Proapoptotic Fas Ligand Is Expressed by Normal Kidney Tubular Epithelium and Injured Glomeruli

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Abstract. Fas ligand (FasL) is a cell membrane cytokine that can promote apoptosis through activation of Fas receptors. Fas receptor activation induces glomerular cell apoptosis in vivo and participates in tubular cell death during acute renal failure. However, there is little information on the expression of FasL in the kidney. This study reports that FasL mRNA and protein are present in normal mouse and rat kidney. In situ hybridization and immunohistochemistry showed that proximal tubular epithelium is the main site of FasL expression in the normal kidney. In addition, increased total kidney FasL mRNA and de novo FasL protein expression by glomerular cells were observed in two different models of glomerular injury: rat immune-complex proliferative glomerulonephritis and murine lupus nephritis. Both full-length and soluble FasL were increased in the kidneys of mice with nephritis. Cultured murine proximal tubular epithelial MCT cells and primary cultures of murine tubular epithelial cells expressed FasL mRNA and protein. Tubular epithelium-derived FasL induced apoptosis in Fas-sensitive lymphoid cell lines but not in Fas-resistant lymphoid cell lines. By contrast, MCT cells grown in the presence of the survival factors of serum were resistant to FasL, and only became partially sensitive to apoptosis induced by high concentrations (100 ng/ml) of FasL upon serum deprivation. Moreover, MCT cells stimulated with inflammatory mediators (tumor necrosis factor-α, interferon-γ, and lipopolysaccharide) increased cell surface Fas expression and were sensitized to apoptosis induced by FasL (FasL 55 ± 5% versus control 8.3 ± 4.1% apoptotic cells at 24 h, P < 0.05). Cytokine-primed primary cultures of tubular epithelial cells also acquired sensitivity to FasL-induced apoptosis. These results suggest that FasL expression by intrinsic renal cells may play a role in cell homeostasis in the normal kidney and during renal injury.

Materials and Methods

Cells, Cytokines, and Antibodies

MCT murine proximal tubular epithelial cells were a generous gift from Eric G. Neilson (University of Pennsylvania, Philadelphia, PA) (18). They were cultured in RPMI 1640 (Life Technologies, Grand

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Island, NY), 10% decomplemented fetal calf serum (FCS), 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in 5% CO₂. In some studies, cells were deprived of serum (0% FCS) for 24 to 48 h. Primary cultures of murine tubular epithelial cells were performed according to previously published techniques (19).

Fas-sensitive A20 and Fas-resistant A20R murine B lymphoma cells were a kind gift from Jürg Tschopp (Lausanne University, Switzerland) (9). L1210 (Fas-negative) and L1210Fas (Fas-transfected) murine leukemia cells were a kind gift from Alberto Anel (Universidad de Zaragoza, Spain) (20).

Polyclonal rabbit anti-FasL antibodies raised against a peptide corresponding to amino acids 2 to 19 mapping at the amino terminus of FasL (13), and the control peptide were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). PE62 polyclonal rabbit anti-FasL antibody was a gift from J. Tschopp (9). The rat IgM monoclonal A11 that binds to the FasL extracellular peptide 196–220 was from Alexis (Laüelfingen, Switzerland) (9), control rat IgM and biotin-anti-rat IgM was from ImmunoKontact (Frankfurt, Germany), and streptavidin phycoerythrin was from Calbiochem (San Diego, CA). Neutralizing anti-FasL antibodies (clone MFL3; Pharmingen, San Diego, CA) were used at a concentration of 10 μg/ml (21). All of these antibodies bind to murine FasL (9,13), and neutralizing MFL3 antibodies inhibit the cytotoxicity of FasL from all strains of mice tested, including SJL mice (21).

Recombinant human FasL (Alexis) was used in the presence of a 10-fold excess of a cross-linking antibody, which by itself was devoid of lethal activity. Cross-linking of FasL restores the biologic activity of soluble FasL and simulates its presence on the cell membrane (22). Human FasL activates the murine Fas receptor (22).

