IL-15, a survival factor for kidney epithelial cells, counteracts apoptosis and inflammation during nephritis

Michiya Shinozaki,1 Junichi Hirahashi,1 Tatiana Lebedeva,1 Foo Y. Liew,2 David J. Salant,3 Ruth Maron,4 and Vicki Rubin Kelley1

1Laboratory of Molecular Autoimmune Disease, Renal Division, Brigham and Women’s Hospital, Boston, Massachusetts, USA
2Department of Immunology and Bacteriology, University of Glasgow, Glasgow, United Kingdom
3Renal Section, Evans Biomedical Research Center, Boston University Medical Center, Boston, Massachusetts, USA
4Center for Neurological Disease, Brigham and Women’s Hospital, Boston, Massachusetts, USA

Received for publication November 6, 2001, and accepted in revised form March 5, 2002.

IL-15, a T cell growth factor, has been linked to exacerbating autoimmune diseases and allograft rejection. To test the hypothesis that IL-15–deficient (IL-15–/–) mice would be protected from T cell–dependent nephritis, we induced nephrotoxic serum nephritis (NSN) in IL-15–/– and wild-type (IL-15+/+) C57BL/6 mice. Contrary to our expectations, IL-15 protects the kidney during this T cell–dependent immunologic insult. Tubular, interstitial, and glomerular pathology and renal function are worse in IL-15–/– mice during NSN. We detected a substantial increase in tubular apoptosis in IL-15–/– kidneys. Moreover, macrophages and CD4 T cells are more abundant in the interstitium and glomeruli in IL-15–/– mice. This led us to identify several mechanisms responsible for heighten renal injury in the absence of IL-15. We now report that IL-15 and the IL-15 receptor (α, β, γ chains) are constitutively expressed in normal tubular epithelial cells (TECs). IL-15 is an autocrine survival factor for TECs. TEC apoptosis induced with anti-Fas or actinomycin D is substantially reduced renal injury in the absence of IL-15. We now report that IL-15 and the IL-15 receptor (α, β, γ chains) are constitutively expressed in normal tubular epithelial cells (TECs). IL-15 is an autocrine survival factor for TECs. TEC apoptosis induced with anti-Fas or actinomycin D is substantially greater in IL-15–/– than in wild-type TECs. Moreover, IL-15 decreases the induction of a nephrotoxic chemokine, MCP-1, that attracts leukocytes into the kidney during NSN. Taken together, we suggest that IL-15 is a therapeutic for tubulointerstitial and glomerular kidney diseases.


Introduction

In most kidney diseases, leukocytes in the kidney mediate tissue injury. The primary site of infiltration is the renal interstitium. The interaction of leukocytes with resident kidney cells stimulates these parenchymal cells to generate cytokines and incite an amplification cascade resulting in inflammation. Tubular epithelial cells (TECs), which comprise the vast majority of parenchymal cells in the kidney, are a rich source of cytokines and chemokines. The dynamics between the kidney-infiltrating leukocytes and the TECs determine the extent of interstitial inflammation. Activated TECs generate numerous cytokines and chemokines that promote renal injury. IFN-γ produced by T cells is the most potent TEC activator. Since IL-15 is a T cell growth factor (1) that is expressed in IFN-γ–activated human kidney TECs (2), we concluded that IL-15 is a pivotal cytokine in kidney inflammation.

The biologic functions of IL-15 are complex. Although the receptors for IL-15 and IL-2 share two subunits (the IL-2 β and common γ chains), the functions of these molecules during T cell–dependent immune responses are distinct and contrasting (3). Specificity for IL-15 is conferred by the α chain of the IL-15 receptor (IL-15R) (4). While IL-15 and IL-2 have somewhat redundant properties in stimulating T cell proliferation, IL-15, unlike IL-2, does not promote T cell apoptosis. Indeed, IL-15 protects T cells from IL-2–triggered apoptosis (5). Moreover, IL-15 is of particular importance as a growth factor for memory CD8 T cells (6). In fact, mice genetically deficient in IL-15 have a reduction in memory CD8 T cells and NK cells (3).

