LPS-evoked IL-18 expression in mesangial cells plays a role in accelerating lupus nephritis

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Objectives. Systemic lupus erythematosus is occasionally accompanied with bacterial infection. Lipopolysaccharide (LPS) from bacteria can accelerate and exacerbate lupus nephritis (LN) in animal models, but some mechanisms underlying the LPS-induced acceleration are still unclear. First, it is not known whether LPS can stimulate mesangial cells (MCs) to secrete the pro-inflammatory cytokine, interleukin (IL)-18. Second, it is also unclear whether LPS and/or IL-18 can induce MC apoptosis. Here, we attempted to clarify the cause-and-effect relationships between LPS stimulation, IL-18 production and MC apoptosis to address the above questions.

Methods. LPS was used to induce accelerated LN in LN-prone mice. LPS and IL-18 were also used to treat cultured MCs isolated from the mice. IL-18 expression and MC apoptosis were investigated by in situ hybridization, the TUNEL method, reverse transcription–polymerase chain reaction (RT–PCR), western blotting, DNA electrophoresis and flow cytometry. NFκB was detected by immunofluorescent staining.

Results. In the LPS-accelerated LN mice, we observed co-existence of IL-18 expression, hyperplasia, apoptosis, and activated apoptotic signal transduction in MCs, as well as marked neutrophil infiltration in the glomerulus, especially around the mesangial region. In cultured MCs, LPS greatly enhanced IL-18 expression, but did not induce apoptosis, while mouse IL-18 did not induce apoptosis or activate apoptotic signal transduction in MCs.

Conclusions. We conclude that LPS can evoke IL-18 production in MCs, but neither LPS nor IL-18 directly induces apoptosis or activates apoptotic signal transduction in the cells. We infer that LPS-induced IL-18 production by MCs could be a mediator by which LPS accelerates and exacerbates LN.

Key words: LPS, IL-18, Lupus nephritis, Mesangial cells, Apoptosis, Accelerated LN.

Introduction

Lupus nephritis (LN) is a frequent organ manifestation of SLE, a disease characterized by the occurrence of many different autoantibodies. LN is also the main cause of morbidity and mortality in SLE. The progression to LN in SLE is very complex [1] and the outcome of LN in SLE is highly variable, ranging from clinically silent nephritis to rapidly progressive crescentic glomerulonephritis with acute renal failure. Many complication factors, including clinical, serological, and histopathological factors, are responsible for the ultimate severity of LN [2].

SLE is occasionally accompanied with bacterial infection [3–6], but the clinical outcomes of bacterial infection in SLE are hard to evaluate due to the interference of medication on patients. Endotoxin, such as lipopolysaccharide (LPS) from Gram-negative bacteria, is a complicating factor which can exacerbate and accelerate LN in mouse models [7]. Both systemic and local effects of LPS are responsible for the LPS-accelerated LN. The systemic effects of LPS are to induce B cell activation, enhance immune complex deposition [7–9] and increase the expression of ICAM-1, which recruits leucocytes to the kidney [10], while one local effect of LPS on glomeruli is to stimulate mesangial cells (MCs) to produce cytokines, which modulate glomerular inflammation and renal cell survival [11, 12]. Interleukin (IL)-18, a relatively recently discovered cytokine of the IL-1 superfamily, has been shown to play pathogenic roles in LN [13]. So far, it is still unclear whether LPS can stimulate MCs to produce IL-18. If it can, this could provide an insight into the mechanism underlying LPS-accelerated LN.

 besides serving as pro-inflammatory mediators, both LPS and IL-18 are apoptosis-inducing factors that affect the survival of many cells. LPS can directly cause apoptosis of alveolar epithelial, endothelial, neuronal and renal tubular cells [14–17], and IL-18 can directly induce apoptosis of cardiac endothelial and renal tubular cells [18–20]. However, it is still uncertain whether LPS and/or IL-18 has an apoptotic effect on MCs and addressing this question is crucial in demonstrating or excluding a mechanism in which LPS accelerates LN by inducing MC apoptosis, because apoptosis could cause MC loss which compromises histological structure of glomeruli. However, moderate apoptosis is beneficial in LN, since apoptosis is an important mechanism for ameliorating MC hyperplasia in LN [21–23].