Models of Renal Injury

Inbred female Wistar rats and albino Swiss mice were obtained from the Fundación Jiménez Díaz animal facilities. Studies were conducted in accord with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Immune complex-mediated proliferative glomerulonephritis was induced in rats that were previously immunized with ovalbumin (Sigma, St. Louis, MO), by daily intraperitoneal administration of 10 mg of ovalbumin (23). Samples from rats with nephritis (n = 3) were obtained when glomerular injury was fully developed (proteinuria >100 mg/d). C57BL/6 (B6) and Balb/c mice were obtained from Iffa-CREDO (Barcelona, Spain). Four-week-old MRL-lpr/lpr mice without nephritis (9,13) and 4-mo-old MRL-lpr/lpr mice with nephritis (n = 3) were a gift from Carlos Martinez-A (Centro Nacional de Biotecnología, Madrid, Spain). Kidney samples were snap-frozen for RNA and protein studies or fixed in 10% buffered formaldehyde and embedded in paraffin for histologic studies.

Studies of Cell Death

Tubular epithelial cells were cultured in 12-well plates. Cells were cultured in serum-free media in the presence or absence of 30 ng/ml murine tumor necrosis factor-α (TNF-α) (Immugenex, Los Angeles, CA), 300 U/ml human interferon-γ (IFN-γ) (Immugenex), and 10 μg/ml bacterial lipopolysaccharide (LPS) (Sigma) for 48 h, with FasL added for the last 24 h. When specified, some cells were also cultured in the presence of 10% FCS. Hypodiploid apoptotic cells were quantified by flow cytometry of permeabilized, propidium iodide-stained cells, as described previously (14). For morphologic assessment of apoptosis, cells were cultured in chamber slides, fixed with methanol: acetone (1:1), and stained with propidium iodide in the presence of RNase A (24).

Reverse Transcription-PCR and Northern Hybridization

Kidney total RNA was isolated using the acid guanidinium-phenol chloroform method (25), and 40 μg was separated in 1% agarose gels containing 2.3% formaldehyde. MCT poly(A) RNA was isolated using the FASTRACK mRNA kit (Invitrogen, San Diego, CA) and 5 μg was loaded. RNA was transferred to nylon membranes (Gene-screen, New England Nuclear, Boston, MA) and prehybridized and hybridized as described previously (16).

Reverse transcription (RT)-PCR for mouse FasL detection was carried out as described previously (16), using the following primers: 5′-CACAACATGTTGGCTACCG, 3′-GCCCATATCCTGTCAGTAG. The resulting PCR product was cloned and sequenced to confirm its identity. It expands several exons, thus excluding the possibility of amplification of genomic DNA. The following primers were used for amplification of rat FasL: 5′-CATAGAGCTGTGCTACC, 3′-ATGGTCAGCAAACGTAAC. The PCR product had 534 bp. Semiquantitative PCR was performed in rat and mouse kidney samples according to published techniques (26). FasL and G3PDH were amplified in the presence of 32P-dCTP (Amersham). The PCR products were separated in 4% polyacrylamide/urea gels, and bands were measured by densitometry and corrected for G3PDH. The optimal number of amplification cycles was chosen on the basis of experiments that established the exponential range of the reaction.

The murine FasL probe has been described previously (16,27). Sense and antisense RNA probes for in situ hybridization were labeled with digoxigenin using a commercially available kit (DIG RNA labeling kit; Boehringer Mannheim, Mannheim, Germany), following the manufacturer’s instructions.

Flow Cytometry Analysis of FasL and Fas Expression

To study cell surface, FasL cells were cultured for 3 h in the presence of 5 mM ethylenediaminetetra-acetic acid (EDTA), which inhibits metalloproteases and decreases the release of FasL from the cell membrane, thus increasing the availability of this cytokine at the cell surface (28). Cells were washed with phosphate-buffered saline (PBS) and resuspended in 0.2% bovine serum albumin/PBS. A total of 5 × 10⁵ cells was incubated with 20 μg/ml monoclonal A11 anti-FasL antibody or control rat IgM in 5% FCS, 0.2% bovine serum albumin, in PBS for 30 min at 4°C, followed by incubation with 1:100 biotin-anti-rat IgM for 30 min at 4°C and by incubation with 1:50 streptavidin phycoerythrin. Cells were analyzed on a cytofluorograph, and debris was excluded from analysis by selective gating based on anterior and right angle scatter. At least 10,000 events were collected for each sample, and data were displayed on a logarithmic scale of increasing fluorescence intensity (14). Mean cell fluorescence was calculated using LYSIS II software.

To study cell surface Fas expression, cells were cultured in the presence of control medium or cytokines for 48 h and stained with Jo-2 anti-murine Fas antibody or control hamster IgG (Pharmingen), as described (14).

