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IFN-γ Receptor Signaling Is Essential for the Initiation, Acceleration, and Destruction of Autoimmune Kidney Disease in MRL-*Fas*^{lpr} Mice[1]

Andreas Schwarting, Takashi Wada, Koji Kinoshita, Gregory Tesch, and Vicki Rubin Kelley[2]

CSF-1 and TNF-α in the kidney of MRL-*Fas*^{lpr} mice are proximal events that precede and promote autoimmune lupus nephritis, while apoptosis of renal parenchymal cells is a feature of advanced human lupus nephritis. In the MRL-*Fas*^{lpr} kidney, infiltrating T cells that secrete IFN-γ are a hallmark of disease. To examine the impact of IFN-γ on renal injury in MRL-*Fas*^{lpr} mice, we constructed a IFN-γR-deficient strain. In MRL-*Fas*^{lpr} mice lacking IFN-γR, circulating and intrarenal CSF-1 were absent, TNF-α was markedly reduced, survival was extended, lymphadenopathy and splenomegaly were prevented, and the kidneys remained protected from destruction. Mesangial cells (MC) that were signaled through the IFN-γR induced CSF-1 and TNF-α in MRL-*Fas*^{lpr} mice. We detected a large number of apoptotic renal parenchymal cells in advanced nephritis and determined that signaling via the IFN-γR induces apoptosis of tubular epithelial cells (TEC), but not MC. By comparison, TNF-α induces apoptosis in MC, but not TEC, of the MRL-*Fas*^{lpr} strain. Thus, IFN-γ is directly and indirectly responsible for apoptosis of TEC and MC in MRL-*Fas*^{lpr} mice, respectively. In conclusion, IFN-γR signaling is essential for the initiation (CSF-1), acceleration (CSF-1 and TNF-α), and apoptotic destruction of renal parenchymal cells in MRL-*Fas*^{lpr} autoimmune kidney disease. ***The Journal of Immunology,*** **1998, 161: 494–503.**

Autoimmune disease in MRL-*Fas*^{lpr} mice is multifaceted, targeting the kidney, joints, eyes, and skin. In MRL-*Fas*^{lpr} mice, kidney destruction is determined by the MRL$^{+/+}$ background genes interacting with a single gene mutation in *Fas* (1). The MRL genes are responsible for autoimmune kidney disease; the *Fas*^{lpr} (*Fas* deficiency) mutation converts a latent, mild nephritis into a rapid and fulminant kidney (2). Conceptually, the absence of *Fas* is responsible for defective peripheral deletion of autoreactive T cells, which are targeted to the kidney and initiate damage. Renal injury in MRL-*Fas*^{lpr} mice is complex and involves interstitial, glomerular, tubular, and perivascular pathology and is mediated by macrophages (Mϕ),[3] T cells, and cytokines (3–6).

Many cytokines are increased in the kidneys undergoing autoimmune destruction in MRL-*Fas*^{lpr} (7–11). The specific cytokines known to initiate or promote kidney injury in MRL-*Fas*^{lpr} mice are CSF-1 and TNF-α (7, 8, 10). CSF-1 is responsible for eliciting and promoting autoimmune kidney destruction. We established that CSF-1 is expressed in the MRL-*Fas*^{lpr} kidney before disease, increases in proportion with advancing kidney damage, and is tightly linked to the extent of kidney pathology (7, 8). Furthermore, gene transfer of CSF-1 into MRL-*Fas*^{lpr} kidney elicits renal injury (12). Although TNF-α increases in parallel to CSF-1, gene transfer of TNF-α fails to elicit autoimmune kidney injury (13). Rather, dual gene transfer of TNF-α and CSF-1 amplifies the extent of renal damage produced by CSF-1 alone (13). Similarly, injecting TNF-α fails to incite renal injury in autoimmune mice before the spontaneous onset of renal injury, but accelerates renal pathology in mice with nephritis (14). Taken together, we envision that CSF-1 elicits autoimmune kidney destruction, while the actions of TNF-α are more distal and require other molecules, notably CSF-1, to accelerate established disease.

T cells are required for autoimmune kidney disease in MRL-*Fas*^{lpr} mice (5, 15–17). During renal injury, CD4$^+$ and double-negative (DN) CD4$^-$CD8$^-$B220$^+$CD21/35$^-$, and a small amount of CD8$^+$ cells infiltrate and accumulate within glomeruli, the interstitium, and perivascular compartments. The DN, CD4$^+$ and CD8$^+$ T cells secrete IFN-γ, a cytokine that has a broad array of functions capable of promoting tissue injury (18, 19). Since IFN-γ is increased in MRL-*Fas*^{lpr} kidneys, and blockade of the IFN-γR signaling prevents glomerulonephritis and prolongs survival (20), we constructed an IFN-γR-deficient MRL-*Fas*^{lpr} strain and determined whether IFN-γ is responsible for CSF-1-, TNF-α-, Mϕ-, and T cell-dependent kidney damage. In addition, since apoptotic renal parenchymal cells are evident in human lupus nephritis (21, 22), we explored the possibility that IFN-γ directly or indirectly is responsible for apoptotic parenchymal cell death. We now report that IFN-γ is required for the production of CSF-1 and TNF-α and induces apoptosis of renal parenchymal cells. MRL-*Fas*^{lpr} mice lacking IFN-γR are protected from fatal lupus nephritis. We propose that IFN-γ released by kidney-infiltrating T cells induces CSF-1 and TNF-α, which, in turn, recruit Mϕ and T cells. This influx of additional T cells into the kidney provides a positive amplification loop that results in apoptotic renal parenchymal cells and culminates in kidney destruction. In conclusion, IFN-γR signaling is essential for the initiation, acceleration, and destruction of the autoimmune kidney in MRL-*Fas*^{lpr} mice.

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[3] Abbreviations used in this paper: Mϕ, macrophage; DN, double negative; TUNEL, terminal deoxynucleotidyltransferase-mediated UTP end labeling; TEC, tubular epithelial cells; MC, mesangial cells; PI, propidium iodide.

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Materials and Methods

Mice

MRL/MpJ$^{+/+}$ (MRL$^{+/+}$) and MRL/MpJ-*Fas^{lpr}*/*Fas^{lpr}* (MRL-*Fas^{lpr}*) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). IFN-γR-deficient mice (129/Sv/Ev × C57BL/6) were provided by Dr. M. Aguet (ISREC, University of Lausanne, Lausanne, Switzerland). The IFN-γR gene was inactivated in these mice by transfecting embryonic stem cells with a replacement vector containing a disrupted murine IFN-γR gene (23). All mice were housed and bred in our pathogen-free animal facility.

*Generation of IFN-γR-deficient MRL-*Fas^{lpr} *mice*

MRL-*Fas^{lpr}* mice lacking the IFN-γR were derived by a series of genetic backcrosses using the cross-backcross-intercross scheme. MRL-*Fas^{lpr}* mice were mated with IFN-γR-deficient (129/Sv/Ev × C57BL/6) mice to yield heterozygous F1 offspring. We intercrossed F1 mice and screened the progeny by PCR amplification of tail genomic DNA for the *Fas^{lpr}* mutation and IFN-γR using specific primers (23, 24). Double homozygous (*Fas^{lpr}*/*Fas^{lpr}*, IFN-γR−/−) N1F1 progeny were backcrossed to MRL-*Fas^{lpr}*/*Fas^{lpr}* mice. B1 progeny, homozygous for the *Fas^{lpr}* mutation and heterozygous for the IFN-γR (IFN-γR$^{+/-}$), were intercrossed, and mice homozygous for the IFN-γR-deficient mutation were selected by PCR typing for continued backcrossing. After three generations of backcross-intercross matings, this breeding scheme generated a colony of MRL-*Fas^{lpr}* mice (95–97.8% MRL background) homozygous and heterozygous for the mutated IFN-γR (25). We analyzed this generation. In this manuscript, we describe IFN-γR−/− as IFN-γR-deficient and IFN-γR$^{+/-}$ as IFN-γR-intact MRL-*Fas^{lpr}* mice.

