The Fas ligand/Fas system in renal injury

Alberto Ortiz, Corina Lorz and Jesús Egido

Unidad de Diálisis, Fundación Jiménez Díaz, Madrid, Spain

Introduction

Cell death by apoptosis regulates cell number during induction and resolution of renal injury [1]. Apoptosis inducers include an expanding family of lethal cytokines, that activate a corresponding family of death receptors [2]. The Fas ligand cytokine (FasL/Apo1L/CD95L) and the Fas receptor (Apo1/CD95) belong to these families [2], and evidence is accumulating of their role in renal injury.

FasL and Fas

FasL is a 40-kDa type II transmembrane protein that can be shed in a soluble form by the action of metalloproteinases [2–5]. Soluble FasL is up to 1000-fold less active than membrane-bound FasL in inducing apoptosis [4,5], but promotes neutrophil chemotaxis [6]. Leukocytes, epithelia and tumour cells express FasL [3]. Fas is a 45-kDa type I transmembrane protein that also exists in soluble form [3].

FasL induces apoptosis through Fas cross-linking [3,4]. However, not all Fas-expressing cells are susceptible to Fas-induced apoptosis [7]. FasL antagonists (soluble Fas, decoy receptor-3, and even soluble FasL), intracellular proteins such as FLIP (an antagonist of caspase 8), and, in certain cells, other protective proteins such as bclxL, protect from the lethality of FasL [2].

The FasL/Fas system has been implicated in the control of the immune response and inflammation, the response to infection, neoplasia, and death of parenchymal cells in several organs [2,3,6,8].

Sources of confusion in the study of the FasL/Fas system

Conflicting reports abound in the FasL/Fas literature. Both molecules are frequently expressed in low amounts, and detection may be difficult. This gave rise to the initial, erroneous concept that their distribution was very restricted. In addition diverse biological effects may be anticipated, and have been reported, that depend on the predominant effect of FasL on cell death of parenchymal cells or leukocytes, on recruitment of inflammatory cells, or additional, not yet well-characterized, functions of Fas or FasL itself. In this regard, Fas activation may be harmful or beneficial to renal injury, depending on whether parenchymal renal cells or the immune response are primarily affected [9,10].

Much of the current information on the biological activity of Fas derives from experiments using agonistic anti-Fas antibodies as activators of Fas. However, antibodies are less effective Fas activators than FasL [5]. Thus, some studies, especially those reporting negative results, should be updated by the use of recombinant FasL.

Information on the physiological role of FasL/Fas also derives from mice with defective proteins. However, these data have certain caveats and should be interpreted with caution. The Fas defect in lpr/lpr (lymphoproliferation) mice is leaky. In these mice low levels of functional Fas are present in lymphoid cells [11] whereas Fas expression in other organs, such as the testis appears to be preserved [12]. Moreover, increased FasL [13] may compensate for lower Fas expression. Mice carrying the lprcg point mutation have intracellular proteins such as FLIP (an antagonist of caspase 8), and, in certain cells, other protective proteins such as bclxL, protect from the lethality of FasL [2].

The gld (generalized lymphoproliferative disease) single point mutation in FasL prevents this cytokine from promoting apoptosis [3]. However, Fas expression is increased in gld mice [14], and may result in autoactivation and triggering of cell death in response to non-FasL lethal stimuli [15]. Taken together these caveats suggest that differences in the response to renal insults between mice with defective FasL/Fas systems and their controls are due to an involvement of the FasL/Fas system, but a negative result does not rule out its participation.

FasL and Fas are expressed in the kidney

Potential sources of renal FasL include infiltrating leukocytes and intrinsic renal cells (Table 1). Murine
Table 1. Participation of FasL/Fas in renal injury.

