a scenario in which the glomerular mesangial and subendothelial deposition of immune complexes and concomitant complement activation triggers mesangial and endothelial cells to locally release chemokines and induce expression of adhesion molecules. Deposited immune complexes and complement may directly induce chemokine production in intrinsic renal cells, but, in addition, locally secreted cytokines, including IL-1, IFN- γ , and TNF- α could be necessary to stimulate the glomerular release of chemokines.^{39,40} These cytokines may be produced either by adjacent glomerular cells or by infiltrating neutrophils and macrophages initially attracted by the glomerular immune complex and complement deposits.^{41,42} Indeed, experimental animal studies have identified a variety of cytokines as important mediators of lupus nephritis (see later). Glomerular release of chemokines and alteration of the endothelial

cell phenotype in turn causes local adhesion, activation, and infiltration of further leukocytes, which are mostly monocytes/macrophages. The concerted interaction and activation of glomerular endothelial, mesangial, and infiltrating inflammatory cells then results in the generation of additional proinflammatory cytokines, lipid mediators of inflammation (eg, leukotrienes, platelet activating factor, and prostanoids), reactive oxygen species, nitride oxide, and enzymes such as proteases, matrix metalloproteinases, plasminogen activator, and granzymes. Together, these disrupt the functional unit consisting of glomerular endothelial, mesangial, and podocytic cells with the consequence of loss of glomerular permselectivity and proteinuria.

Interestingly, when renal chemokine expression was augmented by additional stimuli independent of the initial autoimmune process (eg, through activation of Toll-like receptors), an increased recruitment of inflammatory cells lead to accelerated progression of lupus nephritis toward severe renal damage and early loss of organ function.⁴³ For example, activation of the Toll-like receptor TLR3, which is a receptor for double-stranded RNA, with its synthetic ligand polyinosinic-cytidylic acid aggravated glomerular and interstitial renal disease in MRL/mice, and resulted in an increased glomerular and interstitial infiltration of macrophages and T cells. This was associated with enhanced renal expression of the chemokines CCL2/MCP-1 and CCL5/RANTES, which colocalized to glomerular crescents and interstitial leukocytic cell infiltrates.⁴⁴ In vitro studies confirmed an induced expression of these chemokines in TLR3-positive mesangial cells, macrophages, and dendritic cells on stimulation with the synthetic ligand polyinosinic-cytidylic acid.⁴⁴ Similarly, treatment of MRL/lpr mice with the TLR7 agonist imiquimod or unmethylated bacterial DNA motifs (CpG-DNA), which activate TLR9, markedly induced local chemokine expression in glomeruli, tubular epithelial cells, and renal leukocyte infiltrates, the latter staining positive for TLR7 and TLR9.^{24,45} Imiquimod and CpG-DNA increased renal leukocyte infiltrates and severely aggravated glomerular and tubulointerstitial damage in MRL/lpr mice.^{24,45} Thus, chemo-

kines appear not only to play important roles in the initiation of lupus nephritis, but also may be important mediators of the progression phase, including disease exacerbation triggered by viral or bacterial infections.

A role of chemokines in the progression of lupus nephritis also has been suggested for the B-cell attracting chemokine CXCL13/BLC. In the NZB/W-F1 model, Ishikawa et al⁴⁶ showed a markedly enhanced expression of CXCL13/BLC in the thymus and kidney of mice with established autoimmune disease and lupus nephritis. Myeloid dendritic cells isolated from spleen and thymus were identified as the major producers of CXCL13/BLC.⁴⁶ Subsequent studies colocalized the renal expression of CXCL13/BLC with infiltrating CD11c-positive dendritic cells.⁴⁷ Importantly, renal CXCL13/BLC expression correlated with disease activity, and it was nearly absent during remission of nephritis induced by combined treatment with cyclophosphamide and costimulatory blockade. Even when dendritic cells were present in the residual renal infiltrates during remission, these were negative for CXCL13/BLC.⁴⁷ CXCL13/BLC showed preferential chemotactic activity for CXCR5-positive B1 lymphocytes as compared with B2 cells.⁴⁶ B1 lymphocytes are considered to be a major source of natural antibodies. Therefore, renal accumulation of these cells, together with infiltration of activated, CXCR5-positive CD4 T cells, may lead to local production of autoantibodies that directly mediate progression of renal disease.^{46,47} These data suggest a capacity of chemokines expressed by renal dendritic cells to orchestrate de novo formation of lymphoid-like infiltrates in nephritic kidneys that directly contribute to local autoimmune disease.