Clinical and histologic evaluation

We assessed urinary protein levels weekly using albumin reagent strips (Albustix, Miles, Naperville, IL) and graded them semiquantitatively (0 = none; 1 = 30–100 mg/dl; 2 = 100–300 mg/dl; 3 = 300–1000 mg/dl; 4 = >1000 mg/dl). Serum samples were taken by cardiac puncture at the time of sacrifice. Spleen weight and lymphadenopathy were compared among female IFN-γR-deficient, IFN-γR-intact, and wild-type MRL-*Fas^{lpr}* mice at 6 mo of age. Lymphadenopathy was scored on a scale of 0 to 4 by evaluating the number of nodes (0 = none; 1 = one; 2 = a few; 3 = generalized; 4 = massive). Kidneys were either snap-frozen in OCT compound (Miles) for cryostat sectioning or fixed in 10% neutral-buffered formalin. Formalin-fixed tissue was embedded in paraffin, and 4-μm sections were stained with hematoxylin and eosin and evaluated by light microscopy. We evaluated renal pathology by counting nuclei in the perivascular, glomerular, and interstitial areas. Cryostat-sectioned kidneys were stained for Mϕ with F4/80 (American Type Culture Collection, Rockville, MD; HB198), for T cells with anti-CD4, anti-CD8, and anti-B220 rat anti-mouse mAb according to a previously described immunoperoxidase method (9) (PharMingen, San Diego, CA). To distinguish B220-positive DN T cells from B cells, we performed additional sequential staining using B220 and an Ab against a B cell epitope shared by CD21 and CD35 (7G6, PharMingen). Thus, the unique DN T cells characteristic of MRL-*Fas^{lpr}* mice were defined as CD4$^-$, CD8$^-$, CD21/35$^-$, B220$^+$. Specificity controls included replacement of primary Ab with normal rat IgG or rabbit serum. The number of Mϕ and T cells within the renal lesion was reported as cells per glomerulus and cells per interstitial field, respectively.

To assess intrarenal Ig deposits, 4-μm frozen sections were stained with FITC-conjugated goat anti-murine IgG (Cappel Laboratories, Malvern, PA) for 30 min at 37 C. The amount and extent of IgG were evaluated in at least 50 glomeruli graded from 0 to 3 using coded slides by two investigators.

In situ detection of apoptotic cells

We detected apoptotic cells by enzymatic in situ labeling of apoptosis-induced DNA strand breaks (terminal deoxynucleotidyltransferase-mediated UTP end labeling; TUNEL method). Frozen sections were fixed in 4% paraformaldehyde in PBS, permeabilized with 0.1% Triton X-100 in 0.1% sodium citrate for 2 min on ice, and then labeled with TUNEL reaction mixture containing terminal deoxynucleotidyl transferase and fluorescein-labeled nucleotides (Boehringer Mannheim, Indianapolis, IN) for 60 min at 37°C. Incorporated nucleotides were subsequently labeled with sheep anti-fluorescein Fab conjugated with horseradish peroxidase (1/5; Boehringer Mannheim) for 30 min at 37°C. Bound Ab was detected by development with diaminobenzidene (Vector, Burlingame, CA) to obtain a brown color. To determine the cell phenotype of the apoptotic cells, we performed dual staining. After labeling apoptotic cells with the TUNEL reaction, tissue sections were stained for the presence of F4/80 (Mϕ), CD4, CD8, B220 (T cells), and CD21/35 B220 (B cells) determinants using an avidin-biotin-phosphatase complex-alkaline phosphatase kit (Vector). Phosphatase was developed with Fast Blue reagent (Sigma, St. Louis, MO) and then counterstained with methyl green/alcian blue. The number of apoptotic Mϕ, T cells, B cells, or renal parenchymal cells was evaluated by counting the double-stained cells (TUNEL positive and F4/80$^+$, CD4$^+$, CD8$^+$, CD21/35$^+$, B220$^+$) in individual glomeruli or in the interstitial/perivascular field (100 μm^2). In addition, TUNEL-positive cells were assessed morphologically by light microscopy for features of apoptosis, including condensed and fragmented nuclei. Two blinded observers scored at least 20 random glomeruli and 5 fields (100 μm^2)/specimen.

Cell survival assay

Tubular epithelial cells (TEC) and mesangial cells (MC) from IFN-γR-deficient and intact MRL-*Fas^{lpr}* kidneys at 2 mo of age, before renal injury, were cultured on 96-well flat-bottom tissue culture plates (Costar, Cambridge, MA) at 10 × 10^4 cells/well and incubated with medium, IFN-γ (50 U/ml), or TNF-α (30 ng/ml) for 12 h, then washed with PBS and exposed to medium, IFN-γ (50 U/ml), TNF-α (30 ng/ml), or TNF-α plus IFN-γ for another 24 h. Cells were stained with 0.75% crystal violet for 15 min. Nonadherent cells were removed by two washes with ice-cold PBS, and extinction of stained adherent cells was measured at 490 nm in a Bio-Rad ELISA reader (Bio-Rad, Hercules, CA). Data were reported as a percentage of cell death (OD adherent cells in medium alone minus OD adherent cells plus cytokine)/(OD adherent cells in medium alone). To evaluate whether cell death was mediated by necrosis or apoptosis, nonadherent cells in the supernatant were harvested on slides by cytocentrifugation and examined for apoptosis by the TUNEL method as described above. In addition, nonadherent cells were stained with annexin V and propidium iodide (PI) to distinguish between apoptosis and necrosis. Annexin V gives a membraneous staining pattern in apoptotic and necrotic cells (26), whereas PI only stains nuclei from necrotic cells (27). Unfixed cells were washed with PBS and incubated with a 1/50 dilution of annexin V-fluos (Boehringer Mannheim) and a 1/50 dilution of PI in HEPES buffer for 15 min. Staining was evaluated by fluorescence microscopy (488-nm filter for excitation, 515-nm filter for annexin V detection, and >515-nm filter for PI detection).

In vitro detection of apoptotic cells

To confirm that apoptosis occurs in TEC and MC after cytokine stimulation (see cell survival assay), we assessed adherent TEC and MC after exposure to IFN-γ or TNF-α for apoptotic cells using the TUNEL method, morphologic criteria, and annexin V and PI staining. TEC and MC from IFN-γR-deficient and intact MRL-*Fas^{lpr}* kidneys were cultured on 24-well tissue culture plates (Costar, Cambridge, MA) at 20 × 10^5 cells/well and incubated with medium, IFN-γ (50 U/ml), TNF-α (30 ng/ml), or IFN-γ plus TNF-α for 24 h. Medium was analyzed for endotoxin by amebocyte lysate test (Sigma). We detected apoptosis in adherent-fixed and unfixed cells by TUNEL method, annexin V, and PI staining as described above.

Detection of CSF-1 and TNF-α in serum samples

We quantitated biologically active serum CSF-1 using a previously described Mϕ colony-stimulating assay (28). Briefly, 1 × 10^5 bone marrow cells from C3H$^{+/+}$ mice were added to test serum (30 μl) and plated in Noble agar in supplemented McCoy's 5A medium. Cells were incubated at 37°C in humidified 5% CO_2 atmosphere. Colonies were counted on day 10. The results were reported as CFU per 10^5 bone marrow cells. Test sera or culture supernatants were preincubated with 30 μl of polyclonal rabbit anti-murine CSF-1 Ab for 30 min at room temperature before the assay to establish the CSF-1 specificity.

We measured TNF-α in serum samples from IFN-γR-deficient and IFN-γR-intact MRL-*Fas^{lpr}* mice using the ELISA technique (Genzyme, Cambridge, MA) and reported duplicate samples as picograms per milliliter (mean ± SD). The detection limit of this assay is 15 pg/ml.

CSF-1 and TNF-α in the kidney

We detected CSF-1 and TNF-α in the kidney by the immunoperoxidase technique using polyclonal rabbit anti-human CSF-1 (10 μg/ml) on frozen sections and rabbit anti-murine TNF-α Ab (1/150 dilution) on paraffin sections. Specificity controls included the replacement of primary Ab with normal rabbit IgG and neutralization experiments incubating the anti-human CSF-1 Ab or the anti-murine TNF-α Ab with a 20-fold molar excess of CSF-1 (Genetics Institute, Cambridge, MA) or TNF-α (Genentech, South San Francisco, CA), respectively. CSF-1 and TNF-α were scored

