p38 Mitogen–Activated Protein Kinase Contributes to Autoimmune Renal Injury in MRL-\textit{Fas}^{lpr} Mice

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Abstract. The phosphorylation of p38 mitogen-activated protein kinase (MAPK) is responsible for the production and signal transduction of cytokines and chemokines. This study hypothesized that p38 MAPK activation is required for spontaneous autoimmune renal injury in MRL-\textit{Fas}^{lpr} mice, resembling human lupus erythematosus. FR167653, a specific inhibitor of p38 MAPK, is orally administrated from 3 or 4 mo of age in MRL-\textit{Fas}^{lpr} mice (at doses of 10 or 32mg/kg per day) until 6 mo of age. The phosphorylated p38 MAPK in kidneys of MRL-\textit{Fas}^{lpr} mice was evaluated. The number of phosphorylated p38 MAPK-positive cells was increased in diseased kidneys. The daily oral administration of FR167653 decreased p38 MAPK phosphorylation in kidneys, especially in a group of mice administered FR167653 (32 mg/kg per day) daily from 3 to 6 mo of age. FR167653 reduced the accumulation of macrophages and T cell and prevented kidney pathology, resulting in prolonged survival. In addition, FR167653 reduced expression of MCP-1 and TNF-\alpha in the diseased kidneys and cultured tubular epithelial cells. Furthermore, FR167653 decreased IgG levels in the diseased kidneys and circulation. These results suggest that the phosphorylation of p38 MAPK is required for the pathogenesis of renal injury in MRL-\textit{Fas}^{lpr} mice followed by subsequent expression of renal cytokine/chemokine and IgG production. This study provides evidence that the regulation of p38 MAPK is a novel target for the therapy of renal injury in systemic lupus erythematosus.

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Mitogen-activated protein kinase (MAPK) signaling plays an important role in proliferation and apoptosis in the setting of inflammatory processes (1). The activation of MAPK isoform p38 is involved in apoptosis, stress responses, and inflammation (2). Recent studies revealed that p38 MAPK phosphorylation is essentially responsible for the production of C-C chemokine, monocyte chemoattractant protein (MCP)-1, also termed as monocyte chemoattractant and activating factor (3,4), and for the signal transduction of chemokine receptor, CCR5 (5). Furthermore, p38 MAPK is activated and involved in the pathogenesis of human autoimmune diseases, including the sialoadenitis of Sjögren syndrome (6) and rheumatoid arthritis (7). Thus, the activation of p38 MAPK may contribute to the pathogenesis of autoimmune diseases via the activation of the signal transduction and expression of cytokines and chemokines. However, the role of p38 MAPK remains to be investigated in systemic lupus erythematosus, one of the major progressive autoimmune diseases.

Autoimmune diseases in MRL/\textit{MPJ-lpr/lpr} (MRL-\textit{Fas}^{lpr}) mice resemble human systemic lupus erythematosus, characterized by the dysregulation of both cellular and humoral immunity (8). This strain is particularly valuable for understanding the pathogenesis of autoimmune renal injury. Cytokines are evident before and during autoimmune tissue destruction in MRL-\textit{Fas}^{lpr} mice. It has been previously established that a macrophage (Mφ) growth factor, colony stimulating factor–1 (CSF)-1, interleukin-1\beta (IL)-1\beta and tumor necrosis factor–\alpha (TNF)-\alpha levels are increased simultaneously in the kidney and circulation in MRL-\textit{Fas}^{lpr} mice (9–11), which initiate and promote autoimmune organ destruction. Intrarenal gene transfer of CSF-1 or granulocyte-macrophage colony-stimulating factor (GM-CSF) elicited leukocyte infiltration (Mφ and T cells) in MRL-\textit{Fas}^{lpr} kidneys (12–14). The expression of these cytokines and growth factors are dependent on the activation of nuclear factors, including nuclear factor \kappa B (NF-\kappa B), possibly through activation of p38 MAPK (15). In addition, B cell activation is associated with the activation of p38 MAPK (16). These findings imply that activation of p38 MAPK may be instrumental in progressive autoimmune kidney destruction characteristic of the MRL-\textit{Fas}^{lpr} strain. Thus, we hypothesize that p38 MAPK is responsible for the production and signal transduction of the cytokines and chemokines, thereby ini-
tiating and promoting autoimmune renal injury in MRL-
Fas<sup>lpr</sup> mice.