Of particular importance, IL-15 and IL-2 are not expressed in the same cells. In contrast to the expression of IL-2 in activated T cells, IL-15 is constitutively expressed by a broad array of cell types. Notably, epithelial cells are a principal source of IL-15. In keeping with this data, amplified IL-15 expression at the site of autoimmune disease has been linked to exacerbation of rheumatoid arthritis (7) and inflammatory bowel disease (8). Furthermore, enhanced IL-15 expression is detected in kidneys during human allograft rejection (9). Interestingly, IL-15 blockade with a neutralizing soluble IL-15R or a receptor site antagonist have proven effective in prolonging heart or islet allograft survival (10) and suppressing collagen-induced arthritis (11) and delayed-type hypersensitive reactions (12). Based on these findings, we deduced that preventing signaling via IL-15Rα might be a therapeutic strategy for countering T cell–dependent immune reactions, particularly those involving epithelial cells.
Since T cells mediate immunologic kidney diseases, we hypothesized that IL-15, a potent T cell growth factor produced by kidney TECs, would foster the expansion of activated T cells within the kidney and thus promote T cell–mediated renal injury. To test this hypothesis, we evaluated the role of IL-15 in IL-15–/– mice during accelerated nephrotoxic serum nephritis (NSN), a T cell–dependent model of kidney disease (13). Contrary to our hypothesis, renal damage is more severe and macrophages and CD4 T cells are more abundant in IL-15–/– mice than in IL-15+/+ mice. Although IL-15 is largely expressed by TECs in nephritic kidneys, it is far more abundant in normal kidneys. Why does a failure to express IL-15 lead to heightened renal injury? We now report that IL-15 functions in an autocrine manner as a survival factor for renal TECs. IL-15 serves to protect the kidney during certain immunologic assaults.

Methods

Mice. C57BL/6 mice genetically deficient in IL-15 (IL-15–/– mice) were generated by gene targeting and generously provided by J.J. Peschon (Immunex Corp., Seattle, Washington, USA) (3). Age- and sex-matched C57BL/6 mice, referred to as IL-15+/+ wild-type mice (The Jackson Laboratory, Bar Harbor, Maine, USA), were used as controls. The mice were bred and maintained in a pathogen-free animal facility at Brigham and Women’s Hospital.

NSN. Nephrotoxic serum was prepared as described previously (14). Nephrotoxic serum and nonimmune sheep control serum (Sigma Chemical Co., St. Louis, Missouri, USA) were filter-sterilized. To induce NSN, IL-15–/– and IL-15+/+ mice (8–12 weeks of age) were primed subcutaneously in each flank with 1 mg sheep IgG in Freund’s complete adjuvant (Sigma Chemical Co.). Beginning 5 days later, the mice were challenged with intravenous injections of either 0.1 ml of nephrototoxic serum or control serum for 3 consecutive days. Groups of mice were sacrificed at 6, 11, and 14 days after the initial challenge injection.

Blood urea nitrogen and proteinuria. Blood urea nitrogen (BUN) was measured in the serum prior to the initial challenge and on days 11 and 14 after the initial challenge with nephrotoxic serum, using the urea nitrogen kit from Sigma Chemical Co. Urine protein levels were assessed semiquantitatively using Albustix dipsticks (Bayer Diagnostics, Elkhart, Indiana, USA) on days 0, 5, and 13 after the initial challenge with nephrotoxic serum or control serum. Urinary protein levels were confirmed by analyzing twice on each day.