Immunohistochemistry

Immunohistochemistry was carried out as described previously (29) in paraffin-embedded tissue sections 5 μm thick. Primary antibodies were 20 μg/ml A11 rat monoclonal anti-FasL (Alexis) or 1 μg/ml rabbit polyclonal anti-FasL (Santa Cruz) (13). Sections were counterstained with Carazzi’s hematoxylin. Some sections were subsequently incubated with the proximal tubule marker, fluorescein-conjugated, Tetranogolobus lotus (diluted 1:33) (Sigma) or the collecting tubule marker, fluorescein-conjugated, Dolichos biflorum.
(diluted 1:100) (Sigma) (30). These sections were mounted in 90% glycerol/PBS and photographed immediately. Negative controls included incubation with a nonspecific Ig of the same isotype as the primary antibody in both cases, and competition of the primary antibody with a 10-fold excess of the immunogenic peptide in the case of the rabbit polyclonal. Positive controls included testis (7) and eye (13).

In Situ Hybridization
Paraffin-embedded tissue sections, 5 μm thick, were fixed in 1.5% paraformaldehyde-1.5% glutaraldehyde for 10 min, treated with 5 mM levamisole for 30 min, and deproteinized with 0.2N HCl for 20 min at room temperature followed by digestion with 25 μg/ml proteinase K in 0.1 M Tris, 0.005 M EDTA, 0.5% sodium dodecyl sulfate for 30 min at 37°C. They were subsequently hybridized with 1 ng/ml denatured digoxigenin-11-UTP-labeled FasL riboprobe in hybridization solution (2× SSC, 1× Denhardt’s, 0.1 M sodium phosphate, pH 6.5, 10% dextran sulfate, 40% deionized formamide, 24 mM vanadyl ribonuclease complex, and 0.5 μg/ml yeast tRNA) at 42°C overnight, under sealed coverslips, in a humidified chamber. Slides were washed in 2× SSC for 5 min and 0.2× SSC for 3 min, and incubated with 1:750 alkaline phosphatase-conjugated anti-digoxigenin antibody (Boehringer Mannheim) for 60 min at 37°C. Colorimetric detection of mRNA was performed with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate (X-phosphate) in the dark for 10 to 30 min. Negative controls consisted of matched serial sections hybridized without RNA probe or with sense probe or pretreated with 25 μg/ml RNase A for 1 h (Sigma) before hybridization with the corresponding antisense probe. Testis samples were included as positive controls.

Western Blot
Tissue and cell samples were homogenized in lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, 2 mM ethyleneglycol-bis(β-aminoethyl ether)-N,N'-tetra-acetic acid, 0.2% Triton X-100, 0.3% Nonidet-P40, 0.1 mM phenylmethylsulfonyl fluoride, and 1 μg/ml pepstatin A) and then separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions. After electrophoresis, samples were transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% skim milk in PBS/0.5% vol/vol Tween 20 for 1 h, washed with PBS/Tween, and incubated with 40 ng/ml PE 62, a polyclonal anti-FasL antibody or polyclonal anti-murine Fas (1:500) (Santa Cruz), in 5% milk PBS/Tween for 18 h at 4°C. The blots were washed with PBS/Tween and subsequently incubated with horseradish peroxidase-conjugated antirabbit IgG (1:2500; Amersham). After washing with PBS/Tween, the blots were developed with the chemiluminescence method according to the manufacturer’s instructions (Amersham). FasL (a gift from J. Tschopp)-transfected 293 cell lysates were diluted 10-fold and used as positive controls. As a negative control, the antibodies were preincubated with a 10-fold excess of the immunogenic peptide before Western blotting. Similar results were obtained with a polyclonal anti-FasL antibody from Santa Cruz. Tubulin was used as loading control.

Cytosolic and membrane proteins were obtained by lysing the cells in 2% Triton X-114 precondensed in PBS. After phase separation at 37°C, the lower phase enriched in detergent contains the membrane proteins, and the upper aqueous phase contains the cytosolic proteins. Both phases were separated, precipitated with methanol/chloroform, and resuspended in sample buffer (22).