In order to evaluate potential local mechanisms in LN involving stimulation by LPS, expression of, and stimulation by, IL-18, and MC apoptosis, in vivo and in vitro studies were carried out in an LPS-induced accelerated-LN mouse model and in cultured MCs from the LN-prone mice. The present study clearly demonstrated that LPS-stimulated MCs produce IL-18 and that this correlates well with severe glomerular lesions, but neither LPS nor IL-18 directly induces MC apoptosis, although they might have indirect apoptotic effects on MCs.

Materials and methods

LPS-induced accelerated-LN mouse model

An LPS-induced accelerated-LN mouse model was established in an 8-week-old female New Zealand black/white (NZB/W) f1 mice by twice weekly intraperitoneal injection of LPS (Sigma, St. Louis, MO, USA) for 6 weeks as described previously [7], then blood and urine samples were collected and the mice killed (n = 7 for each day) and kidney samples were taken for the following studies. All experimental measurements were performed in duplicate. All animal experiments were performed with the ethical approval of the Institutional Animal Care and Use Committee of The National Defense Medical Center, Taiwan and performed.
according to the ethical rules in NIH Guide for the Care and Use of Laboratory Animals.

Renal function, urine protein and serum autoantibodies

Urinary was collected in metabolic cages and blood was collected from the retro-orbital plexus. Serum creatinine was measured by colorimetry using a creatinine detection kit (Sigma 640-A) and blood urea nitrogen (BUN) was measured using a urease assay kit (Sigma 640-A) as described previously [24]. Proteinuria was evaluated by measuring total urinary protein using a Pierce BCA protein assay kit (Pierce, IL, USA). Serum autoantibody levels were measured using mouse anti-dsDNA and anti-nuclear antibodies ELISA kits (Alpha Diagnostic Intl. Inc.) and an ELISA plate reader (Bio-Tek, MA, USA).

Immunofluorescence, in situ hybridization, histopathological evaluation and TUNEL analysis

Renal tissues were snap-frozen or fixed in 10% buffered formalin, then cut into sections for detection of deposited immune complexes by immunofluorescence (IF) and for in situ hybridization (ISH), histopathology evaluation and TUNEL analysis. For IF, frozen sections were air-dried, fixed in acetone for 10 min at room temperature and incubated with fluorescent isothiocyanate (FITC)-conjugated goat anti-mouse IgG or C3 antibodies (Cappel; Organon Teknika, Durham, NC, USA) as described previously [24]. For ISH, RNA probes were generated by in vitro transcription (AmpliScribe T7 and SP6 high yield transcription kits, Epicentre, WI, USA). Briefly, mouse IL-18 cDNA was generated from kidney mRNA by the reverse transcription (RT)–polymerase chain reaction (PCR) (IL-18 primers: forward 5'-AGTGTACAAACCGGAGTAATACGG-3' and reverse 5'-TCATCTTTGTTGTGTCCTG-3'). The product from the PCR reaction was inserted into the pGEM-T vector (Promega, WI, USA) and digoxigenin-labelled RNA probes synthesized using T7 RNA polymerase (Roche, IN, Germany). After hybridization with the probes, alkaline phosphatase-conjugated anti-digoxigenin antibody was used to detect the hybridized probe by colour development in NBT/BCIP solution (Roche). For histopathology, formalin-fixed tissues were stained with haematoxylin and eosin (HE). For TUNEL staining, formalin-fixed sections were stained with an ApoTag Plus Peroxidase in Situ Apoptosis Detection kit (Chemicon) according to the manufacturer's instruction.

Isolation of glomeruli

The glomeruli were extracted from the kidneys using a sieving technique as described previously [25, 26]. Briefly, the kidneys were removed, washed with sterile phosphate-buffered saline (PBS) and decapsulated and the cortex was separated from the medulla. A sample of cortex was cut into 1–2 mm³ blocks, which were ground up in NIES solution (Roche). After sieving, the glomeruli were collected from the surface of the 200 mesh net. The glomerular samples were subjected to total RNA extraction using Trizol reagent (Life Technologies, Rockville, MD, USA) according to the manufacturer's instruction for RT-PCR studies and to lysis using RIPA solution [26] for western blotting studies.