Although chemokine expression triggers renal leukocyte influx, the action of inflammatory mediators such as cytokines, complement components, and prostanoids is essential for both driving the autoimmune process (ie, autoantibody production and immune complex formation) and subsequent tissue damage (ie, lupus nephritis) in SLE. In fact, most cytokines investigated have been found to be deregulated in SLE and lupus nephritis. In particular, there is increasing evidence that supports a role for an unabated activation of type I interferon in the

disease pathogenesis.^{48,49} In addition, a significant role for IFN- γ in SLE has been described,³⁵ with knockout of IFN- γ or its receptor in MRL/lpr mice delaying both the onset of disease and the severity of glomerulonephritis.⁵⁰⁻⁵² Interestingly, IFN- γ also limited interstitial macrophage expansion in MRL/lpr kidneys, indicating an additional negative regulatory role for this cytokine.⁵³ IL-1 also may play a pathogenetic role in lupus nephritis. Established MRL/lpr nephritis did not respond to therapy with an IL-1 receptor antagonist in 1 study, but an alternative IL-1 targeting approach using a recombinant IL-1 receptor as a trap for IL-1 ameliorated renal disease in murine models of lupus.^{54,55} Experimental evidence also supports a local proinflammatory role for TNF- α in lupus nephritis,⁵⁶ whereas other reports indicate a dual function of this cytokine, with TNF- α having an additional anti-inflammatory function.^{57,58} Breeding of various knockout alleles onto the MRL/lpr background, neutralizing experiments, or overexpression have identified additional cytokines that mediate lupus nephritis, including IL-6,^{59,60} IL-10,⁶¹ IL-12,⁶² IL-18,⁶³ and colony-stimulating factor-1.⁶⁴ Moreover, studies in transgenic lupus mice illustrated the role of the complement system in mediating renal injury.⁶⁵⁻⁶⁷

WHAT IS THE FUNCTIONAL ROLE OF CHEMOKINES AND CHEMOKINE RECEPTORS IN THE RECRUITMENT OF LEUKOCYTES IN LUPUS NEPHRITIS?

A functional role of single chemokines and chemokine receptors in mediating leukocyte recruitment and renal disease during lupus nephritis has been identified in several experimental studies, mainly using lupus-prone mouse strains with targeted deletion of chemokines or chemokine receptors. MRL/lpr mice genetically deficient in CCL2/MCP-1 lived longer than CCL2/MCP-1-intact MRL/lpr controls, and were protected from the loss of renal function.³⁰ Infiltration of glomerular macrophages, but not T cells, was reduced in CCL2/MCP-1-deficient mice. This was associated with less proteinuria and less glomerular injury (ie, glomerular hypercellularity, glomerulosclerosis, and crescent formation). In the interstitium the accumulation of both macrophages and T cells was reduced, and this was accompanied by

a decrease in tubulointerstitial injury including tubular atrophy and apoptosis. The largest decrease in renal leukocyte infiltrates occurred at the sites of the most abundant MCP-1 expression (ie, the tubulointerstitial compartment).³⁰ Interestingly, perivascular T-cell infiltrates, a characteristic feature in kidneys of MRL/lpr mice, were not altered in CCL2/MCP-1-deficient mice. This is consistent with the minimal expression of CCL2/MCP-1 in the perivascular zone,³⁰ and the absent expression of the CCL2/MCP-1 receptor CCR2 in perivascular T-cell infiltrates.²² Other chemokines such as CCL5/RANTES may be responsible for recruiting infiltrating leukocytes into perivascular lesions, and the accumulation in part may be secondary to local proliferation.³⁰ Thus, a positive feedback loop would be formed because T-cell-expressed CCL5/RANTES will facilitate further recruitment of perivascular lymphocytes. Interestingly, CCL2/MCP-1 appears to mediate not only renal disease but also arthritis in lupus-prone mice. When MRL/lpr mice were treated with a CCL2/MCP-1 antagonist, the onset of arthritis was prevented and, when given after the disease already had developed, a marked reduction in symptoms and histopathology was observed.⁶⁸