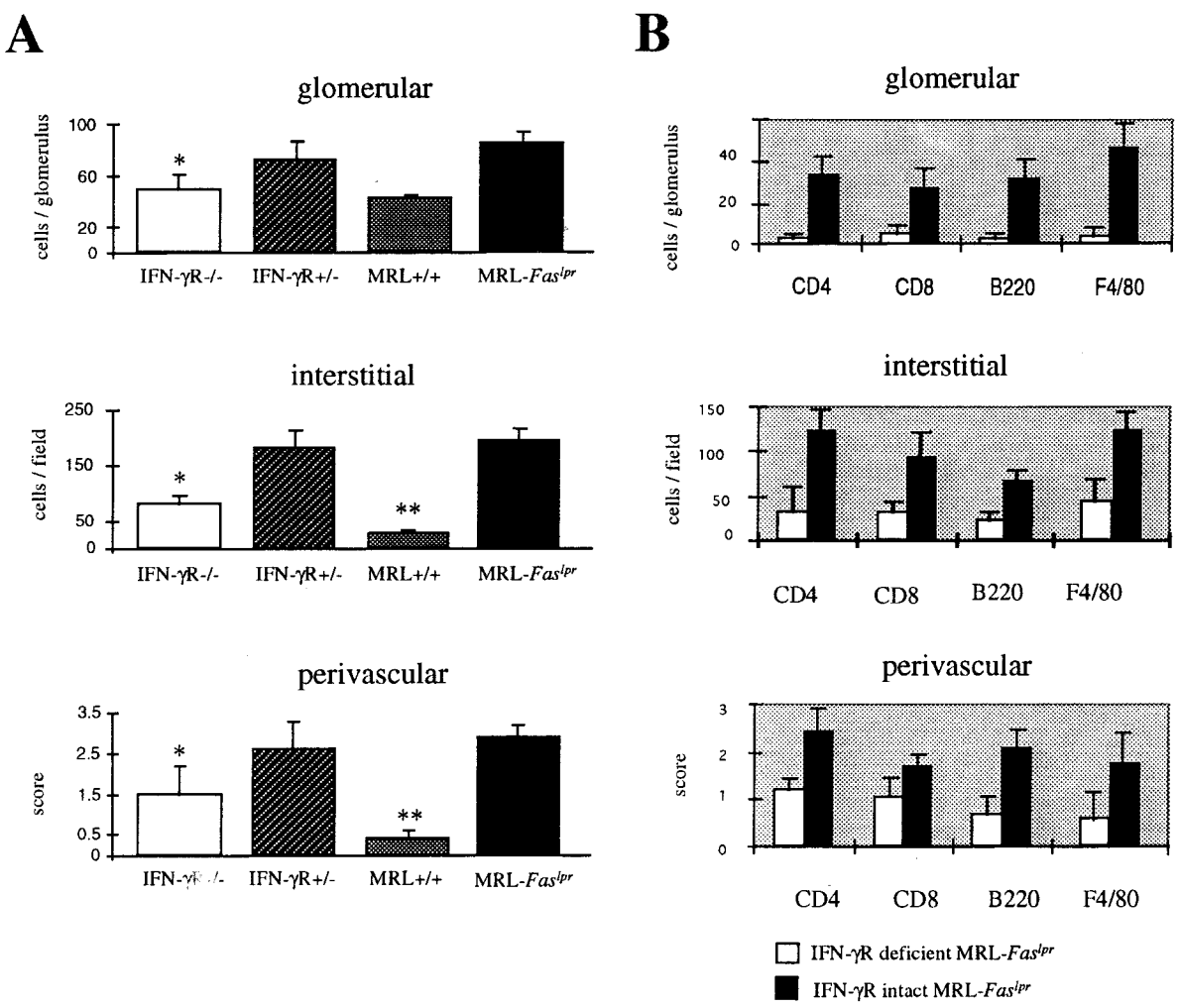

FIGURE 1. *A*, Reduction in kidney-infiltrating cells in IFN-γR-deficient vs IFN-γR-intact MRL-*Fas*^{lpr} mice. Mononuclear cells in glomerular, tubulointerstitial, and perivascular areas are reduced in B3F1 generation IFN-γR-deficient (R−/−) compared with B3F1 generation IFN-γR-intact (R+/−) or wild-type (R+/+) MRL-*Fas*^{lpr} mice. Values are the mean ± SD of infiltrating and parenchymal cells (glomerular, cell counts in 20 random glomeruli; interstitial, 10 random fields (magnification, ×400); perivascular, 10 random inter- and intralobular arteries (magnification, ×400); $n = 5$ in each group; 6 mo of age). * indicates $p < 0.01$, IFN-γR intact vs IFN-γR deficient; ** indicates $p < 0.01$, MRL$^{+/+}$ vs IFN-γR deficient (by Mann-Whitney test). *B*, The kidney infiltration of T cells and Mϕ in MRL-*Fas*^{lpr} mice is dependent on IFN-γ. Mϕ were detected by staining for the presence of F4/80, and T cells were detected by staining for CD4, CD8, and B220 (DN). Values are the mean ± SD. F4/80, CD4, CD8, and B220 cells were counted in 20 glomeruli, 10 random fields (magnification, ×400), and 10 random inter- and intralobular arteries (magnification, ×400; score: 0 = none, 1 = <5 layers surrounding less than a half-circle of the vessels, 2 = 5–10 cell layers surrounding more than a half-circle of the vessels, 3 = >10 layers surrounding vessels; n = 4/group; 6 mo of age). $p < 0.01$, IFN-γR-deficient (R−/−) vs IFN-γR-intact (R+/−) for all phenotypes.

from 0 to 3 (0 = none, 1 = mild, 2 = moderate, 3 = maximum) in >20 random glomeruli and >20 random interstitial fields.

MC in vitro expression of CSF-1

MC were isolated, cultured, and characterized as previously described (29). MC monolayers from IFN-γR-deficient or intact MRL-*Fas*^{lpr} mice 2 mo of age, before renal disease, were treated with 0.25% trypsin and 1 mM EDTA and subcultured into petri dishes containing growth medium (20% heat-inactivated FCS in DMEM, 10 mM HEPES, 1 mM sodium pyruvate, 100 U/ml penicillin, and 100 μg/ml streptomycin). MC were cultured between the 5th and the 10th passage onto coverslides in a six-well plate (Falcon 3046) for CSF-1 immunostaining. MC were rendered quiescent by incubation in medium without serum for 12 h. We then stimulated MC with serum (20–50%) from MRL-*Fas*^{lpr}, MRL$^{+/+}$, or C3H$^{+/+}$ mice 6 mo of age with or without IFN-γ (50 U/ml) or IFN-γ (50 U/ml) alone. CSF-1 was evaluated 24 h later using the immunoperoxidase method as described above.

MC in vitro expression of TNF-α

MC from IFN-γR-deficient and IFN-γR-intact MRL-*Fas*^{lpr} kidneys were stimulated with medium, IFN-γ (50 U/ml), or LPS (1 μg/ml) for 24 h. Supernatants were harvested and measured using the ELISA technique. Duplicate data are reported as picograms per milliliter (mean ± SD).

Northern blot analysis

We isolated total RNA from dissected renal cortexes using RNAzol B (Tel-Test, Friendswood, TX), a modification of the guanidium thiocyanate-phenol-chloroform method (30). Total RNA (20 μg) was electrophoresed through a 1% agarose-formaldehyde gel, blotted to nylon membrane, and hybridized in 50% formamide with ^{32}P-labeled nick-translated probes at 42 C. Hybridized membranes were washed in 2× SSC/0.1% SDS at room temperature and in 0.2× SSC/0.1% SDS at 60 C. The CSF-1 probe was provided by Dr. R. Stanley (Albert Einstein College, Bronx, NY) and consisted of a 594-bp fragment of the plasmid containing the cDNA. The TNF-α probe was a gift from Dr. K. Matsushima (Kanazawa University, Kanazawa, Japan) and consisted of a 500-bp fragment of the plasmid containing the insert. Blots were reprobed with β-actin (*Pst*I fragment of pBA-1) as an internal control for the quantity and integrity of RNA.

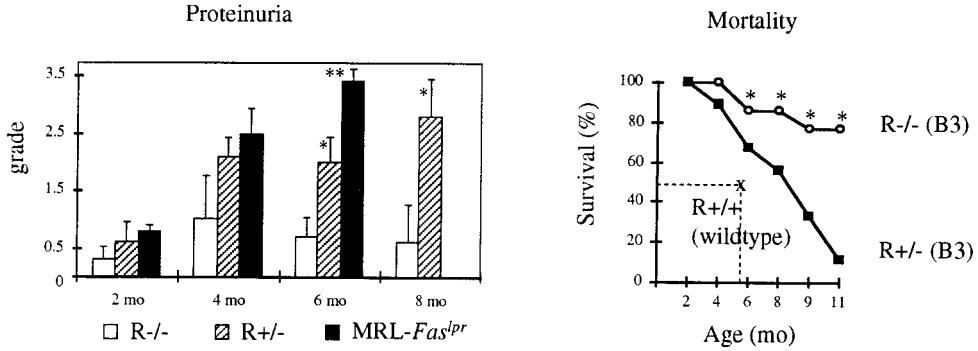

FIGURE 2. Renal injury is prevented and mortality is reduced in IFN-γR-deficient MRL-*Fas*^{lpr} mice. Urinary protein levels were assessed weekly using albumin reagent strips (Albustix, Miles) and were graded semiquantitatively (0 = none; 1 = 30–100 mg/dl; 2 = 100–300 mg/dl; 3 = 300–1000 mg/dl; 4 = >1000 mg/dl). Data given are the mean ± SD (n = 6/group; $p < 0.001$, by Mann-Whitney test). Spleen weight was reduced fivefold and lymphadenopathy was reduced twofold in the IFN-γR-deficient MRL-*Fas*^{lpr} strain (n = 6/group; $p < 0.001$). The IFN-γR-deficient strain survived longer than the IFN-γR-intact strain. Survival is modestly shorter (50% mortality at 5.5 mo of age) in the wild-type MRL-*Fas*^{lpr} mice (X) than in the IFN-γR-intact MRL-*Fas*^{lpr} strain (50% mortality at 8 mo of age). * indicates $p < 0.05$ (by Mann-Whitney test).