To test this hypothesis, we have examined the impacts of p38 MAPK phosphorylation on the systemic autoimmunity resulting in severe renal injury in MRL-Fas<sup>lpr</sup> mice via the daily oral administration of a specific inhibitor of p38 MAPK, FR167653, (1-[7-(4-fluorophenyl)-1, 2, 3, 4-tetrahydro-8-(4-pyridyl)pyrazolo[5, 1-c] (1,2,4) triazin-2-yl]-2-phenylethanedi-one sulfate monohydrate) (17–21). We describe here that the inhibition of p38 MAPK reduced the expression of cytokine/chemokine and IgG levels, which is subsequently followed by a decrease in lethal autoimmune organ destruction, and prolonged survival.

**Material and Methods**

**Mice**

MRL-Fas<sup>lpr</sup> mice aged 1 mo were obtained from Charles River Japan Inc., Atsugi, Japan. All procedures employed in the animal experiments complied with the standards set out in the Guideline for the Care and Use of Laboratory Animals in Takara-machi Campus of Kanazawa University. MRL-Fas<sup>lpr</sup> mice were divided into five groups, and experimental design was described in Figure 1. FR167653 (10 or 32 mg/kg per day), dissolved in drinking water, was orally administrated from 3 or 4 mo of age. Mice in any group were sacrificed at 6 mo of age. Ten MRL/MpJ<sup>H11001/H11001</sup> (MRL<sup>H11001/H11001</sup>) mice were used as negative controls.

**Proteinuria**

We assessed urinary protein levels monthly using dipstick analysis (Bayer Medicals, Tokyo, Japan) and graded them semiquantitatively (0, none; 0.5, 30 to 100 mg/dl; 1, 100 to 300 mg/dl; 2, 300 to 1000 mg/dl; 3, >1000 mg/dl).

**Lymphadenopathy**

Protruding lymph nodes (cervical, brachial, and inguinal) were assessed monthly. Lymph node score based on palpable nodes: 0, none; 1, small, at one site; 2, moderate, at two different sites; and 3, large, at three or more different sites.

**Histopathology**

Kidneys were either snap-frozen in OCT compound for cryostat sectioning or fixed in 10% neutral-buffered formalin. Formalin-fixed tissue was embedded in paraffin, and 4-μm sections were stained with periodic acid-Schiff (PAS) and evaluated by a light microscopy. We evaluated the glomerular, periglomerular, interstitial, and perivascular pathology morphometrically. Glomeruli and periglomeruli were assessed by counting intraglomerular and periglomerular cells at 50 glomerular cross-sections (gcs) per kidney. We evaluated the interstitial pathology by counting the number of infiltrating cells in 20 random interstitial fields (magnification, ×400). The extent of renal pathology was assessed by determining (1) the percentage of crescents at 50 gcs (defined as thickening of Bowman’s capsule wall with two or more cell layers; (2) the percentage of segmental lesions at 50 gcs (exhibiting at least one of the following: necrosis, proliferation, hyalnosis) in glomeruli; (3) the percentage of damaged tubuli (consisting of at least one of the following: dilatation, atrophy, necrosis) in randomly 20 selected microscopic fields (magnification, ×400). The perivascular cell accumulation was determined by scoring the number of cell layers surrounding ten random interlobular and intralobular arteries (0, none; 1, <5 layers surrounding more than half of the vessel; 2, 5 to 10 layers surrounding more than half of the vessel; 3, >10 layers surrounding more than half of the vessel). Scoring was evaluated using uncoded slides.

**Antibodies**

The following primary antibodies were used for immunostaining: rat anti-mouse CD4 IgG2a clone RM4–5 (PharMigen, San Diego, CA) to detect CD4 T cells; rat anti-mouse CD8a (Ly-2) IgG2a clone 53 to 6.7 (PharMigen) to detect CD8 T cells; rat anti-mouse CD45R/B220 IgG2a clone RA3–6B2 (PharMigen) to detect CD4/CD8-negative T cells and B cells; rat anti-mouse monocyte/macrophage IgG2b clone MOMA-2 (BMA biomedicals AG, Augst, Switzerland) to detect M<sub>Φ</sub>; rabbit anti-phosphorylated p38 MAPK polyclonal antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), which react with both α and β isoforms of p38 MAPK as described previously (22); FITC-conjugated anti mouse
IgG (Cappel, Durham, NC) to detect mouse IgG. The secondary antibodies for immunostaining were biotin-conjugated rabbit anti-rat IgG and biotin-conjugated goat anti-rabbit IgG (Vector Labs, Burlingame, CA). ELISA analysis, including serum total IgG, IgG subclasses (Bethyl, Montgomery, TX), and anti-ds DNA antibodies (Alpha Diagnostic, San Antonio, TX), was performed using isotype-specific standards, goat anti-mouse capture antibodies, and horseradish peroxidase-conjugated goat anti-mouse detection antibodies.