Kidney pathology. After removing the kidneys, half of one kidney was bisected, fixed in 10% neutral buffered formalin, and embedded in paraffin. The other half was snap-frozen for immunostaining. The other kidney was used to prepare RNA. Paraffin sections were stained with periodic acid–Schiff reagent for routine histology. Pathology was evaluated using coded slides as previously described (15). We assessed glomerular pathology by evaluating more than 100 glomerular cross sections per kidney. The percentage of glomeruli with crescents (defined as two or more cell layers within Bowman’s space) and segmental lesions (exhibiting at least one of the following: necrosis, proliferation, or hyalinosis) were enumerated. The percentage of damaged tubules (exhibiting at least one of the following: dilation, atrophy, or necrosis) was determined by scoring 400 renal cortical tubules per kidney in randomly selected microscopic fields (×400).

Antibodies. The following primary antibodies were used for immunostaining: rat anti–mouse CD4 IgG2a clone RM4.5 (PharMingen, San Diego, California, USA), to detect CD4 T cells; rat anti–mouse CD8a (Ly-2) IgG2a clone S3-6.7 (PharMingen), to detect CD8 T cells; and rat anti–mouse macrophage IgG2b (prepared from F4/80 hybridoma supernatant HB198; American Type Culture Collection, Rockville, Maryland, USA), to detect macrophages. Rat anti–mouse IL-15 IgG2a clone M49 (Immunex Corp.) was used to detect IL-15 in the kidney. The negative isotype control antibodies were rat IgG2a clone R35-96 and rat IgG2b clone R35-38 (PharMingen).

IL-15 immunostaining. To detect IL-15, formalin-fixed tissue sections were deparaffinized and treated in the microwave in 0.01 M sodium citrate buffer. Sections were incubated with 5% nonfat milk for 30 minutes. Endogenous peroxidase was blocked with 0.6% H2O2. After digestion (with 1 mg/ml bovine testicular hyaluronidase in acetate buffer, pH 5.2, for 1 hour), sections were consecutively treated with avidin and biotin solutions. The sections were incubated with primary anti–mouse IL-15 antibody (1:1,000), then with biotin-conjugated rabbit anti–rat IgG, and subsequently with biotin-conjugated goat anti–rabbit IgG. Positive signals were detected with an avidin-biotin-peroxidase system (Vector Laboratories Inc., Burlingame, California, USA). To examine the specificity of staining, primary antibody was incubated with a 100 M excess of recombinant mouse IL-15 (Research Diagnostics Inc., Flanders, New Jersey, USA). After centrifugation, the supernatant (which no longer contained IL-15 antibody) was used as a negative control.

Identifying kidney-infiltrating leukocytes by immunostaining. Kidney-infiltrating T cells (CD4 and CD8) and macrophages were detected as previously described (15). Intraglomerular infiltrating cells were counted in 20 glomeruli per section. Interstitial cell infiltrates were evaluated by counting the number of leukocytes in the interstitium in ten randomly selected microscopic fields (100 µm²) per kidney.

Ig in kidneys, and serum anti–sheep Ig isotypes. Cryosections of IL-15+/+ and IL-15–/– kidneys (4 µm) were transferred to Superfrost Plus slides (Fisher Scientific Co., Pittsburgh, Pennsylvania, USA), washed with cold PBS, blocked with 1% BSA in PBS, and stained at 1:800 with
The sections were examined by epifluorescent microscopy using a Nikon 40× Plan APO oil-immersion lens. The images were captured with a Spot CCD camera (Diagnostic Instruments Inc., Sterling Heights, Michigan, USA) and exported into Adobe Photoshop. Exposed settings were kept constant. Fluorescence intensity was measured by outlining the perimeter of five glomeruli per section and reading the luminosity from the Histogram command in the pull-down Image menu in Adobe Photoshop. Calibration of the CCD exposure time assured that the settings were in the linear range and well below saturation.

Serum anti–sheep Ig isotypes were measured as previously described (14).

Apoptotic cells in kidneys. Apoptotic cells in the kidneys were identified by the TUNEL method using a TdT-FragEL DNA Fragmentation Detection Kit (Oncogene Research Products, Boston, Massachusetts, USA) according to the manufacturer’s protocol. The apoptotic TECs were counted in 25 randomly selected renal cortical areas (100 μm² per kidney).