A nonredundant role for the CCL2/MCP-1-CCR2 axis in mediating lupus nephritis has been confirmed when CCR2-deficient MRL/lpr mice were studied.⁶⁹ CCR2-deficient animals survived significantly longer than MRL/lpr wild-type controls. CCR2 deficiency reduced lymphadenopathy and glomerular and tubulointerstitial lesion scores in MRL/lpr kidneys. This was accompanied by a reduced infiltration of macrophages and T cells both into the glomerular and tubulointerstitial compartment. In glomeruli, macrophage and T-cell infiltrates were decreased significantly at 20 weeks, whereas the reduced interstitial leukocyte accumulation already was evident at 14 weeks, with a more pronounced decrease at the later time point. Levels of circulating immunoglobulin isotypes and immune complex deposition in glomeruli were comparable in CCR2-deficient and -intact mice. Moreover, IFN- γ mRNA expression was unchanged in spleens from both genotypes. Apparently, the Th1-Th2 balance of the sys-

temic immune response was not affected by the lack of CCR2 in this model. However, anti-dsDNA antibody levels were reduced in the absence of CCR2, and the frequency of CD8-positive T cells in peripheral blood was significantly lower in CCR2-deficient MRL/lpr mice.⁶⁹ Thus, CCR2 deficiency reduced not only renal leukocyte infiltration and injury, but also the systemic T-cell response in MRL/lpr mice. These data suggest an important role for CCR2 both in the general development of autoimmunity and in the renal involvement of the lupus-like disease.

In CCR1- and CCR5-deficient MRL/lpr mice, preliminary studies from our own group revealed striking compartment-specific differences in the function of these 2 receptors in lupus nephritis (unpublished data). Glomerular macrophage and T-cell infiltrates were not reduced in mice lacking the CCR1 at early (14 weeks) and late (20 weeks) time points. In contrast, CCR5 deficiency in MRL/lpr mice resulted in a substantial reduction in glomerular macrophages and T cells at early and late time points of lupus nephritis. Conversely, in the interstitial compartment, macrophage and T-cell infiltration was reduced in CCR1-deficient mice at both time points, whereas CCR5 deficiency did not affect the number of interstitial inflammatory cells, despite the local expression of corresponding chemokine ligands. Consistently, we recently reported an exclusive role of CCR1, but not CCR5, in mediating interstitial leukocyte infiltration in a model of non-immune-mediated interstitial injury induced by ureteral ligation.⁷⁰ Thus, mechanisms of chemokine-mediated leukocyte recruitment into glomeruli or the renal interstitium rely on the non-redundant, compartment-specific action of distinct chemokine receptors, with CCR1 mediating leukocyte recruitment into the interstitium, and CCR5 into glomeruli. In contrast, CCR2 clearly facilitates leukocyte infiltration in both renal compartments.

By using a different experimental approach, Moore et al⁷¹ delivered chemokines locally into the adult kidney of MRL/lpr mice to specifically investigate chemokine function in the effector phase of the renal immune response. As discussed earlier, in addition to CCL2/MCP-1, the

macrophage and T-cell attractant CCL5/RANTES, which binds to CCR1 and CCR5, is upregulated prominently in lupus nephritis, with its increased expression already present before renal injury.^{22,71} In advance of nephritis, CCL5/RANTES was delivered under the renal capsule of MRL/lpr kidneys via gene transfer using genetically modified tubular epithelial cells. This fostered the local recruitment of macrophages and T cells that were instrumental in initiating interstitial nephritis.⁷¹ Thus, it is likely that early expression of CCL5/RANTES in MRL/lpr kidneys is important for initiating renal disease.

In summary, these experimental data indicate that in progressive lupus nephritis: (1) a limited number of chemokines are upregulated in the initiation phase before infiltration of chemokine receptor-positive inflammatory cells, proteinuria, and kidney damage are observed; (2) chemokine generation is restricted to sites of subsequent inflammatory cell infiltration; (3) chemokine receptor expression parallels mononuclear cell infiltration; (4) proinflammatory cytokines are induced late, in parallel with inflammatory cell infiltration and renal injury; (5) sustained or augmented renal chemokine production is associated with progression of disease; and (6) single chemokines and chemokine receptors play a nonredundant, compartment-specific role in mediating renal leukocyte infiltration and subsequent tissue injury. CCR2, CCR5, and their respective chemokine ligands, but not CCR1, are instrumental in glomerular leukocyte accumulation. In contrast, CCR1 and CCR2, but not CCR5, mediate leukocyte infiltration into the tubulointerstitial compartment. Thus, the specific expression of chemokines and chemokine receptors could be an ideal therapeutic target in lupus nephritis because chemokine production precedes renal leukocyte infiltration and cytokine expression associated with progressive kidney disease.⁷²

IS CHEMOKINE OR CHEMOKINE RECEPTOR ANTAGONISM A FEASIBLE CONCEPT FOR THE TREATMENT OF ESTABLISHED LUPUS NEPHRITIS?

