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# Chemokine blockade for lupus model mice

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## 1. ABSTRACT

Over the past decade, accumulating evidence has indicated a crucial role for chemokines and chemokine receptors in the pathogenesis of autoimmune diseases in both human and mouse models. Locally secreted chemokines and their receptors are important mediators of leukocyte recruitment to the tissues, and contribute to the initiation and progression of autoimmune diseases. Thus, blockade of chemokine and chemokine receptor interactions has emerged as a novel therapeutic strategy. MRL/MpJ-*lpr/lpr* (MRL/lpr) and (NZB X NZW) F1 mice, the two strains of mice that develop spontaneous autoimmune disease closely resembling human systemic lupus erythematosus (SLE), are considered to be excellent models for investigating the pathogenesis of the human disease. In addition, similar expression patterns of chemokines and chemokine receptors in inflamed organs are shown in humans and lupus model mice, especially MRL/lpr mice. Therefore, findings obtained from experiments with lupus model mice may be applicable to the treatment of these autoimmune diseases in humans. In this article, we review the role of chemokines and chemokine receptors involved in the pathogenesis of autoimmune diseases and the therapeutic approach of chemokine blockade in lupus model mice.

## 2. MURINE MODELS OF LUPUS

MRL/MpJ-*lpr/lpr* (MRL/lpr) and (NZB X NZW) F1 (NZB/W F1) mice that develop spontaneous autoimmune disease closely resembling human systemic lupus erythematosus (SLE) are considered to be excellent models for investigating the pathogenesis of human disease (Table 1) (1-4).

MRL/lpr mice has a phenotypically characteristic of systemic lymphoadenopathy and splenomegaly with a mutation in the apoptosis-related Fas gene, which result from the accumulation of a large number of polyclonal $Thy1.2^+B220^+CD4^-CD8^-$ T cells in the lymph nodes and spleen (5). These mice coincidentally develop progressive inflammatory diseases of the kidneys, systemic vessels, joints, salivary glands, lungs, liver, pancreas, and skin. These manifestations start at 8-12 weeks of age and are progressive. They are associated with autoimmune traits involving hypergammaglobulinemia, increased autoantibodies such as anti-dsDNA and anti-Sm antibodies and rheumatoid factors (RF), and circulating immune complexes (ICs) (6, 7). In addition, several published data have demonstrated that $CD4^+$ T cells and macrophages play an important role in the production of autoantibodies and disease development (8-11).

**Table 1.** Spontaneous lupus model mice

| | MRL/lpr | (NZBXNZW)F1 |
|---|---|---|
| Glomerulonephritis | +++ | +++ |
| Vasculitis | ++ | - |
| Arthritis | + | - |
| Sialadenitis | ++ | - |
| Pneumonitis | ++ | ± |
| Pancreatitis | + | - |
| Cholangitis | + | - |
| Pericarditis/ myocarditis | + | - |
| Lymphadenopathy/ splenomegaly | +++ | + |
| Anti-nuclear | +++ | +++ |
| Anti—DNA | ++ | ++ |
| Immune complex | ++ | ++ |
| Anti-Sm | + | - |
| Rheumatoid factor | ++ | - |
| Anti-gp70 | ++ | ++ |

The pathological manifestations of systemic autoimmune disease in MRL/lpr mice are characterized by various forms of collagen disease including glomerulonephritis, systemic vasculitis, polyarthritis, and sialadenitis, which resemble lupus nephritis, polyarteritis nodosa, rheumatoid arthritis (RA), and Sjogren syndrome (SS), respectively. In addition, MRL/lpr mice also develop pneumonitis, pancreatitis, and cholangitis.

The glomerulonephritis developing in MRL/lpr mice shows regular histopathological variations, as does human lupus nephritis. The major type is characterized by endocapillary proliferative lesions, and to a lesser extent, granulomatous or crescentic lesions accompanied by severe inflammatory cell infiltrates, segmental wire loop-like lesions and hyaline thrombi. These lesions are generally associated with IC and C3 deposits in the mesangial, subendothelial and/or subepithelial regions.