Primary cultures of MCs

Female NZB/W mice, 6- to 8-months-old, were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and used as the MC source. The establishment of primary cultures of MCs from the NZB/W mice was modified from a previously described protocol [27]. Briefly, glomeruli were purified from minced renal cortex by serial sieving through meshes of different pore-sizes, then the glomeruli suspension was digested for 20 min at 37°C with type IV collagenase and the dissociated glomerular cells cultured in RPMI 1640 medium containing 20% heat-inactivated fetal bovine serum (FBS), penicillin/streptomycin and HEPES (10mM) (GIBCO, Invitrogen, CA, USA). Epithelial cells initially grew faster than MCs, but died after 2–3 weeks and almost pure MCs are then obtained. The cells were plated onto dishes with glass slides and allowed to grow for 16 h in RPMI 1640 medium containing 20% FBS, then for 2 h in RPMI 1640 medium containing 2% FBS. LPS or recombinant mouse IL-18 (MBL, Nagoya, Japan) in RPMI 1640 medium containing 2% FBS was then added for 0, 6, 12 or 24 h and the cells harvested.

Reverse transcriptase (RT)-PCR analysis

Tissue RNA was isolated using TRIzol reagent according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA, USA). For first-strand cDNA synthesis, 3 μg of total RNA was used in a single-round RT reaction in a final volume of 25 μl of a solution containing 0.9 μl of 50 μM oligo(dT)12–18, 1.0 mM dNTPs, 1 × first-strand buffer, 0.4 mM dithiothreitol, 80 U of RNaseout recombinant RNase inhibitor and 300 U superscript II RNase H (Invitrogen, Carlsbad, CA, USA). PCR was performed in a total volume of 15 μl containing 0.9 μl of the cDNA template-containing solution from the RT reaction above, 0.4 μM mouse IL-18 primers (forward: 5'-AGTGTACAAACCGGAGTAATACGG-3' and reverse: 5'-TCATCTTTGTTGTGTCCTG-3') or GAPDH primers (forward: 5'-TCCCGCCCCCTTTGCGGATGATG-3' and reverse: 5'-ACCGGAAAGCCTAGCCATGTG-3'). 1 × PCR buffer, 0.25 mM dNTPs and 1.5 U of KlenTaq DNA polymerase (Ab Peptides Inc. St. Louis, MO, USA). Amplification was carried out at 94°C for 2 min, then for 25 cycles of 94°C for 45 s, 55°C for 1 min and 72°C for 45 s and, finally, at 72°C for 10 min. RT-PCR products were resolved on a 1.5% agarose gel containing 0.1% ethidium bromide and 0.5 × TBE buffer, visualized using a gel documentation system (Bio-Rad, Hercules, CA, USA) and analysed by densitometry.

Western blotting

MCs were lysed using RIPA lysis solution and 10 μl of the lysed sample mixed with an equal volume of 2x sample buffer and run on a 10% SDS-PAGE gel; western blotting was then performed as described previously. The primary antibodies used were rabbit primary antibodies (1/500, Neomarkers Lab Vision Corp.), goat anti-IL-18 (1/100) or β-actin (1/1000) antibodies, rabbit anti-caspase 3 p20 (1/400), BID (1/400) or Bax (1/400) antibodies, or mouse anti-caspase 8 p20 subunit (1/400) or Bel-2 (1/400) antibodies (all from Santa Cruz). The secondary antibodies used were HRP-conjugated rabbit anti-goat IgG (1/10 000, Santa Cruz), goat anti-rabbit IgG antibodies (1/10 000, Pierce, IL, USA) or rat anti-mouse IgG antibodies (1/1000, Santa Cruz), as appropriate.

DNA fragmentation electrophoresis

Cells (10⁶) were treated with LPS for 0, 6, 12, or 24 h or with 10 mM H2O2 for 6 h as an apoptosis positive control [28]. Low molecular weight DNA was then extracted using a G-DEX Genomic DNA Extraction kit (NIRON Biotechnology) according to the manufacturer’s protocol. A mixture of 13.5 μl of the extracted DNA and 1.5 μl of DNA loading buffer (0.5% bromophenol blue, 50% glycerol, 0.5 mM EDTA) was run on an 1.0% agarose gel containing 0.1% ethidium bromide and 0.5 × TBE buffer (0.089 M Tris, 0.002 M EDTA, 0.089 M boric acid) and gel images were obtained using a Photo-Documentation System (Viber Lourmat, France).
Assessment of apoptosis by flow cytometry

The cells were collected by centrifugation and washed twice with PBS, then an Annexin V-FITC Apoptosis Detection kit (BD Pharmingen) was used to assess apoptosis according to the manufacturer’s instructions. Annexin V-positively and negatively labelled cells were counted on a flow cytometer (FACScalibur) equipped with an argon laser (BD Biosciences) operating at an excitation wavelength of 488 nm and an emission wavelength of 520 nm. The resulting histograms were analysed using an analytical software program (WinMDI).