Cell death in cultured TECs. TECs were derived from the kidneys of mice without induced NSN IL-15+/+ and IL-15−/− mice and grown on 96-well flat-bottom tissue culture plates (Corning-Costar Corp., Cambridge, Massachusetts, USA) at 1.0 × 10⁴ cells/well as previously reported (16). To induce apoptosis, TECs were incubated in DMEM/0.5% FCS with or without 0.05 μg/ml actinomycin D (AD) (Sigma Chemical Co.) for 24 hours. Alternatively, TECs were incubated in DMEM/0.5% FCS containing 5 μg/ml LPS and 100 U/ml IFN-γ for 24 hours and then incubated in DMEM/0.5% FCS containing 5 μg/ml LPS, 100 U/ml IFN-γ, 1 μg/ml protein G (Pierce Chemical Co., Rockford, Illinois, USA), and 1 μg/ml anti-Fas antibody (clone Jo2; PharMingen) for an additional 24 hours. TECs incubated in DMEM/0.5% FCS containing 2.5 μg/ml LPS and 100 U/ml IFN-γ served as control. The TECs were stained with 0.1% crystal violet for 10 minutes. Nonadherent cells were removed by washing twice with ice-cold PBS, and the extinction of tubular damage (measured on day 11 after induction (inset; arrow indicates damaged tubule)). Tubular pathology and apoptosis are markedly increased in kidneys during NSN. Tubular damage is increased in IL-15+/+ mice were grown on 24-well tissue culture plates (Becton Dickinson and Co., Franklin Lakes, New Jersey, USA) at 10⁴ cells/well. After stimulation, floating and adherent cells were collected and fixed to glass slides using a Cytospin cytocentrifuge (Shandon Inc., Pittsburgh, Pennsylvania, USA). These TECs were fixed with 4% paraformaldehyde, and apoptotic cells were identified by TUNEL staining using a TdT-FragEL DNA Fragmentation Detection Kit (Oncogene Research Products) according to the manufacturer’s protocol.

RT-PCR analysis of IL-15 and IL-15Rs. Total RNA from the renal cortex, or primary cultured cells were prepared for RT-PCR (18). PCR for IL-15, IL-15Rα chain, IL-2Rβ chain, and the common γ chain was conducted with 1 μl of cDNA in a total volume of 50 μl containing 20 pmol of each primer, 10 mM Tris-HCl at pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM dNTPs, and 2.5 U Taq polymerase (Promega Corp., Madison, Wisconsin, USA). Primer sequences were as follows. IL-15: sense, 5′-GCAGAGGTGGACGACGAC-3′; antisense, 5′-AGCACGGATGGATGATT-3′. IL-15Rα chain: sense, 5′-TCTCCCCACAGTCTACAAAAT-3′; antisense, 5′-GGCCACCCAGGCTCAGTAAAA-3′. IL-2Rβ chain: sense, 5′-GTTGGCGTAGGGTAAAGAC-3′; antisense, 5′-AGCGGAGACGGCGAGAGAC-3′; common γ chain: sense, 5′-CTCCTACTGCCCCCTTCCA-3′; antisense, 5′-TCCATTACTCCACTGTGA-3′.

