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 chemotactic 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/MpJ-lpr/lpr (MRL-Fas<sup>lp</sup>r) 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-Fas<sup>lp</sup>r mice. It has been previously established that a macrophage (Mφ) growth factor, colony stimulating factor-1 (CSF)-1, interleukin-1β (IL)-1β and tumor necrosis factor-α (TNF-α) levels are increased simultaneously in the kidney and circulation in MRL-Fas<sup>lp</sup>r 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-Fas<sup>lp</sup>r kidneys (12–14). The expression of these cytokines and growth factors are dependent on the activation of nuclear factors, including nuclear factor κB (NF-κB), 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-Fas<sup>lp</sup>r 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, 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, hyalinosis) 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

---

**Figure 1.** Experimental design FR167653 is orally administrated from 3 or 4 mo of age in MRL-**Fas**<sup>lpr</sup> mice (group A, at doses of 32 mg/kg per day; groups B, at doses of 10 mg/kg per day) until 6 months of age. All mice were sacrificed at 6 months.

**Figure 2.** FR167653-treated mice survive longer than untreated MRL-**Fas**<sup>lpr</sup> mice. 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 (group 5).
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 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).

**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 Ig capture antibodies (against total IgG and IgG subclasses) in 0.1 M carbonate buffer, pH 9.4 (mouse IgG ELISA quantification kit, Bethyl 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 TCTCATCAGTTCTAGGCC; 3′ primer GGAGTAGACAAGGTACAC) and (5′ primer CTCCTCTCTGAGCTTG-GTG; 3′ primer AAGCCAGCTCTCTTCTTCT) 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 (10⁻⁶ M or 3 × 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-phenolchloroform 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 TTCTGTGCCTGCTGCTGCTCATA; 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.
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
mice consisted of severe proliferative glomerulonephritis, interstitial/perivascular damage. In contrast, renal pathologic changes were attenuated but not abrogated in FR167653-treated MRL-Fas<sup>lpr</sup> 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<sup>lpr</sup> 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<sup>lpr</sup> mice. * P < 0.05 versus untreated MRL-Fas<sup>lpr</sup> mice at 6 mo of age.

**Figure 7.** FR167653-treated mice are protected from kidney-infiltrating macrophages and T cells in MRL-Fas<sup>lpr</sup> mice. There was a significant reduction in the number of Mφ, CD4, and CD8 T cells but not B220 in periglomerular (a), interstitial (b), and perivascular (c) regions; P < 0.05, respectively (Figure 8). 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<sup>lpr</sup> mice (Figure 8d). In groups 3 and 4, glomerular and interstitial phosphorylated p38 MAPK-positive cells also significantly decreased (Figure 8, c and d).

**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<sup>lpr</sup> mice. Phosphorylated p38-positive cells were already detected in kidneys of MRL-Fas<sup>lpr</sup> 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<sup>lpr</sup> mice. Glomerular phosphorylated p38 MAPK-positive cells decreased in groups 1 (Figure 8b) and 2 as compared with untreated MRL-Fas<sup>lpr</sup> 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<sup>lpr</sup> mice (Figure 8d). In groups 3 and 4, glomerular and interstitial phosphorylated p38 MAPK-positive cells also significantly decreased (Figure 8, c and d).

**Reduced TNF-α and MCP-1 Transcripts in FR167653-Treated MRL-Fas<sup>lpr</sup> Mice Kidneys**

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

**FR167653 Reduced Serum IgG and Anti-ds DNA Antibodies in MRL-Fas<sup>lpr</sup> Mice**

To determine whether the inhibition of p38 MAPK also reduced the production of IgG and anti-ds DNA antibodies resulted from B cell activation, we evaluated serum levels of IgG and anti-ds DNA antibodies. As compared with untreated MRL-Fas<sup>lpr</sup> mice, serum levels of total IgG, IgG subclasses, and anti-ds DNA antibodies were significantly reduced in FR167653-treated MRL-Fas<sup>lpr</sup> mice in groups 1, 2 and 3 (Figure 10). In addition to IgG levels, anti-ds DNA antibodies remained significantly lower in groups 1 and 3.

**FR167653 Reduced Glomerular IgG Deposition**

The amounts of glomerular IgG deposition were also reduced in FR167653-treated MRL-Fas<sup>lpr</sup> mice in groups 1 and 2 (Figure 11). 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<sup>lpr</sup> 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-\(Fas^{br}\) mice. We herein report that the inhibition of p38 MAPK reduced autoimmune diseases resulting in a prolonged life span in MRL-\(Fas^{br}\) mice. We also noted that the protection against renal injury in MRL-\(Fas^{br}\) 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-\(Fas^{br}\) 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-1\(\beta\), and TNF-\(\alpha\) for examples, in human mesangial cells (3) and human endothelial cells (4), which are known to promote autoimmune organ destruction in MRL-\(Fas^{br}\) 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-\(\alpha\) were diminished through FR167653 treatment. Therefore, p38 MAPK signaling-dependent chemokine/cytokine production may be responsible for the recruitment and activation of leukocytes in diseased kidneys in MRL-\(Fas^{br}\) 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-\(\beta\) or

---

*Figure 8. Reduced number of phosphorylated p38 MAPK-positive cells in FR167653-treated MRL-\(Fas^{br}\) mice in glomeruli and interstitium. We evaluated phosphorylated p38 MAPK-positive cells in glomeruli and interstitium from MRL-\(Fas^{br}\) mice. A representative photograph showed severe cell proliferation in glomeruli, many of which were phosphorylated p38 MAPK-positive in untreated MRL-\(Fas^{br}\) mice at 6 mo of age (a). Phosphorylated p38 MAPK-positive cells were reduced in FR167653-treated MRL-\(Fas^{br}\) mice (b and c). Also in interstitium, phosphorylated p38 MAPK-positive cells were reduced in FR167653-treated MRL-\(Fas^{br}\) mice in group 1 (d). *P < 0.05 versus untreated MRL-\(Fas^{br}\) mice at 6 mo of age. Original magnification, ×400.*
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>+/−</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-Fas<sup>ipr</sup> 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-Fas<sup>ipr</sup> mice as compared with those of untreated MRL-Fas<sup>ipr</sup> 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 p38 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-Fas<sup>ipr</sup> 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.
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-Fas<sup>lpr</sup> mices is dependent on p38 MAPK activation. The activation of p38 MAPK upregulates cytokines/chemokines and Ig production in MRL-Fas<sup>lpr</sup> 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.

References

23. Pertosa G, Grandaliano G, Gesualdo L, Ranieri E, Monno R, SchenaFP: Interleukin-6, interleukin-8 and monocyte chemotactic peptide-1 gene expression and protein synthesis are indepen-