FasL Bioactivity
FasL killing activity was assessed by incubating $10^5$ to $10^2$ effector MCT cells, 6 h after seeding, with $10^5$ bromodeoxyuridine-labeled target A20R and A20 cells (9) or L1210 and L1210/Fas cells (20) in 48-well plates. Higher effector:target (E:T) ratios were used in preliminary experiments, but were abandoned because tubular cells became overconfluent at E:T ratios > 1. The release of bromodeoxyuridine was then determined after a 14-h coincubation using a cellular DNA fragmentation enzyme-linked immunosorbent assay (Boehringer Mannheim). As a positive control, MCT cells were replaced by 10 ng/ml recombinant FasL. For the calculation of the percentage of dead cells, cell death induced in Fas-sensitive cells by recombinant FasL was considered to be 100%. Flow cytometry confirmed more than 90% apoptosis among A20-Fas-sensitive cells treated with FasL. Results are expressed as mean ± SEM of independent experiments each consisting of quadruplicate wells.
Statistical Analyses

Results are expressed as mean ± SEM. Significance at the 95% level was established using ANOVA and t test. Bonferroni correction for multiple comparisons was used.

Results

Proximal Tubular Epithelium Is the Main Site of FasL Expression in Normal Murine Kidneys

FasL transcripts were detected by RT-PCR in normal mouse and rat kidney (Figure 1). The presence of a FasL transcript was confirmed by Northern blot in normal murine kidney (Figure 1). An approximately 36- to 40-kD FasL protein, corresponding to full-length transmembrane FasL, was the more abundant FasL form in normal mouse kidney (Figure 1). To determine whether intrinsic renal cells account for FasL expression in the normal kidney, the site of FasL expression was explored. FasL transcripts were detected in mouse tubular epithelium by in situ hybridization (Figure 2). Immunohistochemistry using two different anti-FasL antibodies confirmed the expression of FasL protein by normal tubuli (Figure 3). Proximal tubules, identified by their ability to bind the proximal tubule marker Tetragonolobus lotus lectin (30), were the main source of FasL, while collecting ducts were negative. By contrast, FasL was absent from normal glomerular mesangial cells and vessels (Figures 2 and 3). Tubular expression of FasL was noted in the different strains of mice tested (Swiss, Balb/c, B6, MRL lpr/lpr).

Increased Renal FasL and de Novo Glomerular FasL Expression during Glomerular Inflammation

We then addressed the question of whether FasL expression changed in the course of renal inflammation in rodent models. FasL mRNA, as assessed by semiquantitative RT-PCR, was increased in whole kidneys from rats with immune-complex proliferative glomerulonephritis and mice with lupus nephritis (Figure 4). In the course of murine lupus nephritis, both full-length FasL and an approximately 26-kD molecule corresponding to the size of soluble FasL were increased (Figure 4E).

The pattern of FasL expression also changed during glomerular and tubulointerstitial inflammation. De novo FasL expression was noted in the glomerular mesangium, and FasL immunoreactivity was also prominent in tubular cells and among infiltrating interstitial cells in MRL lpr/lpr mice with lupus proliferative glomerulonephritis and tubulointerstitial nephritis (Figure 5).

FasL mRNA and protein are also limited to tubular epithelial cells in normal rat kidney (Figure 6). As was the case during mouse renal inflammation, in rats with immune complex proliferative glomerulonephritis, FasL mRNA and protein were expressed de novo by glomerular cells (Figure 6).

Figure 2. FasL mRNA expression in normal mouse kidney. In situ hybridization. (A) Antisense probe. Note FasL expression in renal tubules, but its absence from glomeruli. (B) Sense probe. Magnification: ×200.

Figure 3. Localization of the tubular segment expressing FasL. Normal mouse kidney (Balb/c) stained with both anti-FasL antibody (A) and the proximal tubule marker Tetragonolobus lotus (B), or both anti-FasL antibody (C) and the collecting tubule marker Dolichos biflorum (D). Magnification: ×200. Arrows localize the same tubuli in A and B or C and D. Proximal tubules are the main source of renal FasL.
Murine Cultured Tubular Epithelial Cells Express FasL