Apoptosis in cultured TECs. Apoptosis in TECs after exposure to apoptotic stimuli (AD or anti-Fas antibody) was assessed using the TUNEL method. TECs derived from IL-15+/+ and IL-15−/− mice were grown on 24-well tissue culture plates (Becton Dickinson and Co., Franklin Lakes, New Jersey, USA) at 10⁴ cells/well. After stimulation, floating and adherent cells were collected and fixed to glass slides using a Cytospin cytocentrifuge (Shandon Inc., Pittsburgh, Pennsylvania, USA). These TECs were fixed with 4% paraformaldehyde, and apoptotic cells were identified by TUNEL staining using a TdT-FragEL DNA Fragmentation Detection Kit (Oncogene Research Products) according to the manufacturer’s protocol. The animals were killed at the indicated times (days 6, 11, and 14), and kidneys were prepared for histology and TUNEL assay. The images were captured with a Spot CCD camera (Diagnostic Instruments Inc.) at 105 cells/well. *P < 0.05. Magnification: a and b, ×100; insets, ×400.
Quantitating intrarenal cytokine mRNA using real-time PCR. MCP-1, RANTES, CSF-1, IFN-γ, or IL-12 (p40) in the renal cortex were analyzed using real-time two-step quantitative RT-PCR. Relative quantitation was performed with SYBR Green PCR reagents (QIAGEN Inc., Valencia, California, USA) and an ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems, Foster City, California, USA) according to both manufacturers’ instructions. Reactions were performed using 100 ng of cDNA. The PCR consisted of HotStarTaq activation for 15 minutes at 95°C, followed by 40 cycles with heating to 95°C for 15 seconds and cooling to 60°C for 1 minute. The mRNA levels were normalized to GAPDH. Sequence-specific PCR primers were purchased from Invitrogen Corp. (Carlsbad, California, USA). The PCR primers used were as follows. GAPDH: sense, 5′-CATGGCCCTCCAGAGGTAGA-3′; antisense, 5′-CCTAGGCCCCCTCTGTATATT-3′. MCP-1: sense, 5′-ACCAGAAGATGATCCCAAT-3′; antisense, 5′-TGCTGGACCCATTTCTTTCT-3′. RANTES: sense, 5′-TGCCAAACCCAGAAGAAGTG3′; antisense, 5′-AAGCTGGCTAGGACTAGACA-3′. CSF-1: sense, 5′-ACCTGGCAGGGACTCACA-3′; antisense, 5′-CAGGCTCTCTCTGCGG-3′. IFN-γ: sense, 5′-AGCTCTTCTCATGGCTGTT-3′; antisense, 5′-TTTGGCCAGTCTCCAGATT-3′. IL-12 (p40): sense, 5′-GACACAAACCAGCACATTGAA-3′; antisense, 5′-CTACCAAGGCACAGGGTCAT-3′.

Results
Renal tubular pathology is markedly increased, while glomerular pathology is modestly worse and BUN is elevated in IL-15−/− mice during NSN. To investigate the importance of IL-15 in kidneys undergoing immune injury, we induced NSN in IL-15−/− and IL-15+/+ mice. Unexpectedly, we detected a striking increase in renal pathology in the IL-15−/− mice compared with IL-15+/+ mice; this was most notable in the tubules on day 11 (Figure 1, a–c). Tubular damage (which included one or more of the following: dilation with flattened or degraded epithelium, atrophy, thickening, and rupture of basement membranes) was increased in IL-15−/− mice on days 11 and 14 (Figure 1, a and c) compared with IL-15+/+ mice (Figure 1, a and c). In addition, peritubular leukocytic infiltrates and casts in tubule lumens were associated with damaged tubules (Figure 1, a and b). The change in glomerular pathology was subtler. We noted an increase in cell number of 30–40% within glomeruli in IL-15−/− mice compared with glomeruli in wild-type mice at 6, 11, and 14 days (Figure 2). On the other hand, we did not detect a difference in the number of glomerular crescents.

Accompanying the increased renal pathology in the IL-15−/− mice was a greater loss of renal function. BUN levels measured on days 11 and 14 after initiation of NSN were elevated in the IL-15−/− mice compared with wild-type mice (Figure 3). Urine protein levels in IL-15−/− and IL-15+/+ mice were increased to a similar degree on days 5 and 13 after NSN induction (data not shown). However, urinary protein is not a suitable functional marker of tubulointerstitial injury in this model, because nephrotoxic serum reacts with several podocyte surface antigens and causes proteinuria by direct podocyte injury in the absence of an inflammatory response (19).