Systemic vasculitis in MRL/lpr mice is histopathologically characterized by granulomatous arteritis, mainly in the renal arteries, but some of the lesions develop in the coronary, pancreatic and hepatic arteries, and in small arteries of the salivary glands, muscles and skin. The arteritic lesions are initiated by an accumulation of $CD4^+$ T cells, but not $Thy1.2^+B220^+CD4^-CD8^-$ T cells, in the periarterial regions, followed by activated macrophages. This seems to be critical for the destruction of the external elastic lamina, followed by arterial intimal thickening.

Arthritis in MRL/lpr mice develops mainly in the foot joints. This feature is characterized by villous proliferation of the synovial lining cells, partly showing granulomatous lesions in the synovial sublining regions. Arthritis is associated with accumulation of inflammatory mononuclear cells including $CD4^+$ T cells and macrophages.

Sialadenitis in MRL/lpr mice is also characterized by the accumulation of inflammatory mononuclear cells including $CD4^+$ T cells and macrophages in the periductular regions, followed by destruction of the parenchyma. In the advanced stage, proliferative lesions in the salivary ductules and replacement of the parenchyma with fatty tissue are observed. It is important to note that chronic dacryoadenitis, pancreatitis, and cholangitis coincidentally develop in addition to sialadenitis.

NZB/W F1 mice also develop renal lesions that are markedly similar to human lupus nephritis. The most striking immunopathological feature of this murine model is the development of autoantibodies to dsDNA and endogenous retroviral gp70 (12-14). The production of these autoantibodies starts at 4-5 months of age and culminates at about 9 months of age. This leads to the deposition of large amounts of IC on the glomerular basement membrane and to the development of diffuse lupus nephritis. Studies on both NZB/W F1 mice and MRL/lpr mice have also demonstrated the involvement of T cells, especially $CD4^+$ T cells, in the pathogenesis of glomerulonephritis (for review, see ref 3). In addition, abnormal expansion of the autoreactive B1 cell population has been also documented in NZB/W F1 mice (15, 16). B1 cells are a specialized cell population that are distinguished from conventional B cells by their origin, and contribute to innate immunity and body cavity immunity by producing natural IgM antibodies in the peritoneal cavity (17-21). However, some IgM antibodies produced by B1 cells are polyreactive with low affinity and broad specificities and cross-react with a variety of self-antigens. Therefore, B1 cells have been considered to be involved in autoantibody production in the development of autoimmune diseases, although it remains to be elucidated whether B1 cells class-switch from IgM to IgG in the development of autoimmune diseases. In NZB/W F1 mice, B1 cells have been reported to be attracted to a target organ by aberrant chemokine production (22-24). B1 cells within the foreign environment of the kidney may elaborate antibodies against renal antigens, thus initiating organ-specific autoimmunity.

## 3. EXPRESSION OF CHEMOKINES AND CHEMOKINE RECEPTORS IN EXPERIMENTAL LUPUS NEPHRITIS

Lupus nephritis is generally considered to be caused by the glomerular deposition of ICs that induce a cascade of inflammatory events ultimately leading to severe tissue damage (Figure 1) (25, 26). Infiltrating mononuclear cells, mainly macrophages and T cells, play an important role in the development of renal injury. Locally secreted chemokines mediate glomerular leukocyte recruitment, effector functions, and subsequent glomerular damage. In the initiation phase, intrinsic renal cells secrete inflammatory chemokines and cytokines such as TNF-α and IL-1β by ICs and complement activation. Up-regulation of adhesion molecules and chemokines on activated glomerular endothelial cells facilitates arrest and transmigration of leukocytes, especially macrophages and T cells, into the glomeruli. The infiltration of macrophages and T cells further enhances the local production of cytokines and chemokines in a positive amplification loop. In particular, intraglomerular macrophages become the major source of growth factors such as fibroblast growth factor, TGF-β and TNF-α. These factors stimulate