Results

*Renal pathology in IFN-γR-deficient MRL-*Fas^{lpr} *mice is prevented*

Renal pathology was diminished in IFN-γR-deficient compared with IFN-γR-intact MRL-*Fas*^{lpr} mice. The numbers of cells in the glomerular, interstitial, and perivascular areas were reduced in IFN-γR-deficient MRL-*Fas*^{lpr} mice compared with those in the IFN-γR intact MRL-*Fas*^{lpr} strain (Fig. 1*A*). By comparison, the numbers of glomerular cells in IFN-γR-deficient MRL-*Fas*^{lpr} mice remained similar to those in normal MRL$^{+/+}$ mice, while the number of cells in the interstitial and perivascular area, although dramatically reduced compared with that in IFN-γR intact MRL-*Fas*^{lpr} mice, was greater than the number in MRL$^{+/+}$ mice. Thus, MRL-*Fas*^{lpr} mice lacking the IFN-γR are protected from renal damage. In addition, we noted that renal pathology in IFN-γR-intact MRL-*Fas*^{lpr} mice (B3 generation) was only modestly less than that in the wild-type MRL-*Fas*^{lpr} strain (Fig. 1*A*). Thus, the degree of renal injury in the B3 generation IFN-γR-intact MRL-*Fas*^{lpr} strain is similar to that in the MRL-*Fas*^{lpr} strain.

Kidney-infiltrating T cells and Mϕ were diminished in IFN-γR-deficient mice compared with those in IFN-γR intact MRL-*Fas*^{lpr} mice (Fig. 1*B*). In particular, glomerular CD4$^+$ and B220$^+$ cells were markedly decreased ($p < 0.001$; Fig. 1*B*). In addition, we established that kidney-infiltrating cells bearing B220 determinants are DN (CD4$^-$CD8$^-$) T cells, since these cells did not express B cell determinants (CD21/35; data not shown). Furthermore, we noted a distinctive cluster pattern of uniform infiltrating mononuclear cells. These distinct mononuclear clusters of CD4$^+$ T cells, noted in the interstitial and perivascular areas in IFN-γR-deficient MRL-*Fas*^{lpr} mice, were not a feature of wild-type or IFN-γR-intact MRL-*Fas*^{lpr} mice (not shown).

There was a reduction in the amount of IgG in the IFN-γR-deficient compared with the IFN-γR-intact or wild-type MRL-*Fas*^{lpr} kidney. Granular deposits of IgG were detected in the mesangium and capillary walls in IFN-γR-intact (2.17 ± 0.57; n = 5) and wild-type (2.53 ± 0.45; n = 3) MRL-*Fas*^{lpr} mice. In contrast, IgG deposits in the kidney were dramatically reduced in IFN-γR-deficient MRL-*Fas*^{lpr} mice (0.75 ± 0.37; n = 6; $p < 0.001$).

*Loss of renal function is prevented and mortality is reduced in IFN-γR-deficient MRL-*Fas^{lpr} *mice*

IFN-γR-deficient MRL-*Fas*^{lpr} are protected from proteinuria. IFN-γR-intact and wild-type MRL-*Fas*^{lpr} mice became proteinuric at 4 mo of age. By comparison, IFN-γR-deficient MRL-*Fas*^{lpr} maintained normal renal function (Fig. 2). The increase in proteinuria was modestly slower in IFN-γR-intact MRL-*Fas*^{lpr} mice compared with that in wild-type MRL-*Fas*^{lpr} mice (Fig. 2). Since the mortality in the wild-type strain is caused by the rapid progressive renal disease, survival was dramatically prolonged in IFN-γR-deficient compared with IFN-γR-intact MRL-*Fas*^{lpr} mice (80 vs 10%, respectively, at 11 mo of age; Fig. 2). We noted that in the B3F1 generation of IFN-γR-intact MRL-*Fas*^{lpr} mice, the 50% mortality was only minimally prolonged compared with that in the wild-type strain (8 vs 6 mo of age, respectively; Fig. 2). Thus, the B3F1 IFN-γR-intact MRL-*Fas*^{lpr} generation has sufficient MRL genes to cause renal disease and is similar to the wild-type MRL-*Fas*^{lpr} strain.

*Splenomegaly and lymphadenopathy are reduced in IFN-γR-deficient MRL-*Fas^{lpr} *mice*

Splenomegaly and lymphadenopathy, hallmarks of disease in MRL-*Fas*^{lpr} mice, were diminished in IFN-γR-deficient MRL-*Fas*^{lpr} mice. Spleen weight was reduced fivefold in IFN-γR-deficient compared with IFN-γR-intact MRL-*Fas*^{lpr} mice (0.25 ± 0.1 g vs 1.1 ± 0.4 g, respectively; n = 6; $p < 0.001$). In addition, lymphadenopathy was reduced twofold in IFN-γR-deficient compared with IFN-γR-intact MRL-*Fas*^{lpr} mice (1.2 ± 0.8 vs 2.7 ± 0.4, respectively; n = 6; $p < 0.001$). It should be noted that the splenomegaly and lymphadenopathy were only modestly increased

Table I. *CSF-1 is not expressed and TNF-α is down-regulated in IFN-γR-deficient MRL-*Fas^{lpr} *kidneys*[a]

	MRL-*Fas*^{lpr}		
	IFN-γR$^{-/-}$	IFN-γR$^{+/-}$	MRL-*Fas*^{lpr}
CSF-1			
Glomeruli	0 ± 0 (6)	2.5 ± 0.2 (4)*	3.0 ± 0 (3)*
Inter/Perivasc	0 ± 0 (6)	1.0 ± 0.5 (4)*	2.0 ± 0.5 (3)*
TNF-α			
Glomeruli	0.8 ± 0.4 (6)	1.8 ± 0.5 (4)**	2.0 ± 0.5 (4)**
Inter/Perivasc	1.3 ± 0.3 (6)	2.5 ± 0.4 (4)**	2.8 ± 0.7 (3)**

[a] CSF-1 and TNF-α staining were performed by the immunoperoxidase method. Intensity of staining graded from 0 (none), 0.5 (trace), 1 (mild), 2 (moderate), and 3 (maximum); Inter/Perivasc, interstitial/perivascular areas; *$p < 0.001$ IFN-γR deficient vs IFN-γR intact; **$p < 0.01$ IFN-γR deficient vs IFN-γR intact MRL-*Fas*^{lpr} mice; 6 mo of age. Numbers in parentheses = n.