Immunofluorescence studies of NF-κB nuclear translocation

MCs were grown on glass slides and fixed with 2% paraformaldehyde for 15 min. For IF staining of NF-κB p65, the sections were incubated overnight at 4°C with rabbit anti-NF-κB p65 antibody (Cell Signalling, MA, USA), then for 2 h at room temperature with FITC-conjugated goat anti-rabbit IgG antibody (Cappel; Organon Teknika, Durham, NC, USA). The percentage of positive MCs for nuclear NF-κB p65 was determined by counting at least 500 cells in each well under a microscope (×400) [29].

Statistics

The data are presented as the mean ± SEM. Student’s t-test was used for the statistical analysis. Differences were considered significant at $P < 0.05$.

Results

Clinical manifestations and autoantibody levels

BUN and creatinine levels were significantly higher in the LPS-treated mice than the control mice (228.61 ± 26.79 vs 32.27 ± 4.76 mg/dl and 0.73 ± 0.06 vs 0.35 ± 0.04 mg/dl, respectively; both $P < 0.05$) (Fig. 1A and B), indicating that LPS accelerated the deterioration of renal clearance function. In parallel, heavier proteinuria was seen in the LPS-treated mice compared with the controls (43.77 ± 1.71 vs 21.84 ± 0.86 mg/ml; $P < 0.05$) (Fig. 1C), revealing accelerated disruption of the renal filtration barrier by LPS.

Anti-nuclear antibody titres in the LPS-treated mice were significantly higher than in controls (OD 1.08 ± 0.04 vs 0.54 ± 0.26; $P < 0.05$) (Fig. 1D), as were anti-ds DNA antibody titres (OD 1.64 ± 0.14 vs 0.384 ± 0.04; $P < 0.05$) (Fig. 1E). In contrast to the control non-LPS-injected mice, diffuse IgG
and C3 deposits were seen in the glomeruli of the LPS-treated mice by IF (Fig. 1F). The higher levels of auto-antibodies in both the serum and glomeruli in the LPS-treated mice show that LPS enhances the autoimmune response [8, 9].

LPS-treated mice show co-existence of IL-18 upregulation, hyperplasia and apoptosis in MCs

tissue sections stained by ISH, HE and TUNEL method are shown in Figure 2A. ISH showed that IL-18 mRNA levels were higher in the mesangial area of LPS-treated mice than in controls, indicating that MCs respond to LPS by IL-18 overexpression. HE staining revealed that, in contrast to the histological profile of the control group, glomeruli in the LPS-treated mice showed marked proliferation and morphologically typical apoptosis (apoptotic bodies indicated by thick arrows) in the mesangial area. To confirm the apoptosis in the glomeruli, TUNEL analysis was performed and showed that TUNEL (+) cells were clearly more abundant in the LPS-treated mice than in control mice.

To further confirm the in vivo coexistence of IL-18 expression and MC apoptosis, RT-PCR and western blot analyses were performed on isolated glomeruli. As shown in Figure 2B, IL-18 mRNA levels in isolated glomeruli from LPS-treated mice were significantly higher than those in control mice (IL-18/GAPDH ratio: 0.35±0.08 vs 0.1±0.04, P<0.05), consistent with the ISH data (Fig. 2A). In addition, cleavage of both procaspases 8 and 3 were significantly increased in LPS-treated mice compared with control mice (Fig. 2C), providing a signalling mechanism accounting for the enhanced apoptosis induced by LPS (Fig. 2A).

LPS treatment of cultured MCs results in IL-18 expression, but not apoptosis

As shown in Fig. 3A, almost pure MC cultures were established, as most of the cells stained positive for two typical MC

![Figure 2](image-url)
biomarkers, α-SMA and vimentin, but not for E-cadherin, an epithelial cell marker. Preliminary tests showed that 50 μg/ml of LPS were just below the threshold for LPS cytotoxic effects on MCs (data not shown). To evaluate effects of LPS on apoptosis and IL-18 expression in MCs, concentrations between 10 and 50 μg/ml were therefore used.

