Involvement of Fas-dependent apoptosis in renal tubular epithelial cell deletion in chronic renal failure

JEFFREY R. SCHELLING and RONALD P. CLEVELAND

Division of Nephrology, Department of Medicine, Rammelkamp Center for Research, and Departments of Medicine and Pathology, Case Western Reserve University School of Medicine, MetroHealth Medical Center, Cleveland, Ohio, USA

Involvement of Fas-dependent apoptosis in renal tubular epithelial cell deletion in chronic renal failure. Renal tubular atrophy predicts a poor prognosis in chronic renal failure, but the molecular mechanisms that regulate tubular atrophy are unknown. Because the Fas apoptosis pathway has been implicated in disease pathogenesis and Fas is expressed in kidney, we hypothesized that Fas-mediated renal tubule epithelial cell (RTC) apoptosis contributes to tubular atrophy in chronic renal failure. Immunohistochemical analyses of renal sections from two murine models of progressive renal disease revealed increases in RTC Fas expression and apoptosis compared with tissue sections from age-matched control kidneys. Increased RTC apoptosis was not accompanied by compensatory hyperplasia, suggesting that RTCs targeted for Fas-dependent apoptotic deletion contribute to tubular atrophy. These data are supported by in vitro studies that showed that interleukin-1α or tumor necrosis factor-α, cytokines that are secreted in chronic renal failure, stimulated increases in Fas expression in cultured RTCs. Both murine kidney cortex and RTCs in culture demonstrated constitutive expression of Fas ligand, a feature that is characteristic of lymphocytes and immune-privileged tissues and previously unrecognized in RTCs. Functional studies revealed that interleukin-1α-stimulated RTC Fas expression was accompanied by increased apoptosis, which was inhibited by blocking anti-Fas ligand antibodies. The data suggest that up-regulated RTC Fas binds to Fas ligand on adjacent RTCs, which then leads to RTC death by fratricide. We propose this pathway as an initiating mechanism of tubular atrophy.

The pathogenesis of chronic renal failure is initiated by glomerular injury in most instances, but it has been documented for 25 to 30 years that tubular atrophy, which is merely a pathologic description for the absence of renal tubule epithelial cells (RTCs), is a better predictor of renal disease progression than glomerular pathology [1–3]. Although tubular atrophy is clearly an important marker of progression, tubular hypertrophy is a common early finding in chronic renal disease, particularly in diabetic nephropathy [4, 5]. Despite intensive investigation regarding the mechanisms of RTC hypertrophy and hyperplasia, the molecular pathways regulating the transition from tubular hypertrophy to atrophy remain unclear.

The most prominent theory that has been forwarded to explain the pathogenesis of tubular atrophy is that RTCs are subjected to chronic ischemia [6, 7]. However, ischemia commonly leads to necrosis, a pathologic feature that is not typically observed in RTCs in most types of chronic renal disease. Furthermore, the processes that render RTC susceptible to ischemic injury would be primarily initiated by downstream effects of glomerular injury, such as obliteration of vasa recta blood supply [6], suggesting that the extent of tubular atrophy should parallel glomerular pathology. However, tubulointerstitial and glomerular histology are not tightly correlated [1, 2], indicating that factors independent of the glomerulus contribute to the pathogenesis of tubular atrophy. Indeed, RTCs have been shown to independently regulate fibrogenic effector pathways in response to injury [8].

An alternative mechanism of tubular atrophy is apoptosis, which has recently been shown to mediate the pathogenesis of many diseases [9], including RTC death [10–13]. The Fas apoptosis pathway is initiated by binding of Fas ligand (FasL) to Fas, which triggers a cascade of intracellular signals that results in apoptotic deletion of Fas-bearing target cells [14]. Constitutive Fas expression has been demonstrated in RTC [15], and Ortiz-Arduan et al have recently shown that RTC Fas expression and function are up-regulated by cytokines in vitro and in an in vivo endotoxic shock model of acute renal failure [16]. The role of Fas-dependent RTC apoptosis was investigated as a potential molecular pathogenetic mechanism of tubular atrophy in this study.

METHODS

Apoptosis assay in kidney tissue

Apoptotic RTC nuclei were detected in kidney frozen sections from FVB/N-p53 [17] and ROP-Os/+ [18] mouse models of progressive renal disease by the immu-
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no fluorescence TUNEL technique [19] according to manufacturer’s instructions (Oncor, Gaithersburg, MD, USA). Kidney sections were incubated with TdT and digoxigen-labeled dUTP and then with FITC-conjugated anti-digoxigen IgG. Positive controls for apoptosis were mouse thymus, and negative controls were kidney sections incubated without TdT. The prevalence of apoptosis was defined as the ratio of TUNEL-positive RTC to total RTC nuclei (as determined by Hoechst 33258 labeling).