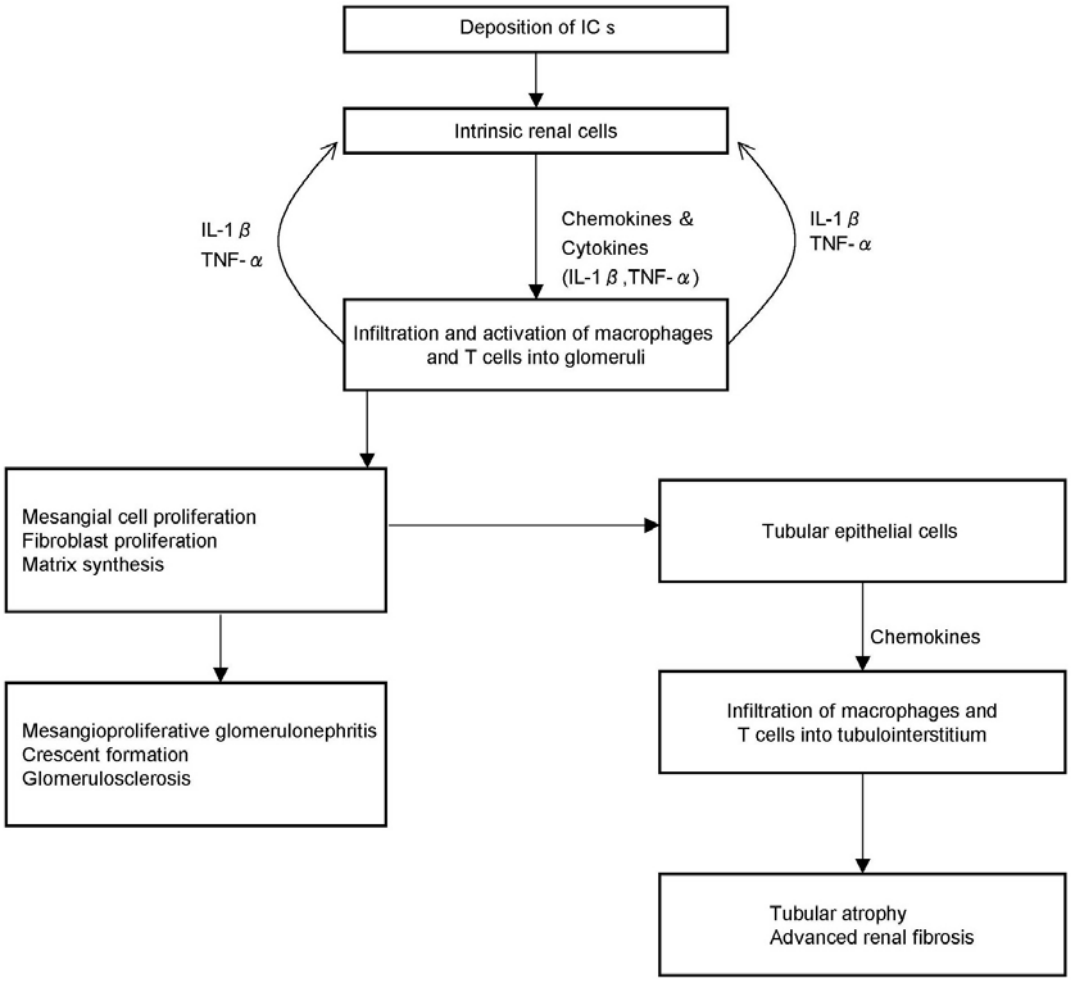


**Figure 1.** Mechanism of the progression of lupus nephritis.

mesangial cell proliferation and matrix synthesis in the glomerulus, leading to focal glomerulosclerosis and mesangioproliferative glomerulonephritis. Following this glomerular damage, there is increased mononuclear cell infiltration into the tubulointerstitium. Subsequent interstitial leukocyte infiltration, fibroblast proliferation, and matrix deposition result in renal fibrosis.