<table>
<thead>
<tr>
<th>FasL expressing cells</th>
<th>Cells sensitive to Fas apoptosis</th>
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<tr>
<td>Mesangial cells</td>
<td>Mesangial cells</td>
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<tr>
<td>Tubular epithelial cells</td>
<td>Primed tubular epithelial cells</td>
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<tr>
<td>Renal fibroblasts</td>
<td>Renal fibroblasts</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>Primed endothelial cells</td>
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<tr>
<td>Leukocytes: lymphocytes, monocytes/macrophages, neutrophils</td>
<td>Leukocytes</td>
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<tr>
<th>Increased renal Fas expression</th>
<th>Increased renal FasL expression</th>
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<tr>
<td>Human/rat allograft rejection</td>
<td>Human/rat allograft rejection</td>
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<tr>
<td>Human proliferative glomerulonephritis</td>
<td>Rat proliferative glomerulonephritis</td>
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<tr>
<td>Murine chronic tubular atrophy: p53 transgensics, ROP-Os/-</td>
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<tr>
<td>Murine endotoxaemia</td>
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<tr>
<td>Rat glomeruli from remnant kidney</td>
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<td>Murine ischaemia-reperfusion</td>
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| Fas agonists promote renal injury     | Fas agonists protect from renal injury           |
| Glomerular injury in mice             | Prolongs rat renal allograft survival            |
|                                       | Overcomes autoimmunity in gld mice               |

| FasL/Fas disruption protects from renal injury | FasL/Fas disruption promotes renal injury |
| Ischaemia reperfusion in B6 lpr mice         | Autoimmune nephritis: MRLlpr, MRLlpr<sup>cg</sup>, MRLgld |
| Unilateral ureteral obstruction in B6 lpr mice | but not other mouse strains with the same mutations |

Mesangial cells, tubular epithelial cells and renal interstitial fibroblasts express FasL [17,18]. Tubular epithelial cells are the main sources of FasL in the normal murine kidney and can promote apoptosis of Fas-sensitive lymphoid cells [Lorz et al. unpublished findings]. By contrast, glomerular cell FasL is only present during proliferative glomerular injury [Lorz et al. unpublished findings]. This may be a consequence of activation by cytokines involved in renal injury, as TNF increases FasL mRNA in cultured murine mesangial cells [17]. Renal FasL is also increased during transplant rejection, where infiltrating leukocytes are the main sources of FasL [19].

Human and murine mesangial cells, human and murine tubular epithelial cells, and murine cortical renal fibroblasts express cell membrane Fas receptors in culture [17,18,20]. LPS, cytokines (TNF-α, IFNγ, IL-1α, IL-1β), nephrotoxins such as CsA, and viruses such as HIV upregulate cell surface Fas in renal cells [17,18,20–24].

Glomerular and/or tubular Fas is increased in experimental endotoxaemia [17], progressive tubular atrophy [21], ischaemia–reperfusion [25], and remnant kidney glomerulosclerosis [26]. In humans Fas is increased in proliferative glomerulonephritis [27] and in tubular epithelium during renal allograft rejection [23].

Fas induces apoptosis in cultured renal cells

Agonistic anti-Fas antibodies kill cultured, non-stimulated mesangial cells and renal fibroblasts [9,17,18,20], while renal microvascular cells and human and murine tubular epithelial cells are relatively resistant [7,17,28] and require priming by LPS or cytokines that increase cell surface Fas to undergo cell death [Lorz et al. unpublished, 23,29]. Down-regulation of Fas in fibroblasts by survival factors protects them from Fas agonist-induced apoptosis [18]. By contrast, survival factors failed to protect mesangial cells from Fas activation [30]. Inhibitors of protein and RNA synthesis also sensitized renal cells to Fas-induced death [7,17]. This suggests that constitutively expressed intracellular proteins protect renal cells from apoptosis. As a whole, these data indicate that the extracellular microenvironment of injured kidneys may sensitize parenchymal renal cells to Fas induced death, leading to cell loss in the course of acute or chronic renal damage.