Proximal tubular epithelial cells were the main source of renal FasL in vivo. We then studied FasL expression in cultured tubular epithelial cells. FasL mRNA was detected in cultured murine proximal tubular epithelial MCT cells by RT-PCR, Northern blot, and in situ hybridization (Figures 7 and 8). RT-PCR also identified a FasL transcript in primary cultures of tubular epithelial cells (Figure 7B). In situ hybridization showed that FasL mRNA expression was higher in MCT cells grown at low cell density than when cells were confluent (Figure 8). Western blot confirmed the presence of a 36- to 40-kD FasL protein in these cells, which, after Triton X-114 extraction, appeared in the membrane fraction (Figure 9A), as corresponds to the full-length transmembrane form of FasL. As was the case for mRNA, nonconfluent cells expressed more FasL protein than confluent cells (Figure 9A), indicating a regulatory process that deserves further study. Flow cytometry showed that low levels of FasL protein are present in the cell surface, where they can be biologically active (mean cell fluorescence 30% higher than control isotype antibody-stained cells) (Figure 9B). The low amount of FasL in the cell surface is consistent with data from other cell types (14,31), where most FasL is bound to intracellular membranes (31). A 36- to 40-kD FasL protein was also present in the membrane fraction of primary cultures of mouse tubular epithelial cells (Figure 9C).

Tubular Epithelial Cell FasL Is Biologically Active

Not all FasL-expressing cells are able to promote apoptosis (11). Thus, we tested the ability of tubular epithelial MCT cell FasL to promote apoptosis. In vitro assays using two different sets of Fas-sensitive and Fas-resistant cells (A20 and A20R, and L1210Fas and L1210) demonstrated that tubular epithelial cell-derived FasL is able to induce cell death in lymphoid cells. A20R cells were generated by continuous culture in the presence of FasL, and have downregulated Fas receptor expression (22). By contrast, L1210Fas were made sensitive to FasL apoptosis by transfecting them with a Fas receptor expression vector (20). Results obtained in A20/A20R cells are shown in Figure 10. An increased rate of cell death was noted among Fas-sensitive A20 cells cocultured with tubular epithelial cells, which was not present among Fas-resistant A20R cells under the same culture conditions. The apoptotic nature of death was confirmed by morphologic evaluation of the cells. Similar results were obtained with Fas-sensitive L1210Fas and Fas-resistant L1210 cells (not shown). Apoptosis induced by tubular epithelial cells in A20 cells was reduced by 73% when neutralizing anti-FasL antibodies were added to the coculture (Figure 10).

FasL Induces Apoptosis in Activated Tubular Epithelial Cells

Tubular epithelial cells have been reported to be relatively resistant to Fas-induced death (15,16). However, Fas deficiency protects tubular epithelial cells from apoptosis in the course of renal ischemia-reperfusion (6). This suggests that sensitivity to FasL in tubular epithelial cells is a regulated process. It has been observed that the survival factors present in serum protect tubular epithelial cells from the lethal effect of TNF-α (32). Indeed, tubular epithelial MCT cells were com-
pletely resistant to FasL-induced apoptosis when grown in the presence of serum (Figure 11A). In addition, nonstimulated MCT cells grown in serum-free media were still relatively resistant to FasL-induced apoptosis. Only the highest concentration of FasL induced a significant, albeit low, amount of apoptosis (Figure 11A) (100 ng/ml FasL 12.8 ± 2.4% versus control 3.2 ± 1.0% apoptotic cells at 24 h, \( P < 0.05 \)). Under these conditions, MCT cells were at least 100-fold more resistant to recombinant FasL than the Fas-sensitive lymphoid cell lines used in our studies, as 1 ng/ml Fasl already induced significant apoptosis in these lymphoid cell lines. As expected from these data, neutralizing anti-FasL antibodies did not modify spontaneous apoptosis in tubular epithelial cells after 24 or 48 h in culture in serum-free media (data not shown). However, during renal injury tubular epithelial cells are exposed to inflammatory mediators. Thus, we studied their sensitivity to FasL in the presence of a cocktail of inflammatory mediators (30 ng/ml TNF-\( \alpha \), 300 U/ml IFN-\( \gamma \), and 10 \( \mu \)g/ml bacterial LPS). Upon activation by inflammatory mediators, the sensitivity of MCT cells to apoptosis induced by FasL was increased: 10 ng/ml FasL already increased the rate of apoptosis, and the death rate of cells exposed to 100 ng/ml FasL was 6.6-fold that of control cells (Figure 11A). The occurrence of apoptosis was confirmed by morphologic criteria (fragmented, condensed, and pyknotic nuclei) of fixed, propidium iodide-stained cells (not shown). MCT cells activated by these inflammatory mediators have an upregulated expression of Fas receptor (Figure 11, B and C), which can contribute to their increased susceptibility to FasL-mediated apoptosis. Serum deprivation also increased Fas expression (Figure 11B), as it does in other renal cells (14).