Apoptotic cells are increased in IL-15−/− mice during NSN. The increase in tubular injury in IL-15−/− compared with IL-15+/+ mice during NSN indicated that IL-15 might be an epithelial cell survival factor. To address this issue, we
enumerated the number of apoptotic cells in the kidney. Apoptotic cells in the kidney were almost exclusively within cortical tubules; IL-15–/– kidneys had twice as many apoptotic cells as did IL-15+/+ kidneys (Figure 1d).

IL-15 expression in the kidney is decreased after the induction of NSN. The increased renal pathology in IL-15–/– mice prompted us to probe for the intrarenal expression of IL-15 in wild-type mice with NSN. As opposed to most cytokines and chemokines, which are not constitutively expressed, we detected an abundant expression of IL-15 in normal TECs that decreases during NSN, and the enhanced TEC pathology and apoptosis in IL-15–/– kidneys during NSN, we hypothesized that IL-15 is a survival factor for epithelial cells.

IL-15 protects TECs from AD-induced death and apoptosis. To test the hypothesis that IL-15 is a survival factor for TECs, we determined whether AD-induced cell death was enhanced in IL-15–/– TECs. We incubated primary cultured TECs derived from IL-15+/+ and IL-15–/– mice with AD (0.05 µg/ml and 0.1 µg/ml) for 24 hours. IL-15–/– TECs were more vulnerable to AD-induced cell death than IL-15+/+ TECs were (Figure 6a). We incubated the IL-15+/+ TECs with soluble IL-15Rα (sIL-15Rα; 200 ng/ml), a molecule that competitively blocks the binding of IL-15 to IL-15Rα, to determine whether enhanced TEC survival was specific to IL-15. The soluble mutant IL-15R, smIL-15Rα (200 ng/ml), which does not block the binding of IL-15 to IL-15Rα, served as a control. The sIL-15Rα increased cell death in IL-15+/+ TECs, while the smIL-15Rα did not alter IL-15+/+ TEC survival (Figure 6b).

To determine whether IL-15 is anti-apoptotic, we exposed IL-15–/– and IL-15+/+ TECs to AD as above and evaluated apoptosis by the TUNEL assay. IL-15–/– TECs had an increase (56%) in apoptosis compared with IL-15+/+ TECs exposed to AD with smIL-15Rα, the control molecule (Figure 6c). Blockade of IL-15R on IL-15+/+ TECs resulted in an amount of AD-induced apoptosis that was similar to that in IL-15–/– TECs (Figure 6c). Furthermore, adding rIL-15 (50 ng/ml and 200 ng/ml) dramatically reduced AD-induced apoptosis of IL-15–/– TECs. Taken together, these results indicate that IL-15 protects TECs from AD-induced apoptosis.

Figure 4
IL-15 transcription is downregulated in the kidneys of mice with NSN. There is a decrease in IL-15 transcript in the kidneys of IL-15–/– mice injected with nephrotoxic serum (measured on day 14 after induction of NSN) compared with transcript in kidneys of uninjectected IL-15+/+ mice, as measured by RT-PCR. Data were analyzed by Bonferroni-Dunn test and are presented as mean ± SEM. n = 3 per group. *P < 0.05.

Figure 5
IL-15 protein is decreased in tubules and glomeruli during NSN in wild-type mice. IL-15 was strongly expressed in the tubules (arrows) and glomeruli (arrowheads) in normal kidneys (a and b). IL-15 expression was markedly decreased within tubules and glomeruli during NSN (day 14) (c and d). Magnifications: a and c, ×100; b and d, ×400.
IL-15 protects TECs from Fas-induced cell death and apoptosis. To eliminate the possibility that IL-15 is a survival factor unique to AD-induced TEC death, we explored the importance of IL-15 in Fas-mediated TEC death. We primed TECs with LPS and IFN-γ to induce Fas expression on TECs, and then incubated the TECs with anti-Fas antibody and protein G to initiate Fas-induced cell death. Fas-induced TEC death was more pronounced (twofold) in IL-15−/− TECs than in IL-15+/+ TECs and was diminished by the addition of rIL-15 (Figure 7a). As in AD-induced cell death, sIL-15Rα (200 ng/ml), but not smIL-15Rα, increased death of IL-15+/+ TECs to the level of IL-15−/− TEC death (Figure 7b). In contrast, CD8 T cells increased in IL-15−/− kidneys but not in IL-15+/+ kidneys compared with pre-NSN levels (measured on day 6, day 11, and day 14; Figure 9c).