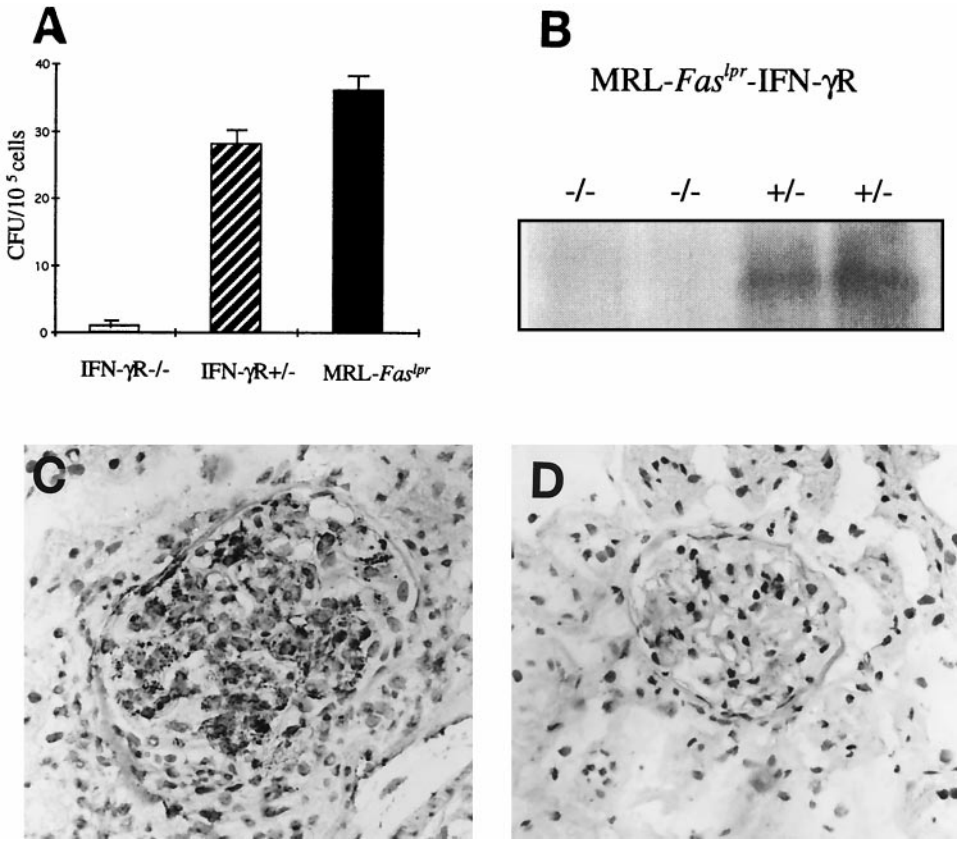

FIGURE 3. CSF-1 is absent in IFN-γR-deficient MRL-*Fas*^{lpr} mice. *A*, Biologically active CSF-1 was abundant in the sera of IFN-γR intact or wild-type MRL-*Fas*^{lpr} mice, but was notably absent in the IFN-γR-deficient MRL-*Fas*^{lpr} strain, as determined by colony-stimulating assay. Values are the mean ± SD (n = 4/group; $p < 0.0001$). *B*, Northern blot analysis. CSF-1 transcripts were absent in IFN-γR-deficient (−/−) MRL-*Fas*^{lpr} kidneys and up-regulated in IFN-γR-intact (+/−) MRL-*Fas*^{lpr} kidneys. *C*, Representative examples illustrating the CSF-1 in glomeruli of IFN-γR-intact MRL-*Fas*^{lpr} kidneys (*C*) and the absence of CSF-1 in IFN-γR-deficient MRL-*Fas*^{lpr} kidneys (*D*), as determined by the immunoperoxidase method. Magnification, ×1000.

in the wild-type MRL-*Fas*^{lpr} mice compared to those in B3F1 generation IFN-γR-intact MRL-*Fas*^{lpr} mice (40 and 22%, respectively).

*CSF-1 is absent in the circulation and kidney of IFN-γR-deficient MRL-*Fas^{lpr} *mice*

We previously established that CSF-1 in the circulation and kidney is detected before renal injury and increases proportionally with progressive renal damage in MRL-*Fas*^{lpr} mice (8). CSF-1 was not detected in the sera of IFN-γR-deficient MRL-*Fas*^{lpr} mice at 6 mo of age, whereas CSF-1 (28 ± 4 and 36 ± 3 CFU, respectively; n = 5) was increased in sera from age-matched IFN-γR-intact and wild-type MRL-*Fas*^{lpr} mice. Similarly, we did not detect CSF-1 transcripts nor did we identify CSF-1 in the kidney sections of IFN-γR-deficient MRL-*Fas*^{lpr} mice. In contrast, CSF-1 was abundant in IFN-γR-intact or wild-type MRL-*Fas*^{lpr} kidneys using either method (Table I and Fig. 3).

*TNF-α is down-regulated in the kidney of IFN-γR-deficient MRL-*Fas^{lpr} *mice*

Our previous studies determined that the increase in TNF-α in the circulation parallels the increase in CSF-1 (10). In the present study, serum TNF-α levels in IFN-γR-deficient MRL-*Fas*^{lpr} mice were reduced compared with those in the IFN-γR-intact MRL-*Fas*^{lpr} strain. In addition, we noted a modest increase in TNF-α in the circulation of wild-type MRL-*Fas*^{lpr} mice compared with that in the IFN-γR-intact MRL-*Fas*^{lpr} strain (Fig. 4), which reflected the slightly slower pace of renal disease as a result of the hybrid genetics. Similarly, we detected a decrease in TNF-α mRNA transcripts in the kidney of IFN-γR-deficient compared with IFN-γR-intact MRL-*Fas*^{lpr} mice at 6 mo of age by Northern blot analysis (Fig. 4). Furthermore, we identified a decrease in TNF-α expression in TEC and infiltrating mononuclear cells in the IFN-γR-deficient compared with the IFN-γR-intact MRL-*Fas*^{lpr} kidney sections (Table I and Fig. 4).

*IFN-γ induces CSF-1 in MC from MRL-*Fas^{lpr} *mice*

Since CSF-1 was absent in the circulation and kidney of IFN-γR-deficient MRL-*Fas*^{lpr} mice, we investigated whether signaling through the IFN-γR is directly responsible for the up-regulation of CSF-1. We stimulated cultured IFN-γR-deficient and -intact MRL-*Fas*^{lpr} MC with rIFN-γ (50 U/ml) for 24 h. IFN-γ induced CSF-1 in IFN-γR-intact MRL-*Fas*^{lpr} MC, whereas unstimulated MC did not express CSF-1 (Table II). In addition, stimulation of IFN-γR-deficient and intact MRL-*Fas*^{lpr} MC with increasing concentrations of serum (20–50%) from nephritic MRL-*Fas*^{lpr} or normal (C3H$^{+/+}$) mice did not induce CSF-1 (Table II). To exclude the possibility that IFN-γ is neutralized by serum factors, we spiked MRL-*Fas*^{lpr} serum with ample IFN-γ to induce CSF-1. Since IFN-γ-supplemented MRL-*Fas*^{lpr} serum induced CSF-1, we suggest that circulating IFN-γ is not responsible for inducing CSF-1 in MRL-*Fas*^{lpr}.

*IFN-γ induces TNF-α in MC from MRL-*Fas^{lpr} *mice*

Primary cultured MC from IFN-γR-deficient or -intact MRL-*Fas*^{lpr} mice produced minimal levels of TNF-α constitutively (Table III). Stimulation with IFN-γ (50 U/ml) increased TNF-α in IFN-γR-intact MRL-*Fas*^{lpr} MC (0 to 265 pg/ml), but did not induce TNF-α in MC isolated from the IFN-γR-deficient MRL-*Fas*^{lpr} strain.

*Renal parenchymal cells from IFN-γR-deficient MRL-*Fas^{lpr} *mice are protected from apoptosis*

Since priming cells with IFN-γ induces apoptosis of hemopoietic cells and may contribute to the extent of Mϕ or T cell accumulation, we investigated the amount of apoptotic cells in situ (31, 32).

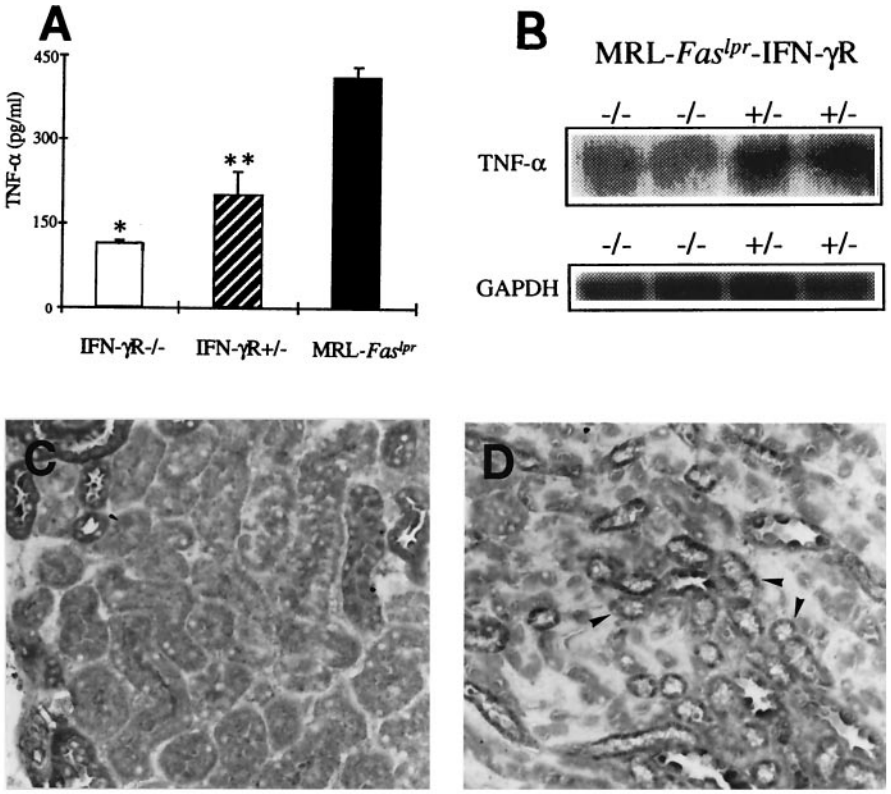

FIGURE 4. TNF-α is down-regulated in IFN-γR-deficient MRL-*Fas*^{lpr}mice. *A,* Serum TNF-α was diminished in IFN-γR-deficient MRL-*Fas*^{lpr} compared with that in IFN-γR-intact or wild-type MRL-*Fas*^{lpr} mice, as detected using the ELISA technique. Values are the mean ± SD (n = 4/group). * indicates $p <$ 0.01, IFN-γR-deficient MRL-*Fas*^{lpr} (R−/−) vs IFN-γR-intact MRL-*Fas*^{lpr} (R+/−); ** indicates $p < 0.01$, IFN-γR-intact MRL-*Fas*^{lpr} vs wild-type MRL-*Fas*^{lpr}. *B,* Northern blot. TNF-α transcripts were reduced in IFN-γR-deficient MRL-*Fas*^{lpr} (−/−) compared with those in IFN-γR-intact MRL-*Fas*^{lpr} (+/−) kidneys. Blots were rehybridized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH). *C,* Immunoperoxidase staining of TNF-α. TNF-α was abundant in cortical TEC from IFN-γR-intact MRL-*Fas*^{lpr} kidneys (*C*), whereas TNF-α was reduced in the renal cortex of IFN-γR-deficient MRL-*Fas*^{lpr} (*D*). Paraffin sections; magnification, ×220.

