Mesangial cell Fas ligand: upregulation in human lupus nephritis and NF-κB-mediated expression in cultured human mesangial cells

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Abstract

Background. Fas ligand (FasL) is a well-known death factor; however, the role of FasL in the regulation of human glomerulonephritis remains unclear.

Methods. We investigated the renal expression and localization of FasL in various forms of human glomerulonephritis by immunohistochemistry, utilizing confocal laser scanning microscopy. We further evaluated cytokine-induced FasL expression via nuclear factor (NF)κB in cultured human mesangial cells (HMC). The level of soluble FasL was measured by a specific enzyme-linked immunosorbent assay (ELISA).

Results. The frequency of glomerular FasL-positive cases was higher in lupus nephritis (37.9%) as compared with other forms of glomerulonephritis (8.7%). The glomerular FasL score in proliferative lupus nephritis was significantly higher than that in nonproliferative forms. Patients with a high apoptosis score, severe microhematuria, proteinuria, or decreased renal function had a high FasL score. Double immunolabelling demonstrated that the most prevalent phenotypes of FasL-positive cells were mesangial cells. In cultured HMC, interleukin (IL)1β, lipopolysaccharide (LPS), or γ interferon (IFN) upregulated membrane-bound FasL. IL1β significantly, and LPS or γIFN weakly activated NFκB, but none of these agents activated NFκB/Rel-related nuclear factor of activated T cells (NFATc) or IFN regulatory factor-1. IL1β-mediated NFκB was completely inhibited in the presence of lactacystin, a potent inhibitor of NFκB. Lactacystin-mediated inhibition of NFκB reduced FasL protein levels. Matrix metalloproteinase (MMP)-7, but not other MMPs (1, 2, 3, 8, or 9), significantly sensitized HMC to release soluble FasL after IL1β stimulation.

Conclusions. The results suggest that: (1) upregulation of mesangial FasL may contribute to the glomerular inflammation in proliferative lupus nephritis in vivo; (2) proinflammatory cytokines, in particular IL1β, produced in nephritis can upregulate FasL via the transcription factor NFκB in HMC; and (3) MMP-7-mediated release of soluble FasL could control the mesangial inflammation.

Key words Apoptosis · Interleukin-1β · Lupus · Matrix metalloproteinase · Nuclear factor κB · Soluble Fas ligand
In a recent report, FasL has been documented to be present in normal tubuar epithelium and injured glomeruli in rat and murine nephritis models. That report suggested that intrinsic renal cell FasL may play a role in cell homeostasis in normal kidney and during renal injury. A fluorescence in situ hybridization study indicated FasL mRNA was expressed in glomeruli in lupus nephritis. However, the role of FasL in glomerular inflammation still remains unclear.

Here we show that glomerular mesangial cells do express FasL in human proliferative lupus nephritis, which exhibits massive mesangial proliferation in vivo. Inflammatory mediators, in particular IL1β, stimulated FasL upregulation through nuclear factor (NF)κB in cultured human mesangial cells in vitro. Our data will give new insights into novel roles of mesangial FasL in the control of glomerular inflammation.

### Patients, materials, and methods

**Patient populations**

Human kidney tissue specimens were obtained by percutaneous needle biopsy from 57 patients, between 1999 and 2001, at our department (Table 1), with their written informed consent. Tissue samples were taken from 29 patients with lupus nephritis and 23 with other glomerular diseases; 9 had crescentic glomerulonephritis (GN), 5 had IgA nephropathy, and 9 had other forms (purpura nephritis, membranous nephropathy, minimal change nephropathy, membranoproliferative GN, and postinfectious GN). As control tissue, histologically normal portions of kidneys that had been resected because of renal cell carcinoma were used (n = 5). The diagnosis was confirmed on the basis of clinical symptoms and immunofluorescence, light, and electron microscopic findings. Classification of lupus nephritis was according to the criteria defined by the World Health Organization (WHO). The clinical data obtained from the patients included serum creatinine and total protein concentration, 24-h creatinine clearance, daily urinary protein excretion, and degree of microhematuria at the time of renal biopsy. Anti-double-stranded DNA autoantibody, plasma complement activity (CH50), and C3 were measured in patients with lupus nephritis as immunoserologic indices. Informed consent was obtained from all patients prior to commencement of the study.

**Light and immunofluorescence microscopic studies**

Percutaneous renal biopsy was performed using a 16-gauge needle under ultrasound observation. The length of each biopsy specimen was approximately 15–20mm, and two or three samples were taken from each patient. The specimens were divided into three portions, and a part of the specimen was fixed in 10% buffered formalin and embedded in paraffin. Sections 3-µm-thick were stained with hematoxylin and eosin (H&E), periodic acid-Schiff, Masson’s trichrome, or periodic acid-silver methenamine stains. Specimens of lupus nephritis were classified according to World Health Organization (WHO) criteria, established by the American Rheumatism Association, as mesangial (class II), focal proliferative (class III), diffuse proliferative (class IV), and membranous (class V) lupus nephritis. In addition, specific histologic features, i.e., activity and chronicity indices, were semiquantitatively graded by the renal pathology scoring system defined by Austin et al. The number of apoptotic cells per glomerulus stained with H&E was assessed as the glomerular apoptosis score. In our previous investigation, the number of apoptotic cells identified in H&E-stained sections by light microscopy significantly correlated with the number of TdT-mediated dUTP-biotin nick end labeling (TUNEL)-positive glomerular cells in lupus nephritis. Another portion of the renal biopsy specimen was processed for immunofluorescence microscopy. In brief, kidney specimens were immediately snap-frozen, and 4-µm-thick sections were prepared. The cryostat sections were stained with anti-human IgG, IgA, IgM, C3, C1q, and fibrinogen conjugated with fluorescein isothiocyanate (FITC; Cappel, West Chester, PA, USA).

**Electron microscopic (EM) studies**

The rest of the renal biopsy specimen was processed for transmission EM as previously described. The speci-