Gene knockouts and transgenics may have disadvantages in identifying chemokines and chemokine receptors as therapeutically targetable

mediators of lupus nephritis. As illustrated in CCR1- and CCR2-deficient mice subjected to nephrotoxic serum nephritis, genetic alterations may be responsible for changing systemic immune responses and leukocyte maturation, thus affecting the induction of the disease model. Moreover, compensatory chemokine and chemokine receptors may evolve from redundant ligands and receptors in genetically deficient mice during development and growth. Thus, for validating the therapeutic potential of chemokine or chemokine receptor blockade the use of specific antagonists in appropriate animal models remains the method of choice. Because proinflammatory chemokines tend to ligate more than 1 chemokine receptor, chemokine receptor blockade may be preferred rather than targeting a single chemokine. However, the specificity of each antagonist needs to be shown for the respective species of the animal model. In fact, many chemokine or chemokine receptor antagonists developed for the human system have no significant specificity for the respective molecule in mice or rats.⁷³ To mimic the clinical setting of affected patients, drug administration to the animal preferentially should be started only after the disease has become manifest in the affected mice.

With this approach the inflammatory chemokine receptor CCR1 was identified as a potential therapeutic target in lupus nephritis. In previous studies we had identified CCR1 as an important mediator of leukocyte infiltration into the interstitial renal compartment of mice with obstructive nephropathy.⁷⁰ Preliminary studies also showed a reduced interstitial leukocyte accumulation in CCR1-deficient MRL/lpr mice (unpublished data, discussed previously). Thus, CCR1 appeared to be a reasonable target to block renal leukocyte infiltration into the interstitium of lupus kidneys. We used BX471, a small molecule antagonist with blocking activity for murine CCR1, to treat female MRL/lpr mice from 20 to 24 weeks of age.³³ CCR1 blockade reduced the numbers of interstitial macrophages and T cells at 24 weeks.³³ The reduction of the interstitial immune cell infiltrates was associated with a reduced number of interstitial myofibroblasts, renal transforming growth factor- β mRNA expression, and interstitial colla-

gen I deposits, indicating a beneficial effect of the CCR1 blockade on the subsequent renal fibrosis. Moreover, the blood urea nitrogen levels in BX471-treated MRL/lpr mice were reduced. Glomerular macrophages, glomerular pathology, and proteinuria were not decreased by the CCR1 antagonist. CCR1 blockade also did not affect serum DNA autoantibody levels.³³ Thus, CCR1 blockade can improve renal function and tubulointerstitial damage in MRL/lpr mice with established proliferative lupus nephritis. In contrast, CCR1 blockade has no effect on the glomerular macrophage infiltration, glomerular pathology, and, hence, proteinuria. These results are in line with the data obtained in the CCR1-deficient MRL/lpr mice. Moreover, a lack of effects of CCR1 blockade on glomerular lesions in the nephrotic syndrome of experimental adriamycin nephropathy recently has been shown, as opposed to ameliorated tubulointerstitial disease.⁷⁴

Because CCR1 blockade cannot prevent glomerular macrophage recruitment one would expect that targeting a chemokine or chemokine receptor that can block the glomerular or even both the glomerular and interstitial recruitment would be even more effective. As already indicated by the beneficial effects seen in CCL2/MCP-1- and CCR2-deficient MRL/lpr mice,^{30,69} the CCL2/MCP-1-CCR2 axis should be promising. In fact, Hasegawa et al³¹ were the first to show that a NH(2)-terminal-truncated CCL2 analogue can block glomerular and interstitial macrophage and T-cell recruitment in MRL/lpr mice with lupus nephritis. They initiated an 8-week course of treatment with the CCL2 analog in 7- and 12-week-old mice, thereby mimicking treatment in prenephritic and early nephritic MRL/lpr mice. Both protocols resulted in a delay of renal damage. Glomerular hypercellularity, glomerulosclerosis, crescent formation, and vasculitis were reduced compared with control mice. CCL2/MCP-1 antagonism from weeks 12 to 20 resulted in a markedly diminished infiltration of macrophages and T cells into glomeruli, the interstitium, and perivascular areas.³¹ These beneficial effects were associated with a decreased production of IFN- γ and IL-2 in the kidney. Of note, levels