Fas actions in the kidney in vivo

Fas activation may lead to glomerular injury. A single injection of agonistic anti-Fas antibodies induced, in a complement-independent manner, acute, self-limited glomerular-cell apoptosis associated with decreased number of mesangial cells, proteinuria, and haematuria in the absence of glomerular inflammation [9]. This was the first time that apoptosis was demonstrated to actually cause glomerular injury. Interestingly, as was the case in vitro, tubular epithelial cells were protected from apoptosis [9].

Renal injury may also improve following modulation of the immune response with Fas agonists. A single injection of the agonistic anti-Fas antibody RK-8 decreased autoimmunity and ameliorated renal injury in MRL-gld/gld mice [10], probably through elimination of autoreactive lymphocytes. The biological activity of this antibody is different from that of Jo-2, previously reported to induced glomerular and liver injury [9], in that RK-8 predominantly damages lymphoid cells, that, in the gld mice, express high levels of Fas [10].

High renal cortex expression of FasL, achieved by means of an adenoviral vector, prolonged renal graft survival in rats [31]. Taking into account the often contradictory experiences published in other transplantation systems [32], this report should be further validated.

Studies involving loss of function mutations

MRL lpr/lpr, MRL lpr<sup>cg</sup>/lpr<sup>cg</sup> and MRL gld/gld mice display lymphoproliferation, autoimmunity and lupus-
like glomerulonephritis [3]. However glomerulonephritis is absent in mice from other strains with the same mutations [3]. Thus, the MRL background favours the development of glomerulonephritis in mice with defective FasL/Fas systems. As autoimmunity and defective FasL/Fas have not been dissociated, it is unknown whether renal damage would be more severe in MRL mice if an intact renal FasL/Fas system were present. In this sense, complete absence of death-inducing Fas receptor in MRL/lpr mice resulted in milder renal injury than in MRL lpr mice, despite similar amount of autoantibody production and glomerular deposition [33]. More recently, Fas-intact MRL+/+ kidneys were implanted into MRL lpr/lpr mice [29]. Fas and FasL were increased in the transplanted kidney, but the extent of nephritis and the amount of apoptosis were similar to those of native MRL lpr/lpr kidneys [29]. The authors speculate that the autoimmune milieu evokes mechanisms that counter FasL/Fas mediated apoptosis [29]. While human Fas mutations also cause an autoimmune lymphoproliferative syndrome (ALPS or Smith–Canale syndrome), these subjects display mainly haematological autoimmunity [34].

Fas induces tubular injury during ischaemia—reperfusion, as tubular damage was decreased in B6 lpr mice when compared with B6 mice [25]. In addition, an intact FasL/Fas system is required to limit certain inflammatory responses, and persistence of systemic inflammation, including renal inflammation, follows murine CMV infection, despite clearance of the virus, in B6 lpr/lpr mice but not in control B6 mice [35].

Summary

The FasL-Fas system regulates renal cell apoptosis, as well as the immune and inflammatory responses. Evidence that FasL and Fas participate in renal injury may be summarized along modified Koch’s postulates (Table 1): (i) FasL is expressed by renal cells and during renal injury, (ii) activation of the Fas receptor promotes apoptosis of cultured renal cells, (iii) Fas agonists induce glomerular injury but they may also decrease renal injury by limiting injurious immunological responses, (iv) mice with disrupted FasL/Fas systems are protected from tubular cell injury during ischaemia—reperfusion, although they develop autoimmune glomerulonephritis if other genetic predisposing factors are present.

FasL/Fas must be considered a new target for therapeutic intervention in renal injury. Therapeutic modulation of Fas should aim not only at protecting intrinsic glomerular or tubular epithelial cells from death, but also at modulating the immune, inflammatory, and fibrogenic responses. Possible therapeutic interventions include Fas agonists, soluble Fas receptors, or other antagonists, and targeting of Fas to undesired cells, such as fibroblasts, in order to decrease their numbers in a physiological manner through apoptosis. Any therapeutic attempt should carefully take into account the possible effects of interference with Fas in other cell systems. Given the complexities of the FasL/Fas system, further studies are warranted.

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