We then checked the sensitivity of primary cultures of tubular epithelial cells to FasL-induced apoptosis. Tubular cells were resistant to FasL under basal conditions; however, they were sensitized by prestimulation with 30 ng/ml TNF-\( \alpha \), 300 U/ml IFN-\( \gamma \), and 10 \( \mu \)g/ml bacterial LPS (Figure 11D). Similar results were obtained with freshly isolated mouse tubules (not shown).

**Discussion**

This article shows that murine tubular epithelial cells are the main renal source of local FasL in the normal kidney. Additional sources of FasL, such as mesangial cells and infiltrating leukocytes, may be present in the glomeruli and interstitial space during renal injury. Renal FasL may potentially regulate the immune response, promote parenchymal cell death, and/or limit inflammatory cell and fibroblast number by promoting apoptosis in leukocytes and renal fibroblasts (1,14).

FasL-induced apoptosis is a complex phenomenon. Not all FasL-expressing cells can kill FasL-sensitive cells (11,33). In leukocytes, FasL is stored in the membranes of intracellular granules and only becomes exposed in the cell surface, where it has killing activity, upon cell activation (31,33,34). Cultured tubular epithelial cells contain full-length FasL in cell membranes, and the amount of FasL is subject to regulation by cell density. Although additional studies are required to characterize the regulation of FasL expression in tubular epithelium,
flow cytometry showed that tubular cell surface FasL was low. The low cell surface expression of FasL is also observed in other FasL-expressing cells (14,31), and is consistent with the potentially dangerous lethal activity of high concentrations of cell surface FasL. However, cell surface FasL from cultured tubular epithelial cells was able to kill lymphoid target cells. The biologic activity was similar to that of melanoma cells expressing FasL (9). The lower killing activity at higher MCT

Figure 6. FasL expression in rat kidney. *In situ* hybridization (A through C) and immunohistochemistry (D through F). (A) Control rat. Antisense probe. (B) Nephritis rat. Antisense probe. (C) Nephritis rat. Sense probe. (D) Control rat stained with anti-FasL monoclonal antibody. (E) Nephritis rat stained with anti-FasL monoclonal antibody. (F) Nephritis rat. Control IgM. Note glomerular FasL-positive cells in proliferative glomerulonephritis, while tubular epithelium is stained in both control and nephritis rats. Magnification: ×400.
cell effector cell density could be attributed to decreased FasL expression under these culture conditions. Expression of FasL by tumor cells is thought to limit the immune response against the tumor (9,10). We might hypothesize that tubular cell FasL keeps in check the renal immune response. Indeed, local sources of FasL during tubulointerstitial renal injury include infiltrating leukocytes and interstitial fibroblasts (16). Indeed, we noted that interstitial cells expressed FasL in lupus mice with combined glomerular and tubulointerstitial nephritis. Moreover, de novo glomerular FasL expression was observed in immune complex-mediated proliferative glomerulonephritis in mice and rats. The cellular origin of glomerular FasL is not clear from our studies. Infiltrating T cells or macrophages may be sources of FasL in this model (23). However, cultured glomerular mesangial cells also express FasL transcripts, and inflammatory cytokines released during glomerular injury, such as TNF-α, increase mesangial cell FasL mRNA levels (16).

During renal damage, FasL may promote apoptosis of renal parenchymal cells. In this sense, glomerular mesangial cells undergo apoptosis when challenged with agonistic anti-Fas antibodies both in vitro and in vivo (4,5). By contrast, nonstimulated tubular epithelial cells of human or murine origin have been reported to be resistant to cell death induced by agonistic anti-Fas antibodies (15,16). This feature is shared by murine tubular epithelial cells in vivo, as systemic injection of agonistic anti-Fas antibodies induced apoptosis in glomerular cells but not in tubular epithelium (4). Recombinant FasL is a more potent inducer of apoptosis than agonistic anti-Fas antibodies (17), and cells that have been previously reported to be resistant to anti-Fas-induced apoptosis were later found to be sensitive to FasL-induced apoptosis (17). Our present studies confirm that nonstimulated murine tubular epithelial cells are quite resistant not only to anti-Fas antibodies (16), but also to recombinant, cross-linked FasL, when grown in the presence of survival factors. Even when the survival factors present in serum were removed, only high concentrations of FasL increased significantly, although mildly, the rate of apoptosis in MCT tubular epithelial cells. This fact, together with the low spontaneous apoptotic rate of cultured tubular epithelial cells, suggests that under basal conditions FasL does not act as an autocrine death factor. Indeed, neutralizing anti-FasL antibodies did not modify the rate of spontaneous apoptosis in these