of anti-DNA antibodies and circulating immune complexes were not affected.³¹ Subsequent studies confirmed these findings by using gene transfer of a plasmid transfection vector encoding a truncated CCL2/MCP-1, which acts as a CCR2-receptor blocker, into the skeletal muscles of 16-week-old MRL/lpr mice.⁷⁵ Treated MRL/lpr mice showed a survival benefit and ameliorated glomerulonephritis with reduced crescent formation. Both glomerular and interstitial macrophages were reduced. However, in this study, CCL2/MCP-1 antagonism did not influence vasculitic lesions.⁷⁵ Additional studies from the same group further defined that a delayed gene transfer with a truncated CCL2/MCP-1 does not affect the presumed role of CCL2/MCP-1 in helper T-cell polarization, but reduces the local Th1-immune response in MRL/lpr kidneys.⁷⁶ This was evident by unaltered production of IFN- γ and IL-12 in splenocytes, whereas glomerular expression of IL-12 and interstitial expression of IFN- γ and IL-12 were reduced significantly.⁷⁶ Clearly, the results of these interventional studies blocking the CCL2/MCP-1-CCR2 axis are consistent with the data obtained in the respective knockout mice and underline the importance of CCL2/MCP-1 and CCR2 in mediating both glomerular and interstitial leukocyte infiltration and subsequent parenchymal injury in lupus nephritis.

Recently, Inoue et al also evaluated CX₃CR1/fractalkine blockade with a truncated CX₃CL1 analog in MRL/lpr mice.³⁸ When the CX₃CL1/fractalkine analog was given to 8-week-old MRL/lpr mice for another 8 weeks the glomerular and tubulointerstitial damage improved, which was associated with a reduced number of CX₃CR1-positive monocytes in the glomerular and interstitial compartment. Again, serum DNA autoantibody levels were not affected. Interestingly, the beneficial effect of the CX₃CL1/fractalkine analog was restricted to autoimmune tissue injury in the kidney, in contrast to lungs and salivary glands, which might be attributed to the expression of CX₃CR1 on glomerular and peritubular endothelial cells.³⁸

Homeostatic chemokines have different functions than inflammatory chemokines.

However, blocking homeostatic chemokines also may have beneficial effects in lupus nephritis, for example, through interfering with systemic immune responses and with the generation of effector leukocytes. Thus, the administration of a CXCL12/SDF-1 antagonist to NZB/NZW mice with established lupus nephritis reduced DNA autoantibody production, glomerular immune complex deposits, proteinuria, and renal injury.⁷⁷

In summary, these interventional animal studies identified blockade of CCR2 or CX₃CR1 as promising therapeutic options to target both glomerular and interstitial infiltrates in lupus nephritis. CCR1 antagonists can only reduce leukocyte infiltration and injury in the tubulointerstitial compartment. Because interference with CCL2/MCP-1 and CCR2 had the most beneficial effects in terms of glomerular and tubulointerstitial pathology, proteinuria, and survival of the lupus mice, these might represent the optimal target in SLE.

HOW DO THE DATA FROM ANIMAL MODELS OF LUPUS NEPHRITIS TRANSLATE TO HUMAN BEINGS?

Descriptive Studies

The experimental data obtained in animal models of lupus are supported by several biopsy studies confirming a similar expression of chemokines⁷⁸⁻⁸⁰ and chemokine receptors^{37,81-84} in human lupus nephritis. These studies localized mRNA and protein expression of the chemokines CCL2/MCP-1, CCL3/MIP-1 α , CCL4/MIP-1 β , and CCL5/RANTES in intrinsic renal cells of glomeruli (podocytes, endothelial, mesangial, and parietal endothelial cells) and the interstitium (mainly in proximal tubular epithelial cells). In addition, a prominent production of these chemokines by infiltrating macrophages and T cells was noted.⁷⁸⁻⁸⁰ Recently, transcriptional phenotyping of glomeruli isolated by laser-capture microscopy from clinical biopsy specimens of patients with lupus nephritis also identified CCL2/MCP-1 as abundantly expressed in nephritic glomeruli, in addition to an increased glomerular expression of CCL3/MIP-1 α .⁸⁵ By immunohistochemistry and in situ hy-