Immunoblot methods

Methods have previously been described in detail [20]. Briefly, kidney cortex was lysed and denatured with boiling sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) buffer (125 mm Tris, pH 6.8, 2% SDS, 5% glycerol, 1% β-mercaptoethanol, 0.003% bromphenol blue) for five minutes. Samples (50 μg/lane) were resolved by 12% SDS-PAGE and transferred to polyvinylidine difluoride (PVDF) membranes. Blots were probed with anti-Fas IgG (1 μg/ml, one hour, room temperature) and then with peroxidase-conjugated IgG (1:5000, one hour, room temp). Band intensity was detected by enhanced chemiluminescence.

Detection of apoptotic cells by flow cytometry

Human renal proximal tubular (HRPT) cells were derived from human proximal RTCs, immortalized by adenov-12/SV40 transformation, and maintained in DMEM–1/10% fetal bovine serum (FBS) [21]. Cells were preincubated with or without interleukin (IL)–1α (20 ng/ml, 24 hr, 37°C). Fas was clustered and activated by co-incubating HRPT cells with agonistic mouse anti-human Fas IgM (CH11 clone, 150 ng/ml, 16 hr, 37°C), fixed in 1% paraformaldehyde, and lifted with 5 mm ethylenediaminetetraacetic acid (EDTA). Apoptotic HRPTs were detected by the immunofluorescence TUNEL technique and flow cytometry as previously described [22]. Background fluorescence was established in HRPT cells incubated with PBS alone, isotype control antibodies, or without TdT. Positive controls were SKW6.4 lymphocytes. Data are expressed as the percentage of apoptotic cells, which was calculated by determining the percentage of gated cells with fluorescence that exceeded background levels.

RESULTS

Using immunohistochemical techniques, increases in RTC apoptosis were observed at multiple time points in ROP-Os/+ and FVB/N-p53 mice compared with tissue sections from age-matched control kidneys (Fig. 1). The prevalence of apoptotic RTCs from Os/+ kidneys ranged from 0.2 to 0.7%, with no compensatory RTC hyperplasia in diseased kidneys (by PCNA staining, data not shown). Figure 2 demonstrates increased kidney cortex expression of Fas in both ROP-Os/+ and FVB/N-p53 mice compared with age-matched controls. The mechanism of Fas overexpression is undoubtedly complex, as IL-1α, tumor necrosis factor (TNF), and hypoxia each stimulated Fas expression in cultured RTCs (data not shown). Because apoptosis and Fas up-regulation coexisted at time points prior to the onset of widespread tubular atrophy, the data suggested that RTCs are targeted for Fas-dependent apoptotic deletion, and this process contributes to tubular atrophy. These studies also indicate that, in addition to known proinflammatory effects of IL-1 and TNF, these cytokines may indirectly promote tubular atrophy via Fas-dependent RTC apoptosis.

Surprisingly, both murine kidney cortex and RTCs in culture demonstrated constitutive expression of RTC FasL (data not shown), a feature that was previously assumed to be restricted primarily to lymphocytes and immune-privileged tissues and was unrecognized in RTCs. Furthermore, RTC Fas overexpression in conjunction with constitutive RTC FasL expression raised the possibility...
of a Fas–FasL interaction between adjacent cells, resulting in lymphocyte-independent “fratricidal” RTC apoptosis, as previously described in the pathogenesis of alcoholic cirrhosis and autoimmune thyroiditis [23, 24]. Functional studies in cultured RTCs revealed that IL-1 stimulated increased apoptosis, which was inhibited by co-incubation with blocking anti-FasL antibodies (Table 1), indicating that Fas-dependent RTC fratricide is a plausible mechanism of tubular atrophy.

**DISCUSSION**

Although it has been recognized for decades that tubular atrophy is one of the most accurate pathologic predictors of renal disease progression [1, 2], the molecular mechanisms that regulate this process remain unclear. We demonstrated that RTCs undergo increased apoptosis in two murine models of progressive chronic renal failure, suggesting that apotic RTC deletion contributes to tubular atrophy. These data are in agreement with previous reports of RTC apoptosis in both animals and humans with chronic renal disease [10, 12, 13, 25]. From a teliologic perspective, an apoptotic mechanism of tubular atrophy is appealing because, in contrast to ischemia, apoptosis efficiently promotes cell removal with minimal inflammation. Although the apoptosis values (0.2 to 0.7%) may seem meager, particularly compared with more susceptible in vitro systems, it has been estimated that apoptosis at a continuous rate of only 0.2% would result in a 50% decrease in cell number by six months [26]. The prevalence of apoptotic RTCs, without concomitant increases in RTC hyperplasia, suggests that net RTC deletion by apoptosis may be quantitatively important in the pathogenesis of tubular atrophy.