DNA ladder electrophoresis showed that 10–50 μg/ml of LPS did not induce apoptosis (DNA fragmentation) of MCs; results using 50 μg/ml of LPS are shown in Fig. 3B. However, both RT-PCR and western blotting showed that the same concentrations caused significant IL-18 expression over time (Fig. 3C and 3D). Compared with basal levels, a significant increase in IL-18 mRNA was seen after 12 or 24 h of treatment with 10 μg/ml of LPS, while levels in control MCs remained unchanged (Fig. 3C) (IL-18/GAPDH ratio at 12 and 24 h 0.509 ± 0.012 and 0.414 ± 0.007, respectively, vs 0.164 ± 0.087 and 0.170 ± 0.095 in controls; P < 0.05). Similarly, a marked increase in IL-18 protein levels was seen under the same conditions, while levels in control MCs did not change significantly (Fig. 3D) (IL-18/β-actin ratio at 12 and 24 h 0.403 ± 0.015 and 0.450 ± 0.034, respectively, compared with undetectable levels in the controls; P < 0.05).

**Inability of IL-18 to induce apoptosis in cultured MCs**

Using flow cytometry with annexin V as the probe to detect early apoptosis, no significant difference in the percentage of annexin-positive cells was seen between IL-18 (0 – 400 ng/ml) treated and control MCs at 6, 12, or 24 h (P > 0.05); the data for the highest concentration are shown in Fig. 4A. DNA fragmentation, a late event in apoptosis, was also examined by agarose electrophoresis and, again, no apoptosis was seen at 6, 12 or 24 h of IL-18 (0–400 ng/ml) treatment; the data for the highest concentration are shown in Fig. 4B.

To completely rule out an apoptotic effect of IL-18 on MCs, various apoptotic signalling proteins were evaluated by western blot analyses. The expression of typical apoptotic and anti-apoptotic proteins (p 20 subunit of procaspase 8, Bid, Bcl-2, BAX, p 20 subunit of procaspase 3 and cleaved PARP) in MCs treated with 400 ng/ml of IL-18 for up 24 h did not differ significantly from that in control MCs (P > 0.05); the data for the 24 h
treatment are shown in Fig. 4C and D. These results confirm that no apoptotic or anti-apoptotic pathways were activated or suppressed by IL-18.

**LPS, but not IL-18, evokes NF-κB nuclear translocation in cultured MCs**

Since nuclear factor κB (NF-κB) is a transcriptional regulator that plays a central part in signal transduction by both LPS (Toll-like) receptors and the IL-18 receptor, NF-κB activation were evaluated in MCs stimulated with LPS or IL-18. No significant difference in NF-κB nuclear translocation was seen between MCs treated with 400 ng/ml of IL-18 for up to 24 h and control MCs (data not shown), contrasting with the substantial NF-κB nuclear translocation seen in MCs treated for 6 h with 10 μg/ml of LPS (62 ± 5.5% compared with 11.5 ± 2.1% in controls; \( P < 0.05 \)) (Fig. 5).

**Discussion**

In the accelerated-LN mouse model, LPS administration resulted in increased serum auto-antibody levels (Fig. 1D and E), increased immune complex deposition (Fig. 1F), aggravated renal histological profiles (Fig. 2A), and accelerated and exacerbated the clinical syndrome (Fig. 1A–C). These changes can be partly explained by the increased IL-18 expression by MCs induced by LPS (Fig. 2A and 2B), as IL-18 is a pro-inflammatory cytokine that can recruit and activate neutrophils [30, 31], which, in turn, can result in damage to glomeruli (Fig. 6D). Indeed, marked infiltration of neutrophils was seen around MCs in the LPS-treated mice compared with control mice (Fig. 2A, neutrophils indicated by thin arrows), consistent with the results of previous studies [7–9].