bridization, renal expression of chemokine receptors generally was localized to infiltrating inflammatory cells, with the exception of a CXCR3 isoform that, as discussed earlier, was detected in mesangial cells.³⁷ Available data indicate that glomerular macrophages express CCR2, whereas glomerular CCR5 expression was relatively low.^{81,82} In contrast to CCR5, a glomerular expression of CCR1 was not detected,⁸³ which is consistent with the experimental data discussed earlier that indicated an absent role of CCR1 in mediating glomerular leukocyte infiltration. In the tubulointerstitial compartment, macrophages were reported to express CCR1, CCR2, and CCR5.⁸¹⁻⁸³ CCR2 also is expressed on a subset of interstitial T cells, with the majority of T cells being positive for CCR5 and CXCR3.^{37,81,82} In crescentic glomerulonephritis, leukocytes in both the glomerular and interstitial compartments are almost uniformly positive for CX₃CR1, the CX₃CL1/fractalkine receptor. In contrast to CCR1, CCR2, and CCR5, CX₃CR1 expression is not restricted to special leukocyte subpopulation or a single renal compartment.⁸⁴ Recently, expression of the CXC chemokine receptor CXCR1 was found in infiltrating, mainly polymorphonuclear, leukocytes in and around the glomeruli and the tubulointerstitium.⁸⁶ These data point to a functional, albeit incompletely understood, role of a CXCR1-mediated infiltration of polymorphonuclear leukocyte in the pathogenesis of lupus nephritis. Moreover, smooth muscle cells of arterioles were commonly positive for CXCR1, with its expression apparently reduced in nephritic kidneys compared with control tissue.⁸⁶ The latter finding suggests an additional homeostatic role of CXCR1 signaling in normal kidney.

Increased levels of chemokine protein and mRNA, especially CCL2/MCP-1, can be detected in the urine of patients with active disease.^{78,87-89} Consistent with the increased renal expression of CXCL10/IP-10 in MRL/lpr mice,³⁵ a recent study reported increased urinary mRNA levels of CXCL10/IP-10 and its cognate receptor CXCR3 in patients with class IV lupus nephritis that correlated with disease activity.⁹⁰ Serum levels of CXCL10/IP-10 in addition to CCL3/MIP-1 α , CCL2/MCP-1, CCL5/RANTES, and CXCL12/SDF1 are increased significantly in

SLE patients.⁹¹ Moreover, patients with SLE showed abnormal T-cell expression of several chemokine receptors, including increased CCR1 and CXCR2 expression. Interestingly, patients with renal involvement also had increased surface expression of CCR3 and CXCR3, but lower serum levels of soluble CXCL10/IP-10, compared with SLE patients without renal disease or controls.⁹¹ In human cutaneous lupus lesions, infiltrating leukocytes consisted of mainly CXCR3-expressing cells, and the CXCR3 ligands CXCL9/MIG, CXCL10/IP-10, CXCL11/I-TAC, and CCL5/RANTES were the most abundantly expressed chemokines in these lesions.⁹²

In summary, these data indicate that chemokines are important mediators of renal leukocyte infiltration in human disease. Apparently, locally expressed CCL2/MCP-1 attracts CCR2-positive macrophages into injured glomeruli, and potentially also into the tubulointerstitial compartment. Glomerular expression of CCL3/MIP-1 α , CCL4/MIP-1 β , and CCL5/RANTES also may mediate the infiltration of CCR5, but not CCR1-positive subsets of macrophages or T cells. In contrast, the prominent infiltration of interstitial T cells in lupus nephritis apparently is facilitated by tubulointerstitial expression of CCL3/MIP-1 α , CCL4/MIP-1 β , CCL5/RANTES, and CXCR3 ligands such as CCL10/IP-10, which act on T-cell expressed CCR5 and CXCR3. CCR1 and CCR5 appear important for the recruitment of macrophages into the tubulointerstitium. The uniform expression of CX₃CR1 on infiltrating renal leukocytes suggests that CX₃CL1/fractalkine acts as an adhesion molecule early in the extravasation process, without cell type- or compartment-specific differences.