Figure 7. FasL expression in murine cultured tubular epithelial cells. (A) RT-PCR. MCT, proximal tubular epithelial cell line. (B) RT-PCR. TEC, primary cultures of mouse tubular epithelial cells. (C) Northern blot, poly(A) mRNA (MCT cells). MWM, molecular weight markers.

Figure 8. In situ hybridization for FasL in MCT cells. (A) Antisense probe. (B) Antisense probe. (C) Sense probe. (D) RNase-treated, antisense probe. Magnification: ×200 in A and C; ×400 in B and D.
cells. This is also the case for other cell types with constitutive expression of FasL (2).

Several factors may contribute to the relative resistance of tubular epithelium to FasL-induced death. The amount of cell surface Fas receptor is one of them. Indeed, the basal expression of Fas is low in tubular epithelial cells, below the limit of detection of the flow cytometry technique we used. Inflammatory stimuli that increase Fas expression prime mesangial cells to undergo apoptosis induced by anti-Fas antibodies (16). In this regard, increased Fas expression has been noted in tubular epithelial cells in the ischemia-reperfusion model of renal injury and during chronic tubular atrophy (6,37). Under these
circumstances, tubular epithelial cells may undergo apoptosis upon Fas stimulation, in a manner analogous to other FasL-expressing epithelia, such as thyrocytes, during thyroid inflammation (2,37). Indeed, Fas deficiency protected tubular epithelial cells from apoptosis induced by renal ischemia-reperfusion (6).

In vitro, a combination of inflammatory mediators that may be present in the kidney during renal injury increased tubular epithelium Fas expression and sensitivity to FasL-induced apoptosis both in MCT cells and primary cultures of tubular epithelial cells. By contrast, the survival factors present in serum decreased tubular cell Fas and protected against FasL-induced apoptosis. In addition to the amount of Fas receptors, endogenous intracellular proteins may protect from Fas-induced death, as inhibition of protein synthesis promoted anti-Fas-induced apoptosis in nonstimulated cultured human tubular epithelial cells (15). Candidate proteins include members of the Bcl2 family of anti-apoptotic proteins, such as BclxL. Constitutive expression of BclxL by tubular epithelial cells and its absence from glomerular cells (38,39) may be one of the factors that contributes to their different susceptibility to FasL apoptosis in vivo. Indeed, both TNF-α and serum deprivation decreased BclxL levels in tubular epithelial cells (32).

It is noteworthy that both full-length transmembrane FasL as well as the soluble form of the cytokine were increased in murine lupus nephritis. Local expression in the cell surface is important for the ability of FasL to induce cell death, as the apoptotic-inducing capacity of naturally, enzymatically processed soluble FasL is reduced by 1000-fold compared with cell surface transmembrane FasL (22). Soluble FasL may even function as a death antagonist, by competing with the more active cell membrane FasL (17). The presence of soluble FasL during renal inflammation may limit the lethal activity of transmembrane FasL. Indeed, in contrast to results in acute renal failure (6), in the course of lupus nephritis Fas deficiency did not protect tubular epithelial cells from apoptosis (40).

We have explored the relationship between renal FasL and apoptosis. However, FasL may have additional functions. Recent evidence suggests that soluble FasL can promote neutrophil chemotaxis (41). In addition, FasL itself can transduce intracellular signals (42). It is currently unknown whether this is another pathway for FasL to influence tubular epithelial cell biology.

In summary, FasL is expressed by tubular cells in normal kidney and de novo in the glomeruli during renal injury. Renal...
cell FasL promotes apoptosis of lymphoid cells. It had been previously demonstrated that Fas activation induced apoptosis of mesangial cells in vitro and in vivo. We now show that while under basal conditions tubular epithelial cells are relatively resistant, recombinant FasL induces apoptosis of tubular epithelial cells activated by inflammatory stimuli. Taken together, these results suggest that FasL and Fas play a role in normal kidney homeostasis and renal injury that deserves further study.

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