Several studies on chemokine and chemokine receptor expression have been reported in animal models of lupus nephritis (22, 27-33). In MRL/lpr mice, De Lema *et al.* have observed a limited number of chemokines and chemokine receptors being upregulated during progressive lupus nephritis (27). Out of nine chemokines tested by RNAse protection assay, CCL2/MCP-1, CCL4/MIP-1β, CCL5/RANTES and CXCL10/IP-10 were induced. Immunohistochemical and *in situ* hybridization analyses localized the expression of CCL2 and CCL5 to the glomeruli, tubular epithelial cells, and interstitial mononuclear cell infiltrates. The localization of CCL2 in MRL/lpr kidneys is consistent with our results and other report (28, 29). The renal expression of chemokine receptors was restricted to CCR1, CCR2 and CCR5, the respective chemokine receptors of the coexpressed chemokine ligands (27). In contrast, CCR3 and CCR4 were absent in MRL/lpr kidneys. Chemokine receptor expression was restricted to infiltrating mononuclear cells and could not be detected in intrinsic renal cells (27, 30). Macrophages prominently expressed CCR2, but also CCR1 and CCR5. In contrast, renal T cells, especially $CD8^+$ T cells, were CCR5 positive with relatively low levels of CCR1 and CCR2 expression. Moreover, we found an increased CX3CL1/fractalkine expression in MRL/lpr mice during lupus nephritis (31). Significant expression of CX3CL1 was localized predominantly in the glomerular endothelial cells but was occasionally observed in the mesangial cells and, to a lesser extent, in the interstitial microvasculature. This was supported by the finding that infiltration of CX3CR1-expressing cells increased after induction of CX3CL1 expression in the kidney of MRL/lpr mice. Taken together, glomerular, interstitial, and perivascular infiltrates of chemokine receptor-expressing leukocytes generally co-localized with sites of chemokine expression and renal injury.

In NZB/W F1 mice, Zoja *et al.* have reported that CCL2 expression was also increased in intrinsic glomerular cells, tubular epithelium, and infiltrating mononuclear cells,

as well as MRL/lpr mice (32). Expression of CXCL12/SDF-1 and CXCL13/BLC have been shown in NZB/W F1 mice during the development of lupus nephritis (22, 33). CXCL12 is produced by podocytes and, to a lesser extent, endothelial and mesangial cells, whereas CXCL13 is produced by infiltrating CD11c-positive dendritic cells. Pathological changes in the kidneys of NZB/W F1 mice with nephritis are associated with an increased *in situ* production of CXCL12 and CXCL13. These two chemokines play an important role in the homing and expansion of B1 cell population. Therefore, the production of these 2 chemokines in the glomeruli suggests recruitment of B1 cells to inflamed glomeruli, in which B1 cells may produce autoantibodies.

## 4. EXPRESSION OF CHEMOKINES AND CHEMOKINE RECEPTORS IN HUMAN LUPUS NEPHRITIS

To clarify the involvement of chemokines and their cognate receptors in human lupus nephritis, both urinary and serum levels of chemokines were measured in patients (34-41). Urinary CCL2 levels in lupus nephritis patients with active lesions were significantly higher than those with inactive lesions (34-39). CCL2 was mainly detected in glomerular endothelial cells, mesangial cells, tubular epithelial cells, and infiltrated mononuclear cells by both immunohistochemistry and *in situ* hybridization (42, 43). These observations suggest that CCL2 is involved in the pathogenesis of lupus nephritis, especially through the recruitment of macrophages and T cells. Recently, increased urinary mRNA levels of CXCL10 and its cognate receptor CXCR3 in patients with class IV lupus nephritis have been also reported to correlate with disease activity (39).

Serum levels of CCL2, CCL3, CCL5, and CXCL10 were significantly elevated in the serum of SLE patients (41). Moreover, immunohistochemistry and *in situ* hybridization of renal biopsy specimens from lupus nephritis patients showed that CCL2, CCL3, CCL4, CCL5, and CXCL10 were also expressed in intrinsic renal cells of glomeruli and interstitium (42-46). Macrophages in the glomeruli express CCR2, whereas the majority of T cells in the interstitium express CCR5 and CXCR3. These findings indicate that chemokines are important mediators of renal leukocyte infiltration in human lupus nephritis. Locally expressed CCL2 attracts CCR2-expressing macrophages into injured glomeruli. In contrast, the prominent infiltration of interstitial T cells which express CCR5 and CXCR3 is facilitated by expression of CCL3, CCL4, CCL5, and CXCL10. In crescentic glomerulonephritis, leukocytes in both the glomeruli and interstitium were almost uniformly positive for CX3CR1, suggesting that CX3CL1 acted as an adhesion molecule in the transmigration process (47).