**Immunohistochemical Examination**

Tissues for immunoperoxidase staining were snap-frozen in OCT (Miles Scientific, Naperville, IL) and stored at −80°C. We located Mφ, CD4+, CD8-, and B220-positive cells, characteristics of MRL-Fas<sup>lpr</sup> mice using monoclonal antibodies. These cells were identified using the avidin-biotin complex immunoperoxidase technique. Using a light microscope, we counted Mφ, CD4+, and CD8-positive T cells and B220-positive cells in periglomerular areas of 20 randomly selected glomeruli that were expressed as cells/glomerulus (gcs). Interstitial Mφ, CD4-, and CD8-positive T cells and B220-positive cells were counted in 10 randomly selected fields of cortical interstitium with a light microscope (magnification, ×400) and described as cells/field.

Similarly, we graded the perivascular Mφ, CD4+, and CD8-positive T cells in 10 interlobular and intrafollicular arteries.

In addition, we detected phosphorylated p38 MAPK within the kidneys. The presence of phosphorylated p38 MAPK was demonstrated immunohistochemically in paraffin-embedded tissue specimens at 6 mo of age by the indirect avidin-biotinylated peroxidase complex method with rabbit anti-phosphorylated p38 MAPK polyclonal antibodies (10 µg/ml). A normal rabbit serum was used as a negative control.

To evaluate the phosphorylated p38 MAPK-positive cells in the kidneys, we counted phosphorylated p38 MAPK-positive cells in glomerular areas of 20 randomly selected glomeruli that were expressed as cells/glomerulus (gcs). Interstitial phosphorylated p38 MAPK-positive cells were counted in ten randomly selected fields of cortical interstitium with a light microscopy (magnification, ×400) and described as cells/field.

The Deposition of IgG

To determine IgG deposits in diseased kidneys, 4-µm frozen sections were stained with FITC-conjugated antibodies detecting murine IgG at 37°C for 30 min. The amount and extent of fluorescence was evaluated in at least 50 glomeruli graded from 0 to 3 (0, none; 1, mild; 2, moderate; 3, severe).

Profile of Serum Immunoglobulins and anti-ds DNA Antibodies

ELISA plates were coated overnight at 4°C with 5 µg/ml goat anti-mouse IgG capture antibodies (against total IgG and IgG subclasses) in 0.1 M carbonate buffer, pH 9.4 (mouse IgG ELISA quantification kit, Bethesda Laboratories, Inc., Montgomery, TX; Mouse anti-ds DNA antibodies ELISA quantification kit, Alpha Diagnostic, San Antonio, TX). Wells were blocked for 1 h with assay diluent (0.5% BSA in 0.1 M borate buffer, pH 8.0). We added standards of IgG, IgG subclasses, or anti-ds DNA antibodies to the plates (100 µl/well), performing a series of threefold dilutions, and assessed serum samples. Standards and serum samples were incubated overnight at 4°C, and bound IgG was detected with goat anti-mouse detection antibodies conjugated with horseradish peroxidase and enzymatically developed. Absorbance was measured at 450 nm. Serum anti-ds DNA antibodies were showed OD index (sample OD/standard OD).

Intrarenal Transcripts of MCP-1 and TNF-α

Total RNA was extracted from the cortices, and analyses were performed using reverse transcriptase PCR (RT-PCR), as described previously (22). Reverse transcription was performed using a RT-PCR kit (Perkin Elmer, Foster City, CA) for total RNA obtained and combined from mice in each group (1 µg of RNA per mouse). The complementary DNA product (1 µg) was amplified by PCR. Primers (5’ primer CTCTCATCGTTCTAGGCC; 3’ primer GGAGTA-GACAAGGTACAAC) and (5’ primer CCTCTCTTGTAGCTTG-GTG; 3’ primer AAGCCAGCTCTCTTGAGCT) were used to detect TNF-α and MCP-1. Ten microliters of PCR products were analyzed using 2% agarose gel electrophoresis and stained with ethidium bromide. The housekeeping gene GAPDH was used for PCR controls. Transcripts (TNF-α, MCP-1)/GAPDH ratios were evaluated.