IL-18 is produced by many cell types [32], but it is not known if it is expressed in MCs. As shown by ISH in Figure 2, IL-18 expression was detected in MCs and was greatly enhanced by LPS treatment (Fig. 2A and B). Furthermore, the LPS-induced IL-18 expression was a direct, rather than an indirect, effect, as LPS also stimulated the expression of IL-18 mRNA and protein in cultured MCs (Fig. 3). Our *in vitro* experiment is the first to directly demonstrate that IL-18 is produced in untreated MCs and that this expression is enhanced by LPS. In MCs, LPS might directly control gene expression of IL-18 as the promoter activity...
upstream of exon 1 in IL-18 gene is regulated by LPS through MyD88-dependent signalling pathway [32–34]. However, our data did not exclude a possibility that other cytokines can mediate the LPS-induced IL-18 up-regulation, which needs further systematical investigations. Besides, the data suggest that an experiment on cultured MCs for IL-18 expression could be confounded by the LPS effect due to LPS impurity in experimental reagents.

The locally produced IL-18 might act in a paracrine and/or an autocrine manner to influence LN progression (Fig. 6). Paracrine roles of IL-18 can be deduced from previous reports, i.e. IL-18 secreted by MCs can boost local inflammation by acting on nearby infiltrating inflammatory cells [30, 31] or can penetrate the capillary to exert an apoptotic paracrine effect on downstream tubular cells [19]. However, whether IL-18 has an autocrine effect on MCs is not known. Microscopically, IL-18 overexpression, hyperplasia and apoptosis of MCs were seen to co-exist in the glomeruli of LPS-accelerated LN mice (Fig. 2A). Excessive apoptosis of MCs can distort the glomerular structure and lead to renal dysfunction, whereas moderate apoptosis can ameliorate MC hyperplasia and maintain the normal glomerular structure [21–23]. Clarifying whether LPS or IL-18 can directly induce MC apoptosis could provide an insight into the pathogenesis of LPS-accelerated LN.

Using primary cultures of MCs from LN-prone mice, the direct effects of LPS and IL-18 on apoptosis of the cells could be precisely evaluated in the absence of the inflammatory cells (Fig. 2A) and auto-antibodies (Fig. 1F) seen in the glomeruli of the LPS-induced accelerated-LN mouse model. To determine the role of LPS in the induction of apoptosis, various concentrations of LPS were used to treat MCs, including the dose of 50 μg/ml, which was 5 times the dose required to induce a significant increase in IL-18 mRNA and protein in MCs (Fig. 3C and 3D) and is significantly higher than the concentrations required to induce apoptosis in other cells [14–16]. In addition, apoptotic effects of IL-18 on MCs were evaluated by treating the cells with various concentrations (0–400 ng/ml) of IL-18, the highest concentration used being 4 or 40 times higher than that used previously to induce apoptosis of cardiac endothelial cells or tubular cells, respectively [18, 19]. Our in vitro experiments showed that LPS or IL-18 was unable to induce MC apoptosis (Figs 3B and 4) and ruled out the possibility that LPS or IL-18 directly cause the apoptosis of MCs observed in the LPS-treated mice (Fig. 2). These results strongly suggest that, in LPS-exacerbated LN, the enhanced MC apoptosis is unlikely to be caused by a direct effect of circulating LPS and locally produced IL-18, but by other mechanisms [28, 35–38].

A common pathway for signal transduction of LPS and IL-18 after receptor binding is recruitment and activation of MyD88/IRAK, degradation of 1kB, and, finally, induction of NFκB nuclear translocation [32]. Our data showed that LPS induced marked NFκB activation in MCs, whereas IL-18 did not (Fig. 5), explaining the LPS-enhanced IL-18 expression (Figs 2 and 3) and inability of IL-18 to induce MC apoptosis (Figs 4A and B). To completely exclude a role of IL-18 in MC apoptosis, our in vitro data showed that IL-18 was unable to activate the extrinsic (procaspase 8), intrinsic (Bel-2 and Bax) and common (procaspase 3 and PARP) pathways of apoptosis in MCs (Fig. 4C and D), despite the in vivo co-existence of IL-18 overexpression and procaspase activation (Fig. 2B and C).

Taken together, our results demonstrate that LPS enhanced IL-18 expression in MCs both in vivo and in vitro, but neither LPS nor IL-18 directly induced apoptosis in MCs isolated from LN-prone mice. We suggest that the MC-produced IL-18 could act like a paracrine cytokine to exacerbate glomerular inflammation and is probably involved in the apoptosis of the downstream tubular cells, but cannot act as an autocrine cytokine to influence MC survival. We infer that LPS-induced IL-18 up-regulation in MCs might be a mechanism, operating locally in the kidney, responsible for LPS-accelerated LN.

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