## 5. BLOCKADE OF CHEMOKINES AND CHEMOKINE RECEPTORS IN EXPERIMENTAL LUPUS NEPHRITIS

Similar expression patterns of chemokines and chemokine receptors are shown in human and mouse models of lupus nephritis, especially MRL/lpr mice (for review, see refs 25 and 26). Therefore, findings obtained from experiments with MRL/lpr mice may be applicable to the treatment of lupus nephritis in humans. Several approaches have been used to identify potential roles for chemokines in autoimmune diseases in animal models (for review, see refs 25 and 26). One is the administration of chemokine-neutralizing antibodies. However, this method is not suitable for long-term observation in the MRL/lpr mice because repeated administration of chemokine-neutralizing antibodies induces ICs. The use of gene knockouts and transgenics for chemokines and chemokine receptors is also an excellent strategy. However, the results are sometimes difficult to interpret because of differences in the genetic backgrounds of the animals used and possibility of the modification of immunological functions themselves during the process of host maturation. In particular, genetic background can profoundly influence the susceptibility to disease, the type of inflammatory infiltrate, and the eventual course of disease. Adequate backcrossing is important because a significant part of the experimental findings assigned to the knockout phenotype are often explainable by the different genetic backgrounds used (48). In addition to small molecular chemokine antagonists, $NH_2$-terminally truncated or modified chemokine analogues have been described to act as receptor antagonists (for review, see ref 49). The function of the chemokine domain has been analyzed by crystal structure determination and nuclear magnetic resonance. It has been observed that the N-terminal region is essential for functional activity and that the loop immediately following the cysteine sequence, as well as the N-terminal region, plays an important role in receptor binding. Truncation or modification of a few N-terminal amino acids in several chemokines, such as CCL2, CCL5 and CXCL1, has been reported to lead to significant changes in functional activity and receptor binding. We established a new system allowing long-term observation of the effect of chemokine antagonists on autoimmune diseases in MRL/lpr mice by using the non-metastatic fibroblastoid cell line MRL/N-1 transfected with the secreting form of $NH_2$-terminally truncated chemokine analogues (28). This system allows us to observe the effect of chemokine antagonists in these mice for more than 2 months.

CCL2, a potent chemoattractant for monocytes and for T cells, is secreted from many kinds of renal cells, including endothelial, mesangial, tubular epithelial and interstitial cells and macrophages, in response to stimulation with inflammatory cytokines and ICs (for review, see ref 26). In lupus nephritis, production of CCL2 in the many types of renal cells is triggered by the deposition of IC and complement activation, and subsequently leads to mononuclear cell infiltration. Moreover, after CCL2 production by local cells, the infiltrating macrophages become a source of CCL2, resulting in an amplification loop. To examine whether the $NH_2$-terminally truncated CCL2 analogue, referred to as CCL2 antagonist, blocks these process, we inoculated CCL2 antagonist-transfected MRL/N-1 cells into MRL/lpr mice ages 7 weeks (before the onset of lupus nephritis) and 12 weeks (at the early stage of lupus nephritis) (28). After

8 weeks, both groups of CCL2 antagonist-treated mice showed markedly diminished infiltration of macrophages and T cells into the glomeruli, interstitium, and perivascular areas, resulting in significant amelioration of glomerular hypercellularity, glomerulosclerosis, crescent formation, and vasculitis compared with control MRL/lpr mice. This amelioration was associated with the reduction of IFN-γ and IL-2 production in the kidney. No reduction of circulating IC and anti-dsDNA antibodies was observed in the CCL2 antagonist-treated MRL/lpr mice. In addition, there was no difference in the intrarenal deposition of IgG and C3 between control and CCL2 antagonist-treated MRL/lpr mice. These observations support the finding that CCL2 is expressed secondary to the deposition of IC. Our results were confirmed by a study in which a gene on a plasmid transfection vector encoding a truncated CCL2, referred to as the 7ND construct, was transferred into the skeletal muscles of 16-week-old MRL/lpr mice (50). The 7ND construct improved survival of MRL/lpr mice with lupus nephritis. This was due to decreased renal inflammation with less inflammatory cell infiltrates and reduced IL-12 and IFN-γ production in the kidneys of MRL/lpr mice (51). These findings are also consistent with the data obtained in both CCL2- and CCR2-deficient MRL/lpr mice (29, 52). These mice showed a marked decrease in macrophage and T cell recruitment with concordant reductions in kidney pathological features and proteinuria, despite the similarly high levels of IgG and C3 deposition compared with control MRL/lpr mice. This finding provided strong evidence that a genetic deficit in CCL2 or its receptor CCR2 can delay at least the initiation of lupus nephritis. Moreover, we showed that the CCL2 antagonist ameliorated not only the initiation but also the progression of lupus nephritis. This indicates that the administration of CCL2 antagonist is both a preventive and a therapeutic regimen.