Cell Culture and Treatment by IL-1β and TNF-α

Human renal proximal tubular epithelial cells (TEC) (lot No. CC-2553; Cambrex, East Rutherford, NJ) were grown in Renal Epithelial Cell Growth Medium (REGM) BulletKit (Cambrex) with 100 µg/ml streptomycin and 100 U/ml penicillin in a humidified atmosphere (5% CO2/95% air) at 37°C. Cultured TEC were trypsinized, suspended in
REGM, and seeded onto six-well collagen type-IV-coated plates (Asahi Technoglass Co., Tokyo, Japan). TEC were pretreated with or without FR167653 (1 \times 10^{-6} M or 3 \times 10^{-7} M) for 1 h, followed by the stimulation with both IL-1β (10 ng/ml) and TNF-α (20 ng/ml). TEC were harvested 24 h and 48 h after the stimulation of IL-1β and TNF-α. Total RNA was extracted from TEC using RNAzol B (Tel-Test, Friendswood, TX), a modification of the acid guanidium isothiocyanate-phenol-chloroform method (23).

**Detection of MCP-1 Transcripts in Cultured TEC**

To determine the effects of FR167653 on MCP-1 transcripts, total RNA was analyzed by reverse transcription PCR (RT-PCR). cDNA was reverse-transcribed from total RNA (1 μg RNA per mouse) using an RT-PCR kit (Takara Shuzo Co. Ltd, Tokyo, Japan). The cDNA product was amplified by PCR. Primers for MCP-1 (5’ primer TTCTGTGCCTGCTGCTCATA; 3’ primer GAGTGAGTGTTCAAG-TCTTCG) were used. Ten microliters of PCR products were analyzed using 2.0% agarose gel electrophoresis and stained with ethidium bromide. The housekeeping gene GAPDH was used for PCR products.

**Detection of MCP-1 in Supernatants of Cultured TEC by ELISA**

MCP-1 levels in supernatants of cultured TEC were determined by ELISA when stimulated by the combination of IL-1β and TNF-α with or without FR167653 treatment (24).

**Statistical Analyses**

The data represent the means ± SEM. Statistical significance was determined by ANOVA and Kruskal-Wallis analyses. Data was analyzed using Kaplan-Meier life table method for survival curves.

*Figure 5.* Renal pathology is reduced in FR167653-treated MRL-Fas<sup>br</sup> mice. MRL-Fas<sup>br</sup> mice developed severe proliferative glomerulonephritis and interstitial/perivascular damage (group 5). Renal pathology was decreased in FR167653-treated MRL-Fas<sup>br</sup> mice, particularly in group 1. PAS staining; original magnification, ×200.
Results

FR167653-Treated MRL-Fas<sup>lpr</sup> Mice Survive Longer than the Untreated

Survival in the FR167653-treated MRL-Fas<sup>lpr</sup> mice (groups 1 to 4) was extended as compared with untreated MRL-Fas<sup>lpr</sup> mice (Figure 2). The 50% mortality in untreated MRL-Fas<sup>lpr</sup> mice was 6 mo of age. In comparison, FR167653-treated MRL-Fas<sup>lpr</sup> mice in groups 1 to 4 had a prolonged lifespan.

FR167653-Treated MRL-Fas<sup>lpr</sup> Mice Are Protected from Proteinuria

Proteinuria was prevented in FR167653 treated MRL-Fas<sup>lpr</sup> mice (Figure 3). From 3 to 6 mo of age, increase in proteinuria was diminished in mice of group 1, whereas levels of proteinuria were elevated in untreated MRL-Fas<sup>lpr</sup> mice. In group 2, proteinuria, which was already evoked at the initiation time of administration of FR167653, was also significantly reduced by the administration of FR167653 at 6 mo of age. In addition, at 6 mo, urinary protein excretion was significantly reduced in group 1 than that in group 2 (P < 0.05). In group 3, proteinuria was also significantly prevented.

Lymphadenopathy Is Ameliorated in FR167653-Treated MRL-Fas<sup>lpr</sup> Mice

Untreated MRL-Fas<sup>lpr</sup> mice showed severe lymphadenopathy at 6 mo of age. The incidence and severity of lymphadenopathy remained diminished in FR167653-treated MRL-Fas<sup>lpr</sup> mice as compared with these of untreated MRL-Fas<sup>lpr</sup> mice (Figure 4).