We detected up to 2% apoptotic cells in IFN-γR-intact MRL-*Fas*^{lpr} mice (Table IV and Fig. 5). In contrast, the number of apoptotic cells within glomerular, interstitial, and perivascular areas was markedly decreased (0.3%) in IFN-γR-deficient MRL-*Fas*^{lpr} mice (Table IV and Fig. 5). By comparison, apoptotic cells were not detected in the kidney of age-matched normal (MRL$^{+/+}$) mice (Table IV). We determined the phenotype of the apoptotic cells by dual and sequential staining. Calculating the percentage of different apoptotic cell types per total number of apoptotic cells revealed that the majority of apoptotic cells (~75%) were renal parenchymal cells, while few T cells (CD4, CD8, DN), Mϕ (F4/80), or B cells (CD21/35) were apoptotic (data not shown).

IFN-γ and TNF-α induce cell death in TEC and MC, respectively, in vitro

TEC. Exposure of MRL-*Fas*^{lpr} TEC to IFN-γ resulted in as much as 20% cell death after 24 h (Fig. 6*A*). Coincubation of IFN-γ and TNF-α induced the same number of dead TEC as IFN-γ alone, while stimulation with TNF-α alone did not induce TEC death (Fig. 6*A*). Priming of TEC with IFN-γ for 12 h with subsequent exposure to TNF-α resulted in a higher increase in TEC death than stimulation with IFN-γ alone (Fig. 6*A*). By comparison, IFN-γ did not induce cell death in IFN-γR-deficient MRL-*Fas*^{lpr} TEC. Thus, signaling through the IFN-γR is responsible for TEC death.

MC. While IFN-γ did not induce death in MRL-*Fas*^{lpr} MC, TNF-α induced MC death (18%) in IFN-γR-deficient and IFN-γR-intact MRL-*Fas*^{lpr} strains (Fig. 6*B*). Taken together, IFN-γ induces TEC death, and TNF-α induces MC death in MRL-*Fas*^{lpr} mice.

Apoptosis is involved in IFN-γ- and TNF-α-induced death of renal parenchymal cells

Serum deprivation is considered to efficiently initiate cell death by apoptosis (33). Therefore, serum deprivation was used as a positive control for apoptotic cell death in MRL-*Fas*^{lpr} TEC and MC. Serum deprivation of MRL-*Fas*^{lpr} TEC resulted in twofold more dead TEC in IFN-γR-deficient and IFN-γR-intact MRL-*Fas*^{lpr} strains compared with TEC death induced by IFN-γ (Fig. 6*A*). Similarly, serum starvation of MRL-*Fas*^{lpr} MC induced more MC death than that induced by TNF-α (Fig. 6*B*). This finding prompted us to examine whether apoptosis is involved in the

Table II. *IFN-γ, but not MRL-*Fas^{lpr} *sera, induces CSF-1 in MRL-*Fas^{lpr} *mesangial cells*[a]

Sera[b]			MRL-*Fas*^{lpr}	
MRL-*Fas*^{lpr}	C3H[c]	IFN-γ[d]	IFN-γR$^{-/-}$	IFN-γR$^{+/-}$
+	−	−	0 ± 0	0 ± 0
−	+	−	0 ± 0	0 ± 0
−	−	+	0 ± 0	2.3 ± 0.6
+	−	+	0 ± 0	2.2 ± 0.4

[a] CSF-1 scored from 0 (none), 0.5 (trace), 1 (mild), 2 (moderate), to 3 (maximum) as described in *Materials and Methods.*
[b] Sera, pooled from 5 MRL-*Fas*^{lpr} mice, 6 mo of age.
[c] Sera, pooled from four age-matched C3H mice.
[d] rIFN-γ: 50 U/ml.

Table III. *IFN-γ induces TNF-α in MRL-*Fas^{lpr} *mesangial cells*[a]

	MRL-*Fas*^{lpr}	
(pg/ml)	IFN-γR$^{-/-}$	IFN-γR$^{+/-}$
Media	14	0
IFN-γ	0	265*

[a] TNF-α was detected by ELISA. Mesangial cells from IFN-γR-deficient and intact MRL-*Fas*^{lpr} mice stimulated with rIFN-γ at 50 U/ml or media alone. *$p <$ 0.001 vs media alone, Mann-Whitney test.

Table IV. *IFN-γR signaling is required for increased apoptosis in MRL-*Fas^{lpr} *kidney*[a]

	MRL^{++}	MRL-*Fas*^{lpr} IFN-γR$^{-/-}$	MRL-*Fas*^{lpr} IFN-γR$^{+/-}$
Apoptosis			
Glomeruli	0 ± 0	0.5 ± 0.6	4 ± 2.7*
Inter/Perivasc	0 ± 0	0.8 ± 0.3	7 ± 4.6*

Apoptotic cells determined by counting TUNEL-positive cells/15 glomeruli and in 100 μm^2 Inter/Perivasc (interstitial/perivascular) area (×400). *$p < 0.001$ IFN-γR$^{+/-}$ vs IFN-γR$^{-/-}$; $n = 4$ in each group, Mann-Whitney test.

mechanisms of TEC death induced by IFN-γ and TNF-α in MRL-*Fas*^{lpr} mice. We studied the number of apoptotic cells in dead (nonadherent) TEC by a combination of the TUNEL method, morphologic criteria, and annexin V and PI staining (Table V). We detected 29% apoptotic (TUNEL- and annexin V-positive, but PI-negative) TEC among nonadherent TEC after incubation with IFN-γ (Table V). In contrast, cell death was rarely detected in IFN-γR-deficient and IFN-γR-intact TEC after incubation with medium alone (Table V). To ensure that apoptosis is involved in IFN-γ-induced TEC death, we also assessed adherent TEC and MC after exposure to IFN-γ or TNF-α by the TUNEL method, morphologic criteria, and annexin V and PI staining. We detected 2 to 4% apoptotic TEC in cultured monolayers after 12 h. Adding IFN-γ to TEC increased apoptosis in adherent IFN-γR-intact MRL-*Fas*^{lpr} TEC five- to sixfold, whereas IFN-γ did not induce apoptosis in IFN-γR-deficient MRL-*Fas*^{lpr} TEC (Table V).

Discussion

The IFN-γR-deficient MRL-*Fas*^{lpr} strains constructed by this and another laboratory (20) both show prolonged survival and the kidneys are protected from destruction compared with MRL-*Fas*^{lpr} strains with intact IFN-γR (20). This study is an in-depth analysis that focuses on the intrarenal pathologic events within the entire kidney (glomerular, interstitial, tubular, and perivascular areas) and analyzes the renal parenchymal and kidney-infiltrating cells and cytokines known to promote kidney destruction. By comparison, Le Hir's laboratory restricted their study to the Ab-mediated immune events and reported a decrease in circulating autoantibodies implicated in glomerular damage (IgG2a and IgG3) (34, 35). We now report that the kidney infiltration of T cells and Mϕ is prevented in IFN-γR-deficient MRL-*Fas*^{lpr} mice compared with that in the IFN-γR-intact MRL-*Fas*^{lpr} strain. In addition, signaling through the IFN-γR is responsible for CSF-1, TNF-α, and apoptosis in renal parenchymal cells during autoimmune kidney disease in MRL-*Fas*^{lpr} mice. Thus, several mechanisms contribute to the impact of IFN-γ during distinct points in the pathogenesis of autoimmune renal injury: the induction phase, since CSF-1 is responsible for inciting and promoting injury (7, 8, 10); the accelerating phase, since TNF-α is known to promote established kidney injury (10, 13); and the advanced phase, since apoptosis of renal parenchymal cells is a feature of severe disease.