Next, we investigated whether $NH_2$-terminally truncated CX3CL1 analogue, referred to as CX3CL1 antagonist, ameliorates lupus nephritis in MRL/lpr mice using a similar experimental design to that used for the CCL2 antagonist (31). CX3CL1 is a unique chemokine that contains a CX3C motif and exists in both soluble and membrane-anchored forms (53-56). The membrane-anchored form of CX3CL1 is comprised of a chemokine head tethered to the cell surface by a mucin stalk, followed by a single transmembrane-spanning domain. The soluble form is a fragment of the extracellular portion, which is cleaved at a conserved motif proximal to the membrane by metalloproteinases. CX3CL1 and its receptor, CX3CR1, are known to mediate both cell adhesion and cell migration. Membrane-anchored CX3CL1 can be markedly upregulated on primary endothelial cells by inflammatory cytokines such as TNF-α and IL-1β, and rapidly induces firm adhesion of CX3CR1-expressing cells under both static and flow conditions. In contrast, like other soluble chemokines, soluble CX3CL1 induces calcium mobilization and migration of CX3CR1-expressing cells. In humans, CX3CR1 is expressed on macrophages and cytotoxic effector lymphocytes such as natural killer (NK) cells and cytotoxic T cells. In mice, its expression in circulating blood cells is restricted to macrophages and NK cells (57). During the development of lupus nephritis in MRL/lpr mice, CX3CL1 is markedly induced on endothelial and mesangial cells in response to the production of inflammatory cytokines such as TNF-α and IL-1β. The production of these cytokines is triggered by the deposition of ICs and complement activation in many types of renal cells. For example, under high blood-flow conditions in the glomerular capillaries, endothelium-derived membrane-anchored CX3CL1 would play an essential role in the recruitment and accumulation of CX3CR1-expressing leukocytes, especially macrophages, since soluble CX3CL1 generated on the surface of the endothelium would be washed away. In contrast, soluble CX3CL1 generated by mesangial cells has been suggested to bind to extracellular matrices and complement the action of endothelium-derived membrane-anchored CX3CL1. Treatment of MRL/lpr mice with CX3CL1 antagonist before the onset or during the early stages of lupus nephritis significantly reduced glomerular hypercellularity, glomerulosclerosis, crescent formation and vasculitis compared with control mice (31). This seemed to be due to a marked reduction in macrophage accumulation. However, no reduction in circulating ICs or anti-dsDNA antibodies was observed in the CX3CL1 antagonist-treated MRL/lpr mice. These findings show that the CX3CL1 antagonist inhibited not only the initiation but also the progression of lupus nephritis in MRL/lpr mice as well as the case of CCL2 antagonist.

In these two reports, we demonstrated that both the CCL2 antagonist and CX3CL1 antagonist ameliorated the initiation and progression of lupus nephritis in MRL/lpr mice. Here we discuss the interaction of CCL2 and CX3CL1 during the development of renal damage. CCL2 is secreted from many kinds of renal cells, including endothelial, mesangial, tubular epithelial and interstitial cells, and macrophages in response to stimulation with proinflammatory cytokines and ICs. On the other hand, CX3CL1 is expressed predominantly in activated glomerular endothelial cells but is only occasionally observed in the mesangial cells. Treatment with either CCL2 antagonist or CX3CL1 antagonist failed to prevent macrophage infiltration completely. In addition, CCR2-positive macrophages and T cells were observed in the glomerulus, tubulointerstitium and perivascular region in the CX3CL1 antagonist-treated MRL/lpr mice. Blockade of either the CCL2-CCR2 system or the CX3CL1-CX3CR1 system with neutralizing antibodies also reportedly fails to prevent macrophage infiltration completely in various animal models of nephritis (for review, see ref 58). In addition, the anti-CCL2 antibody suppressed leukocyte infiltration and ameliorated proteinuria in the early stage of murine nephrotoxic nephritis, while treatment with the anti-CX3CL1 antibody was less effective at the early stage than at the late stage (59). These findings suggest that the differential expression of CCL2 and CX3CL1 may sequentially recruit distinct subsets of monocytes/macrophages and T cells to the glomerulus and tubulointerstitium during the development of renal damage. Therefore, a combination of the CX3CL1 antagonist and CCL2 antagonist may be more effective as therapy for lupus nephritis.