Renal Pathology Is Reduced in FR167653-Treated MRL-Fas<sup>lpr</sup> Mice

To determine the impact of p38 MAPK inhibition, we evaluated the extent of renal pathology in FR167653-treated MRL-Fas<sup>lpr</sup> mice in comparison with untreated MRL-Fas<sup>lpr</sup> mice at 6 mo of age (Figures 5, 6, and 7). Renal pathology in untreated
Kidney disease in MRL-Fas lpr mice consists of severe proliferative glomerulonephritis, interstitial/perivascular damage. In contrast, renal pathologic changes were attenuated but not abrogated in FR167653-treated MRL-Fas lpr mice (Figure 5), as compared with that of MRL++ mice (Figures 5, 6, and 7). We noted reductions in the numbers of glomerular, periglomerular, interstitial, and perivascular cells. In particular, mice in group 1 exhibited a diminishment in glomerular and interstitial cell infiltration as compared with untreated MRL-Fas lpr mice (23.7% reduction, CD4, and CD8 T cells but not B220 in periglomerular (a), interstitial (b), and perivascular (c) lesions in FR167653-treated MRL-Fas lpr mice. *P < 0.05 versus untreated MRL-Fas lpr mice at 6 mo of age.

Reduced Number of Phosphorylated p38 MAPK-Positive Cells in Kidney

We evaluated the number of phosphorylated p38 MAPK-positive cells in kidneys from MRL-Fas lpr mice. Phosphorylated p38-positive cells were already detected in kidneys of MRL-Fas lpr mice at 1 mo of age, when autoimmune organ diseases were hardly observed (data not shown). At 6 mo of age, the number of phosphorylated p38-positive cells was 39.6 ± 3.8 in glomeruli (Figure 8c) and 79.7 ± 3.3 in interstitium (Figure 8d) in untreated MRL-Fas lpr mice. Glomerular phosphorylated p38 MAPK-positive cells decreased in groups 1 (Figure 8b) and 2 as compared with untreated MRL-Fas lpr mice (24.8 ± 1.3 in group 1, 26.8 ± 1.4 in group 2; P < 0.05, respectively) (Figure 8c). In interstitium, phosphorylated p38 MAPK-positive cells also significantly decreased in group 1 (46.6 ± 2.6; P < 0.05) and 2 (51.6 ± 1.4; P < 0.05) as compared with untreated MRL-Fas lpr mice (Figure 8d). In groups 3 and 4, glomerular and interstitial phospho-phytated p38 MAPK-positive cells also significantly decreased (Figure 8, c and d).

Reduced TNF-α and MCP-1 Transcripts in FR167653-Treated MRL-Fas lpr Mice Kidneys

It is previously reported that TNF-α and MCP-1 transcripts are upregulated with the deposition of renal injury in MRL-Fas lpr mice are required for autoimmune kidney disease (10,25). In kidneys of FR167653 treated MRL-Fas lpr mice in groups 1 to 4, TNF-α and MCP-1 transcripts were reduced as compared with untreated MRL-Fas lpr mice (Figure 9).

FR167653 Reduced Serum IgG and Anti-ds DNA Antibodies in MRL-Fas lpr Mice

To determine whether the inhibition of p38 MAPK also reduced the production of IgG and anti-ds DNA antibodies resulting from B cell activation, we evaluated serum levels of IgG and anti-ds DNA antibodies. As compared with untreated MRL-Fas lpr mice, serum levels of total IgG, IgG subclasses, and anti-ds DNA antibodies were significantly reduced in FR167653-treated MRL-Fas lpr mice in groups 1 to 4, TNF-α and MCP-1 transcripts were reduced as compared with untreated MRL-Fas lpr mice (Figure 9).

FR167653 Reduced Glomerular IgG Deposition

The amounts of glomerular IgG deposition were also reduced in FR167653-treated MRL-Fas lpr mice in groups 1 and 2 (Figure 10). However, their IgG levels in circulation and glomeruli were still higher than those of MRL++ mice (Figures 10 and 11). These results suggest FR167653 treatment may inhibit the B cell activation, characteristic to autoimmune disorder of MRL-Fas lpr mice.