We suggest that signaling through the IFN-γR is responsible for CSF-1 production by MC in MRL-*Fas*^{lpr} mice. This is based on the following: 1) CSF-1 is absent in the kidney and circulation of IFN-γR-deficient MRL-*Fas*^{lpr} mice; 2) CSF-1 is primarily a MC

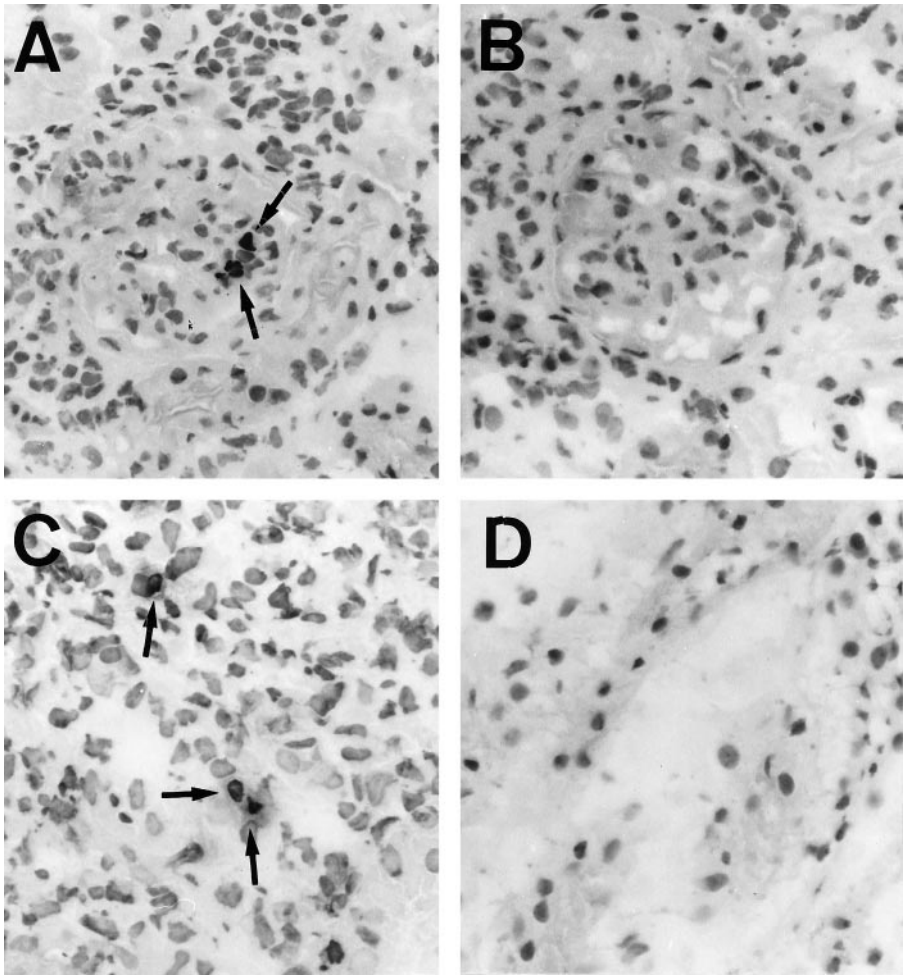

FIGURE 5. IFN-γR deficiency in MRL-*Fas*^{lpr} mice reduces the number of apoptotic cells in the kidneys. Apoptotic cells were determined by TUNEL staining. Representative examples of apoptotic cells (condensed nuclei, marked with arrows) in a glomerulus (*A*) and in the interstitium (*C*) of IFN-γR-intact MRL-*Fas*^{lpr} kidneys are shown. By comparison, the number of apoptotic cells was reduced in glomeruli (*B*) and interstitium (*D*) of IFN-γR-deficient MRL-*Fas*^{lpr} kidneys. Magnification, ×330.

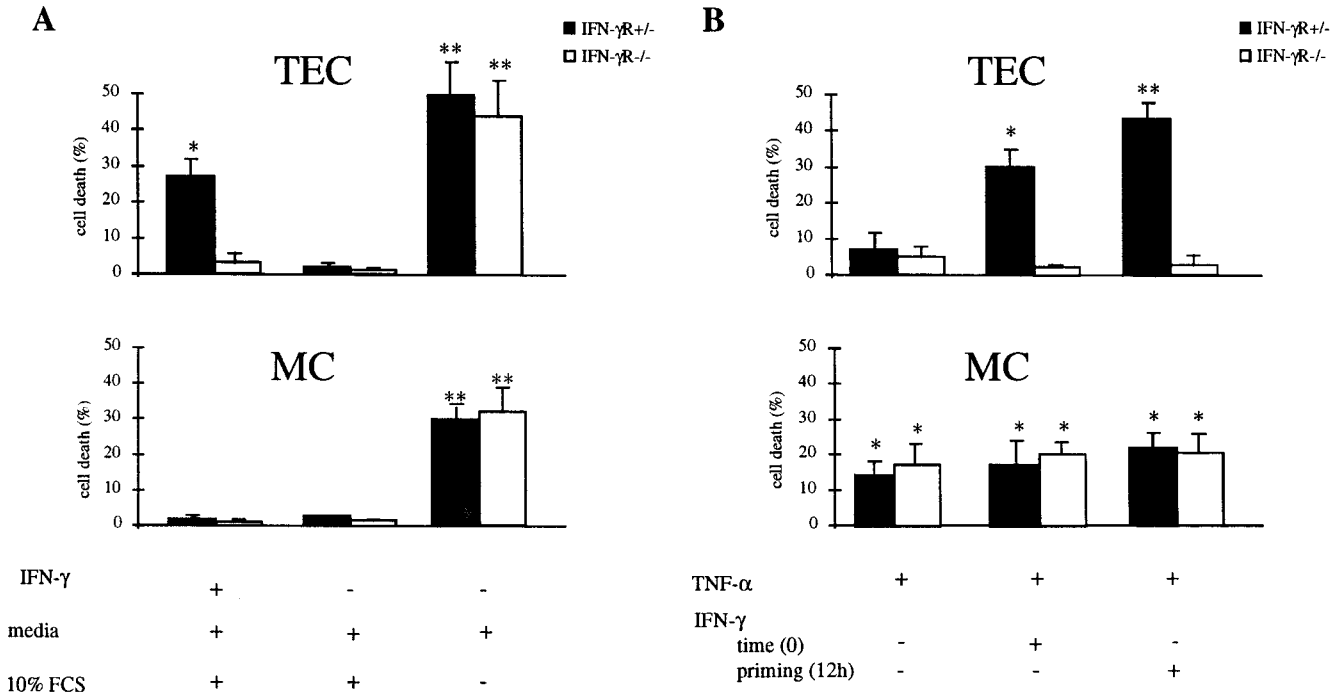

FIGURE 6. IFN-γ and TNF-α induce renal parenchymal cell death in MRL-*Fas*^{lpr} mice. TEC and MC from IFN-γR-deficient and IFN-γR-intact MRL-*Fas*^{lpr} mice were primed with IFN-γ (50 U/ml) or TNF-α (30 ng/ml) for 12 h and exposed to IFN-γ, TNF-α, or IFN-γ plus TNF-α for 24 h. The proportion of dead cells determined by the crystal violet assay was expressed as the percentage of adherent cells cultured in medium minus the percentage of adherent cells after stimulation. *A*, Exposure of IFN-γR-intact MRL-*Fas*^{lpr} TEC to IFN-γ resulted in as much as 30% cell death after 24 h (*A*). In contrast, IFN-γ did not induce death in MRL-*Fas*^{lpr} MC (*A*). Serum deprivation caused the death of 40 to 50% TEC or MC (*A*). TNF-α induced MC death (18%) in IFN-γR-deficient and IFN-γR-intact MRL-*Fas*^{lpr} strains (*B*). Priming of TEC with IFN-γ enhanced the induction of death by TNF-α (*B*). Values are the mean ± SD (eight wells per experiment) for three separate experiments. * indicates $p < 0.001$ compared with cells in medium alone; ** indicates $p < 0.05$ compared with IFN-γ alone (by analysis of variance).

product (7); and 3) IFN-γ stimulates MRL-*Fas*^{lpr} MC to generate CSF-1. On the other hand, IFN-γ also indirectly induces CSF-1. TNF-α and/or circulating Ig complexes could increase intrarenal CSF-1, since TNF-α and Ig complexes are abundant in the serum of MRL-*Fas*^{lpr} mice, and each is capable of inducing CSF-1 in MC (36). However, it is intriguing that incubation of MC with sera from nephritic MRL-*Fas*^{lpr} mice did not induce CSF-1. This could indicate that systemic exposure of TNF-α or Ig complexes is insufficient to cause MC to secrete CSF-1. On the other hand, transplanting a normal MRL$^{+/+}$ kidney into a MRL-*Fas*^{lpr} recipient after removal of nephritic kidneys induces CSF-1 in the kidney, suggesting that a circulating component is required for triggering CSF-1 production (37). It is also possible that this experiment was technically flawed. It is possible that the level of any of these components (IFN-γ, TNF-α, and Ig complexes) in the serum sample was insufficient or inactivated and thus failed to induce CSF-1. Alternatively, it is possible that the circulating component in the transplant experiment (37) was an autoreactive T cell that released IFN-γ locally within the kidney. We favor this explanation, since TEC genetically modified to secrete CSF-1 and/or TNF-α implanted in the kidney, delivering these cytokines locally and not systemically, elicited renal injury in MRL-*Fas*^{lpr} kidneys (12, 13). This suggests that high titers of cytokines localized within the kidney are required for promoting renal injury.