CCR1 blockade has been demonstrated to be effective on lupus nephritis in MRL/lpr mice (30). CCR1, the receptor for CCL4 and CCL5, has been reported to be expressed in high levels on glomerular macrophages and low level on interstitial T cells in MRL/lpr mice (27). Since the expression of CCL4 and CCL5 is induced in intrinsic glomerular and tubulointerstitial cells, the presence of CCR1 on infiltrating leukocytes is consistent with the pattern of chemokines induced. Therefore, CCR1 was also considered as a therapeutic target for lupus nephritis. BX471, a small molecular antagonist with blocking activity against murine and human CCR1, was administrated into MRL/lpr mice (30). Daily injections of BX471 were started at 20 weeks of age in female MRL/lpr mice. Female MRL/lpr mice of this age suffer from advanced diffuse proliferative lupus nephritis and proteinuria associated with early tubulointerstitial inflammation and tubular injury. After 4 weeks of treatment, the parameters of renal function and renal histopathology were evaluated. CCR1 blockade reduced the numbers of interstitial macrophages and T cells. This reduction of the interstitial immune cell infiltrates was associated with reduced myofibroblasts, renal TGF-β mRNA expression, and interstitial collagen-I deposits, indicating a beneficial effect of the CCR1 blockade on the subsequent renal fibrosis. In addition, the blood urea nitrogen levels in BX471-treated MRL/lpr mice were reduced. Interestingly, glomerular pathology, including the number of glomerular macrophages as well as proteinuria, remained unaffected by CCR1 blockade. CCR1 blockade had also no effect on serum levels of anti-dsDNA antibodies. These findings show that blocking CCR1-dependent interstitial immune cell infiltrates can improve renal function and tubulointerstitial damage in MRL/lpr mice with advanced diffuse proliferative lupus nephritis. In addition, these data exclude a role of CCR1 for glomerular macrophage recruitment.

Lymphoid chemokines regulate the homing of immune cells to lymphoid organs rather than to inflammatory tissues. Therefore, blocking lymphoid chemokines may have beneficial effects on lupus nephritis by interfering with systemic immune responses and the generation of autoreactive cells. In fact, a CXCL12 antagonist showed beneficial effects on lupus nephritis in NZB/W F1 mice by reducing anti-dsDNA antibodies, IC deposits, and subsequent glomerular lesions and proteinuria (33).

## 6. EXPRESSION OF CHEMOKINES AND CHEMOKINE RECEPTORS IN EXPERIMENTAL AUTOIMMUNE SIALADENITIS

MRL/lpr mice spontaneously develop progressive sialadenitis, which becomes noticeable at 8 to 10 weeks of age. Small focal infiltrates of inflammatory cells (< 50 cells) are predominantly located around the blood vessels within the salivary glands. After 12 weeks of age, larger mononuclear cell infiltrates, especially those composed of $CD4^+$ T cells, become apparent in the periductular regions, extending to the parenchyma and resulting in parenchymal destruction. MRL/lpr mice show severe parenchymal destruction with ductular proliferation, granulomatous lesions, and/or fibrosis by the age of 20 weeks (60, 61).