Transcripts and Protein Levels of MCP-1 Were Diminished by FR167653 Treatment in TEC

The simultaneous stimulation of IL-1β and TNF-α enhanced MCP-1 transcripts in cultured human TEC (Figure 12a). How-
ever, the enhanced transcripts of MCP-1 were decreased by treatment with FR167653 (Figure 12a). In addition, MCP-1 levels in supernatants were also diminished by the simultaneous incubation of FR167653. In contrast, cultured TEC with media alone faintly expressed MCP-1 (Figure 12, a and b).

**Discussion**

In this report, we have tested the roles of p38 MAPK in autoimmune renal injury in MRL-Fas<sup>lpr</sup> mice. We herein report that the inhibition of p38 MAPK reduced autoimmune diseases resulting in a prolonged life span in MRL-Fas<sup>lpr</sup> mice. We also noted that the protection against renal injury in MRL-Fas<sup>lpr</sup> mice resulted from a reduced infiltration of leukocytes, a diminished expression of cytokines known to promote renal injury, and a reduced production of Ig. Thus, we conclude that the activation of p38 MAPK is required to promote cytokine/chemokine and Ig production, which in turn result in lethal autoimmune renal injury in MRL-Fas<sup>lpr</sup> mice.

During inflammation, p38 MAPK, activated in various cell types, is closely related to apoptosis, stress responses, and inflammation (2). In addition, p38 MAPK phosphorylation participates in the production of MCP-1, IL-1β, and TNF-α for examples, in human mesangial cells (3) and human endothelial cells (4), which are known to promote autoimmune organ destruction in MRL-Fas<sup>lpr</sup> mice (9,10). We report here that activated p38 MAPK-positive cells, increased in the diseased kidneys, were reduced by the administration of FR167653, concomitantly with the reduction of severity of autoimmune renal injury. Moreover, intrarenal transcripts of MCP-1 and TNF-α were diminished through FR167653 treatment. Therefore, p38 MAPK signaling-dependent chemokine/chemokine production may be responsible for the recruitment and activation of leukocytes in diseased kidneys in MRL-Fas<sup>lpr</sup> mice. In addition to induction of the recruitment of leukocytes to the sites of inflammation, p38 MAPK is presumed to be involved in cell proliferation where transforming growth factor-β or
platelet-derived growth factor may work (26). In this study, the long-term administration of FR167653 had impacts on the prevention of end-stage organ damage. We recently reported that the administration of FR167653 at the onset of glomerulonephritis ameliorated glomerulosclerosis and interstitial fibrosis via the inhibition of MCP-1 (22). In addition, a recent study revealed that MCP-1 mediates collagen deposition in experimental glomerulonephritis by TGF-β (27). In turn, TGF-β may contribute to the secretion of tubular MCP-1 in nephrotic syndrome (28). Thus, once the activation of p38 MAPK is inhibited by FR167653, the upregulation of TNF-α and MCP-1 is reduced, possibly thereby leading to the prevention of cell infiltration and proliferation and long-term organ injury. To understand whether the effects of p38 MAPK are directly on renal cells or whether this is indirectly due to a reduction in IgG levels, we investigated (1) serum levels of immunoglobulins and anti-ds DNA antibodies and IgG depositions in diseased kidneys and (2) the inhibitory impacts on MCP-1 production in cultured TEC. We detected reduced serum levels of Ig and anti-ds DNA antibodies and a decrease in the amount of Ig deposition in the kidney. Moreover, the inhibition of p38 MAPK resulted in reduction of subclasses of immunoglobulins in serum. Supporting this notion, a recent study revealed that p38 MAPK activation is indispensable for B cell activation leading to Ig production (29). In addition, Ig deposition in the kidney promotes and accelerates autoimmune renal destruction (30). In addition to reduction of B cell activation, FR167653 exhibited direct effects on tubular epithelial cells, resulting in reduction of chemokine, MCP-1. Thus, our data suggest that inhibition of p38 MAPK activation via FR167653 is effective for autoimmune renal injury through the decrease both in Ig production and activation of renal resident cells, such as TEC. We have uncovered the therapeutic effects of p38 MAPK, responsible for promoting and escalating autoimmune renal injury. We report the inhibition of p38 MAPK from 3 mo of age, the onset of autoimmune disease in MRL-Fas<sup>br</sup> mice, and reduced autoimmune renal injury. Moreover, FR167653 treat-
ment begun from 4 mo of age was less effective compared with those begun from 3 mo of age, but it still significantly reduced escalating renal injury. These suggest that p38 MAPK inhibition may dampen the promotion and escalation of autoimmune attack in kidneys, even when tissue damage had been already commenced. This implies a possible impact on already-established human autoimmune diseases where p38 MAPK activation followed by upregulation of cytokines and/or chemokines plays an important role in the pathogenesis. Collectively, these data suggest that p38 MAPK has a promising future for the therapeutic target in human autoimmune renal injury.