Table V. *IFN-γ induces apoptosis in MRL-*Fas^{lpr} *TEC*[a]

	Media	IFN-γ	TEC MRL-*Fas*^{lpr} (%) IFN-γR$^{-/-}$	TEC MRL-*Fas*^{lpr} (%) IFN-γR$^{+/+}$
Adherent	+	−	3.8 ± 1.2	3.2 ± 1.4
	+	+	2.4 ± 2.0	23.0 ± 2.3*
Detached	+	−	NC	NC
	+	+	NC	29.0 ± 14.0

[a] Apoptotic cells detected by TUNEL method in adherent and detached TEC as described in Materials and Methods. IFN-γ was at 50 U/ml.* $p < 0.001$ IFN-γR$^{+/+}$ vs IFN-γR$^{-/-}$ or media alone, ANOVA. NC, no cells.

TNF-α was markedly reduced, but unlike CSF-1 was still detectable in the absence of IFN-γR signaling in MRL-*Fas*^{lpr} mice. This is not surprising, since TNF-α is regulated by two distinct mechanisms: neonatal up-regulation related to the *Fas*^{lpr} mutation and an increase in mature mice that is proportional to the severity of lupus nephritis (9, 10). In the IFN-γR-deficient MRL-*Fas*^{lpr} strain, the *Fas*^{lpr} mutation alone may be sufficient to sustain some TNF-α production.

We established that IFN-γ halts the expansion of Mϕ in CSF-1-incited interstitial nephritis in MRL-*Fas*^{lpr} mice by blocking proliferation and enhancing apoptosis (32). Therefore, we initially anticipated that IFN-γR-deficient MRL-*Fas*^{lpr} mice would have more Mϕ in the kidney during spontaneous renal disease. On the contrary, we identified fewer Mϕ in the IFN-γR-deficient than in the IFN-γR-intact MRL-*Fas*^{lpr} strain. How can we explain this paradox? We suggest that IFN-γ overrides CSF-1-induced proliferation of Mϕ in the kidneys of MRL-*Fas*^{lpr} mice. Since CSF-1 is absent in the IFN-γR-deficient MRL-*Fas*^{lpr} strain, Mϕ are not recruited and do not proliferate in the kidney, and therefore the impact of IFN-γ is not evident.

It is also important to appreciate that T cells did not proliferate in IFN-γR-deficient MRL-*Fas*^{lpr} kidneys. This would seem counterintuitive. We previously reported that DN T cells secreting IFN-γ reduced the proliferation of T cells stimulated by TEC and

are thus self regulatory (38). Furthermore, IFN-γ prevents proliferation of the murine Th2 cell (39) and limits alloimmune responses in murine cardiac and skin transplantation by down-regulating the proliferation of activated T cells (47). Yet we did not find an increase in T cell proliferation in IFN-γR-deficient MRL-*Fas*^{lpr} kidneys. This is understandable and analogous to our finding with Mϕ. T cells, like Mϕ, are not drawn into the kidney in IFN-γR-deficient MRL-*Fas*^{lpr} mice; thus, the antiproliferative impact of IFN-γ on T cells cannot be appreciated.

It has been reported by Le Hir's laboratory that targeted deletion of IFN-γR- and IFN-γR-intact MRL-*Fas*^{lpr} mice developed lymphadenopathy (20). However, we note a difference in the lymphadenopathy comparing IFN-γR-deficient and IFN-γR-intact MRL-*Fas*^{lpr} mice. We suggest that this apparent discrepancy is related to the criteria used to evaluate lymphadenopathy. In our study using a semiquantitative analysis, lymphadenopathy in IFN-γR-deficient MRL-*Fas*^{lpr} mice was reduced compared with that in IFN-γR-intact or wild-type MRL-*Fas*^{lpr} mice, but did not return to the baseline. In contrast, Le Hir's study used an all or none criteria. In agreement with our data, a recent study claimed that lymphadenopathy was reduced in IFN-γ-deficient MRL-*Fas*^{lpr} mice compared with that in IFN-γ-intact MRL-*Fas*^{lpr} mice (40). Furthermore, since DN T cells are responsible for lymphadenopathy in MRL-*Fas*^{lpr} mice, we suggest that IFN-γ regulates this T cell population. While these data support the concept that IFN-γ fosters the accumulation of DN T cells in lymph nodes, the mechanism remains to be elucidated.

Most importantly, we report for the first time an increase in apoptosis in renal parenchymal cells in MRL-*Fas*^{lpr} mice. Renal parenchymal cells can express Fas and Fas ligand in MRL$^{+/+}$ mice (41). However, Fas/Fas ligand interactions cannot be responsible for mediating apoptosis in the *Fas*-deficient MRL-*Fas*^{lpr} strain. We detected apoptosis in the kidneys of 2% of the MRL-*Fas*^{lpr} mice. This is remarkable, since apoptotic cells are rapidly phagocytosed by Mϕ, epithelial cells, and fibroblasts and are therefore extremely difficult to detect in tissue (42). The extensive amount of apoptosis in MRL-*Fas*^{lpr} kidneys may be the result of increased apoptosis of renal parenchymal cells overwhelming the phagocytic capacity of the kidney and/or impaired phagocytosis of apoptotic cells. We have now also established the novel finding that IFN-γ is responsible for the in vitro apoptosis of renal parenchymal cells. This was determined using several criteria that distinguish apoptosis (morphology, TUNEL, and annexin V) from necrosis (morphology, annexin V, and PI) (26, 27, 33). In addition, we have reported that Mϕ from MRL-*Fas*^{lpr} mice proliferate more readily and are induced to undergo apoptosis more easily than Mϕ from other strains (manuscript in preparation). Thus, the increase in apoptotic cells in the MRL-*Fas*^{lpr} kidney represents renal parenchymal cells and includes Mϕ. Taken together, we propose that the intrarenal increase in IFN-γ in MRL-*Fas*^{lpr} mice released by T cells in the kidney triggers apoptosis of renal parenchymal cells. We speculate that the combined increase in apoptosis and defective clearance of these dead cells explains the substantial number of apoptotic cells in the MRL-*Fas*^{lpr} nephritic kidney.

We suggest that IFN-γ-induced apoptosis of renal parenchymal cells is detrimental to the kidney. In the IFN-γR-deficient MRL-*Fas*^{lpr} kidney protected from renal destruction we identified only few apoptotic cells. By comparison, apoptotic renal parenchymal cells were readily present in the IFN-γR-intact MRL-*Fas*^{lpr} kidneys in advanced renal injury. This is in agreement with studies in human lupus nephritis reporting a correlation between the increase in apoptotic renal parenchymal cells and the extent of glomerulosclerosis and deterioration of renal function (21). On the other hand, it has also been proposed that apoptosis is a protective mechanism that removes excess proliferating renal parenchymal cells and resolves proliferative glomerulonephritis (22). However, this putative homeostatic mechanism fails to prevent fatal lupus nephritis in MRL-*Fas*^{lpr} mice. By extension, it would be interesting to determine whether IFN-γ is responsible for inducing apoptosis in patients with lupus nephritis.

The focus of our study was the impact of IFN-γ at different stages of kidney disease in MRL-*Fas*^{lpr} mice. Nevertheless, renal injury in MRL-*Fas*^{lpr} mice is complex and involves cytokine/growth factors and Ab-dependent mechanisms (43). It is important to note that IFN-γ promotes the switch to the IgG subclasses implicated in glomerular injury (IgG2a and IgG3) (34, 35). Therefore, the decrease in circulating IgG2a and IgG3 anti-DNA Abs in IFN-γ-deficient and IFN-γR-deficient MRL-*Fas*^{lpr} mice may be responsible for protecting renal injury in MRL-*Fas*^{lpr} mice (20, 40, 44). In addition, circulating IgG may induce CSF-1 expression by mesangial cells, which, in turn, elicits renal injury (36). However, T and B cells are abnormal in MRL strains (45, 46); therefore, the regulation of autoantibodies by cytokines is unclear. Thus, the interrelationship between cytokines and autoantibodies in MRL-*Fas*^{lpr} mice requires additional studies.

In conclusion, we have identified several pathogenic mechanisms regulated by IFN-γ that are central to autoimmune kidney disease in MRL-*Fas*^{lpr} mice: the inductions of CSF-1, TNF-α, and apoptosis in renal parenchymal cells. We propose that kidney-infiltrating T cells release IFN-γ, which induces CSF-1 and TNF-α and recruits Mϕ and autoreactive T cells. Autoreactive kidney-infiltrating T cells in MRL-*Fas*^{lpr} mice continue to supply IFN-γ and thus fosters a positive amplification loop responsible for renal parenchymal cell apoptosis and culminates in kidney destruction.

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