Multiple apoptosis signaling pathways have been described, but the Fas system was the focus of investigation because it has recently been implicated in the pathogenesis of tissue injury in multiple organs [23, 24, 27–30]. Furthermore, Fas-dependent apoptosis has been demonstrated in some [16], but not all [31], studies with cultured RTCs. Although previous reports have demonstrated basal kidney Fas expression [15], a unique finding in the current studies is enhanced RTC Fas expression in chronic renal failure. The observation that RTC Fas expression was increased in vivo does not contradict the conventional paradigm whereby Fas-expressing cells undergo apoptosis following binding to lymphocyte FasL. However, in vivo and in vitro data demonstrated FasL expression in RTCs, a finding that has heretofore been restricted primarily to lymphocytes and immune-privileged tissues. Therefore, up-regulation of RTC Fas, in conjunction with constitutive RTC membrane FasL expression, increases the probability of RTC Fas–RTC FasL binding. This interaction could then result in lymphocyte-independent “suicide” or “fratricide” [23]. Our in vitro data support this type of Fas-dependent RTC death; blocking anti-FasL antibodies had no effect on baseline RTC apoptosis, indicating that low-level basal RTC Fas expression was insufficient for functional Fas–FasL interaction between RTCs. However, IL-1 induction of RTC Fas expression was associated with increased apoptosis, which was inhibited by blocking anti-FasL antibodies. These data suggest that enhanced RTC Fas expression increases the likelihood of Fas-FasL binding, which promotes RTC deletion by fratricide.

In conclusion, we have provided evidence in murine models of chronic renal failure that RTCs are induced to express Fas, constitutively synthesize FasL, and undergo apoptosis by Fas-dependent fratricide. Because apoptosis may represent a final common RTC death pathway and tubular atrophy is a strong predictor of disease progression, we speculate that efforts to abort Fas-dependent RTC apoptosis may contribute to improved prognosis in diseases where definitive treatment is not currently available.

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Reprint requests to Jeffrey R. Schelling, M.D., MetroHealth Medical Center, Division of Nephrology, 2500 MetroHealth Drive, G531, Cleveland, Ohio 44109-1998, USA.

E-mail: jrs15@po.cwru.edu

**REFERENCES**


**Table 1. Renal tubular cells (RTC) undergo Fas-dependent fratricidal apoptosis**

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>% Apoptotic RTC</th>
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<tbody>
<tr>
<td>No additions</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>Anti-FasL IgG</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>IL-1</td>
<td>16.3 ± 1.4</td>
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<tr>
<td>IL-1 + anti-FasL IgG</td>
<td>1.5 ± 0.3a</td>
</tr>
<tr>
<td>IL-1 + isotype control IgG</td>
<td>16.4 ± 5.0</td>
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Human renal proximal tubule cells (HRPT) were cultured in serum-free media for 48 hr ± IL-1 (20 ng/ml, 24 hr, 37°C) ± blocking anti-human FasL antibodies (anti-FasL, 10 µg/ml, 24 hr, 37°C). Apoptotic cells were detected by immunofluorescence TUNEL and flow cytometry as described in the Methods section. Results are expressed as mean % apoptotic cells above background (fluorescence was restricted primarily to lymphocytes and immune-privileged tissues. Therefore, up-regulation of RTC Fas, in conjunction with constitutive RTC membrane FasL expression, increases the probability of RTC Fas–RTC FasL binding. This interaction could then result in lymphocyte-independent “suicide” or “fratricide” [23]. Our in vitro data support this type of Fas-dependent RTC death; blocking anti-FasL antibodies had no effect on baseline RTC apoptosis, indicating that low-level basal RTC Fas expression was insufficient for functional Fas–FasL interaction between RTCs. However, IL-1 induction of RTC Fas expression was associated with increased apoptosis, which was inhibited by blocking anti-FasL antibodies. These data suggest that enhanced RTC Fas expression increases the likelihood of Fas-FasL binding, which promotes RTC deletion by fratricide.

In conclusion, we have provided evidence in murine models of chronic renal failure that RTCs are induced to express Fas, constitutively synthesize FasL, and undergo apoptosis by Fas-dependent fratricide. Because apoptosis may represent a final common RTC death pathway and tubular atrophy is a strong predictor of disease progression, we speculate that efforts to abort Fas-dependent RTC apoptosis may contribute to improved prognosis in diseases where definitive treatment is not currently available.