To understand the time course of autoimmunity, we assessed protruding lymph nodes monthly (Figure 4). Massively enlarged lymph nodes reflect the activation of systemic autoimmunity in MRL-Faslpr mice. Lymphadenopathy appeared from 2 mo of age and increased until 6 mo of age. The incidence and severity of lymphadenopathy diminished in FR167653-treated MRL-Faslpr mice as compared with those of untreated MRL-Faslpr mice during the time course examined. This means that the activity of systemic autoimmunity remained low by FR167653 treatment.

These studies further support the concept that the inhibition of 38 MAPK is still insufficient to completely prevent autoimmune renal injury. Other MAPK may be responsible for recruiting infiltrating cells and cellular proliferation. For example, the classic extracellular signal-regulated kinases (ERK) are identified in the context of growth factor-related signaling in the various types of cultured renal cells (31). In addition, increased renal ERK activation is associated with proliferative anti-glomerular basement membrane glomerulonephritis in vivo (32). Therefore, we would identify most important therapeutic targets in systemic autoimmunity MRL-Faslpr mice to achieve complete protection from renal injury.

FR167653 was originally developed as an inhibitor of IL-1β and TNF-α (17–20); however, the precise molecular pharmacologic actions of FR167653 remain still unclear. In this study, we showed that FR167653 inhibited p38 MAPK activation.

![Figure 11. Intraglomerular IgG deposition is reduced in FR167653-treated MRL-Faslpr mice. Significant amounts of IgG were detected in diseased glomeruli in MRL-Faslpr mice, which were reduced by treatment of FR167653. * P < 0.05 versus untreated MRL-Faslpr mice at 6 mo of age. † P < 0.05 versus FR167653-treated MRL-Faslpr mice from 4 mo of age.](image)

![Figure 12. MCP-1 transcripts and MCP-1 protein are suppressed by FR167653 treatment. Total RNA was extracted from IL-1β- and TNF-α-stimulated TEC. IL-1β and TNF-α enhanced the transcription of MCP-1 in cultured human TEC. However, the enhanced mRNA expression of MCP-1 was decreased by the treatment with FR167653 (a). In addition, elevated MCP-1 levels in supernatants were significantly decreased by FR167653 (b). Results are representative of three experiments. * P < 0.05 versus IL-1β- and TNF-α-stimulated TEC without FR167653 treatment at the same hour in cultured period.](image)
We previously reported that FR167653 does not affect the activation of other MAPK, such as ERK and JNK (22). Supporting this notion, a recent study showed that FR167653 specifically inhibited p38 MAPK activation, especially p38α using an immune-complex kinases assay (20). Moreover, the well-identified specific p38 MAPK inhibitor SB203580 is structurally similar to FR167653, and the structural basis for the specificity of pyridinil-imidazole inhibitors of p38 MAPK is noted (33). In addition, SB242235 specifically inhibits p38α and p38β (34). Concomitantly, we showed the reduction of p38 MAPK phosphorylation, especially p38α and p38β, through FR167653, because antibodies that used for p38 MAPK react with both α and β isoforms of p38 MAPK. Therefore, we speculate that FR167653 may specifically inhibit p38 MAPK activation in this model. However, the inhibitory effects of FR167653 on cyclooxygenases (COX) still remain uncertain. Takahashi et al. (20) reported FR167653 did not have inhibitory effects of COX-2, while Kawano et al. (35) described the suppressive effects of FR167653 on prostaglandin synthesis via the inhibit of COX-2, which may play a role in active lupus nephritis (36). Therefore, further studies will be required to determine the detailed molecular mechanisms of FR167653.

In conclusion, we have determined that autoimmune renal injury and Ig production in MRL-FasL mice is dependent on p38 MAPK activation. The activation of p38 MAPK upregulates cytokines/chemokines and Ig production in MRL-FasL mice. In turn, these recruit T cells and MΦ into kidneys, thereby perpetuating progressive autoimmune tissue destruction. Thus we suggest that p38 MAPK is an appealing therapeutic target for combating autoimmune renal injury in systemic lupus erythematosus.

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