Th1-associated cytokines, especially IFN-γ, have been reported to play an important role in the progression of autoimmune sialadenitis in MRL/lpr mice (61-64). The three Th1-associated chemokines, CXCL9/Mig, CXCL10, and CXCL11/I-TAC, were predominantly expressed in the ductal epithelium adjacent to lymphoid infiltrates in the salivary gland from the early stage of autoimmune sialadenitis in MRL/lpr mice (61). The notion that these chemokines are expressed in the ductal epithelium is also supported by the finding that a marked infiltration of $CXCR3^+$ T cells was present around the duct. Although the trigger of autoimmune sialadenitis is unknown, from the finding that IFN-γ-producing cells appeared in the salivary glands preceding the expression of these Th1-associated chemokines, we can consider the possibility that IFN-γ production induces the expression of these chemokines in the ductal epithelium. In contrast, expression of the Th2-associated chemokines CCL17/TARC and CCL22/MDC in the salivary glands was negligible. MRL/lpr mice also began to show a significant increase of CCL2 in the salivary glands at 12 weeks of age (65, 66). CCL2 was expressed mainly in infiltrating macrophages and interstitial cells, but no expression was observed in the ductal epithelium.

In humans, several investigators have suggested that polarized Th1 balance is associated with the pathogenesis of SS (67-69). In addition, the Th1-associated chemokines CXCL9, CXCL10, and CXCL11 have also been reported to be predominantly expressed in the ductal epithelium, leading to the accumulation of T cell infiltrates in the salivary glands of patients with SS (70, 71).

## 7. BLOCKADE OF CHEMOKINES AND CHEMOKINE RECEPTORS IN AUTOIMMUNE SIALADENITIS OF MRL/LPR MICE

Since the development of autoimmune sialadenitis through the expression of Th1-associated chemokines and their common receptor CXCR3 in patients with SS closely resembles that in MRL/lpr mice, our findings in MRL/lpr mice may be applicable to the therapy of autoimmune sialadenitis in humans. Treatment of MRL/lpr mice with the CXCL10 antagonist in the early stage of sialadenitis significantly reduced periductal mononuclear cell infiltration and parenchymal destruction compared with control mice (61). This was due to a significant reduction in the infiltration of $CXCR3^+$ T cells, predominantly Th1 cells, resulting in decreased IFN-γ production. Treatment with the CCL2 antagonist significantly reduced sialadenitis, whereas CX3CL1 antagonist did not have any effect on sialadenitis, compared with control MRL/lpr mice. However, the reduction of CCL2 antagonist was less than that of CXCL10 antagonist.

## 8. BLOCKADE OF CHEMOKINES AND CHEMOKINE RECEPTORS IN ARTHRITIS IN MRL/LPR MICE

Arthritis in MRL/lpr mice has similar characteristics to human RA including cell infiltration, pannus formation, bone and cartilage breakdown, and the

presence of serum RF (for review, see refs 3 and 4). Mononuclear cell infiltrates, especially macrophages and $CD4^+$ T cells, are prominent, and their products, such as cytokines that amplify the inflammatory response and enzymes that destroy connective tissue, are readily detected in the diseased joints of MRL/lpr mice. CCL2 is produced by both synovial cells and infiltrated macrophages. Thus, the inhibition of CCL2 function could control inflammation by preventing accumulation of macrophages and $CD4^+$ T cells in the joints. Gong *et al.* have shown that a 67-amino acid sequence of CCL2, which acts as a CCL2 antagonist *in vivo*, ameliorated arthritis in MRL/lpr mice, and furthermore that a combination of CCL2 and CXCL1 inhibition with chemokine antagonists resulted in a larger reduction of arthritis than CCL2 blockade alone (72, 73).

## 9. CONCLUSIONS

Accumulating evidence indicates a critical role of chemokines and chemokine receptors in the development of autoimmune diseases in both humans and animal models. Similar expression patterns of chemokines and chemokine receptors in lupus nephritis, autoimmune sialadenitis, and arthritis are shown in humans and lupus model mice, especially MRL/lpr mice. Therefore, findings obtained from experiments with MRL/lpr mice may be applicable to the treatment of these autoimmune diseases in humans. In this article, we reviewed several chemokine antagonists which can ameliorate the development of lupus nephritis, sialadenitis, and arthritis in lupus model mice. However, additional members of the chemokine and chemokine receptor family may represent promising targets for therapy. With an increasing number of chemokine receptor antagonists currently being developed and evaluated in clinical trials, we expect that in the future, selective blockade of chemokine functions will offer the promise of a new therapeutic strategy for the treatment of autoimmune diseases.

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