Genetic Data

The functional relevance of chemokines in the pathogenesis of lupus nephritis also is suggested by genetic polymorphisms of chemokine or chemokine receptor genes that have been associated with the development of lupus nephritis in human beings. In the 5' flanking region of the CCL2/MCP-1 gene an A/G polymorphism at position -2518 upstream from the transcription site was reported to be associated

with circulating CCL2 levels.⁹³ In 1 study, the A/A genotype was found more commonly in controls than in SLE patients. Moreover, the A/A genotype was observed in only 23% of the SLE patients with lupus nephritis compared with 58% of those without nephritis.⁹⁴ Peripheral blood mononuclear cells isolated from SLE patients with the A/G and G/G genotypes secreted substantially more CCL2/MCP-1 than cells obtained from patients with the A/A genotype.⁹⁴ Thus, a CCL2 -2518 A/G or G/G genotype may predispose to the development of SLE, and SLE patients with these genotypes may be at higher risk of developing lupus nephritis, presumably because of an increased CCL2/MCP-1 secretion that augments leukocyte recruitment and activation. Consistent with the earlier-described findings, a preliminary study reported greater monocyte interstitial infiltrates in patients with lupus nephritis who were heterozygous or homozygous for the -2518 G allele.⁹⁵ However, other investigators could not find an association between the -2518 (A/G) polymorphism in the CCL2/MCP-1 gene and the susceptibility to SLE or lupus nephritis.^{96,97} The reason for these discrepant results may lie in the different ethnic backgrounds and the relatively small number of patients examined, resulting in insufficient statistical power to reach valid conclusions. Regarding other chemokines or chemokine receptors, a recent study in Chinese children with SLE did not find a significant difference in the frequency of the -2518 (A/G) CCL2/MCP-1 and a -403 (G/A) CCL5/RANTES gene polymorphism between patients and controls.⁹⁸ However, significant differences were observed in the distribution of a -28 (C/G) CCL5/RANTES gene polymorphism, with the CCL5/RANTES -28G allele leading to increased expression of CCL5/RANTES. This genotype was more frequent in SLE patients and was associated significantly with higher initial levels of antinuclear antibodies, lower levels of complement C3, and a higher incidence of central nervous system lupus.⁹⁸ Another Chinese study suggested a probable link between the G/A polymorphism at the CCL5/RANTES -403 locus with renal involvement in SLE.⁹⁹ Interestingly, a significant increase in the frequency of the $\Delta 32$ deletion in the CCR5 gene, a CCL5/RANTES

receptor, was observed in SLE patients with biopsy-proven nephritis, and a higher severity index was found among patients bearing the $\Delta 32$ CCR5 allele.¹⁰⁰ This is somehow contradictory to other studies that found an association between the $\Delta 32$ mutation and improved renal outcomes, for example, better long-term graft survival in renal transplant recipients.¹⁰¹ Thus, there are inconsistencies between these genetic analyses and there are problems with their statistical validity (too few cases for genetic analysis). Nonetheless, genetic variants between SLE patient groups may extend to the chemokine system, which may influence the response to a particular antichemokine therapy.

CONCLUSIONS

Treatment of lupus nephritis with immunosuppressive agents has significantly increased renal and overall survival of patients with SLE. Considering the risk of infections, sterility, and malignancy associated with this type of treatment, the search for potentially more selective immunosuppressive targets has identified chemokine and chemokine receptors as promising candidates for therapeutic intervention. Prophylactic and, more importantly, therapeutic blockade of selected chemokines and receptors in experimental animal models have proven the therapeutic potential of chemokine antagonism in lupus nephritis. Biopsy specimens and urinary excretion data from patients with lupus nephritis point toward CCL2/MCP-1-CCR2 and CXCR3 as major targets for human glomerular lesions, and CCR1 and CCR5 for subsequent interstitial lesions. With an increasing number of human chemokine receptor antagonists being developed and evaluated in clinical trials, specific blockade of chemokine functions offers the promise for a new therapeutic strategy in the treatment of lupus nephritis that is both selective and efficacious. In view of potential additional benefits of therapeutic strategies targeting pathogenic antibody and immune complex formation, costimulation, adhesion molecules, and cytokines,¹⁰² clinical trials ultimately will prove whether chemokine antagonism may develop into one of several adjuvant biologic therapies of human lupus nephritis.

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