Glomerular leukocytic infiltrates are increased in IL-15−/− kidneys. To determine whether leukocytes were responsible for increasing the numbers of cells in glomeruli, we stained for the presence of macrophages, CD4 T cells, and CD8 T cells. Initially, we detected more macrophages (day 6) and CD4 T cells (days 6 and 11) in IL-15−/− glomeruli than in IL-15+/+ glomeruli (Figure 10, a and b). In contrast, as in the renal interstitium, the IL-15−/− kidneys during NSN, we detected an initial (on day 6 and day 11) enhanced increase in macrophages, and a more enduring increase in CD4 T cells measured on days 6, 11, and 14 in the interstitium versus wild-type kidneys (Figure 9, a and b). In contrast, CD8 T cells increased in IL-15−/− kidneys but not in IL-15+/+ kidneys compared with pre-NSN levels (measured on day 6, day 11, and day 14; Figure 9c).

Renal interstitial leukocytic infiltrates are more prominent in IL-15−/− mice during NSN. Leukocytic infiltrates in the renal interstitium are prominent in NSN (see Figure 9). In the IL-15−/− kidneys during NSN, we detected an initial (on day 6 and day 11) enhanced increase in macrophages, and a more enduring increase in CD4 T cells measured on days 6, 11, and 14 in the interstitium versus wild-type kidneys (Figure 9, a and b). In contrast, CD8 T cells increased in IL-15−/− kidneys but not in IL-15+/+ kidneys compared with pre-NSN levels (measured on day 6, day 11, and day 14; Figure 9c).

IL-15 protects TECs from Fas-induced cell death and apoptosis. To eliminate the possibility that IL-15 is a survival factor unique to AD-induced TEC death, we explored the importance of IL-15 in Fas-mediated TEC death. We primed TECs with LPS and IFN-γ to induce Fas expression on TECs, and then incubated the TECs with anti-Fas antibody and protein G to initiate Fas-induced cell death. Fas-induced TEC death was more pronounced (twofold) in IL-15−/− TECs than in IL-15+/+ TECs and was diminished by the addition of rIL-15 (Figure 7a). As in AD-induced cell death, sIL-15Rα (200 ng/ml), but not smIL-15Rα, increased death of IL-15+/+ TECs to the level of IL-15−/− TEC death (Figure 7b). In addition, we determined that IL-15−/− TECs had an increase in Fas-induced apoptosis that was more than IL-15+/+ TECs (Figure 7c). Moreover, adding rIL-15 (200 ng/ml) reduced TEC apoptosis in IL-15−/− TECs to the level detected in IL-15+/+ TECs (Figure 7c). Thus, IL-15 is a survival factor for Fas- and AD-induced TEC apoptosis.

IL-15 and IL-15R are expressed by normal TECs. To determine whether IL-15 could act as an autocrine molecule, we probed kidneys and primary TECs from normal C57BL/6 mice for IL-15 and IL-15R expression. IL-15 transcripts were detected in the normal kidneys and TECs using RT-PCR (Figure 8a). The transcripts of the IL-15R, comprised of the IL-15Rα, IL-2Rβ, and common γ chains, were identified in TECs (Figure 8b). Therefore, normal TECs express both IL-15 and all three chains of IL-15R.
numbers of CD8 T cells in IL-15−/− glomeruli remained normal, while there was an increase in CD8 T cells in IL-15+/+ glomeruli (days 6, 11, and 14; Figure 10c). Taken together, this data indicates that macrophages and CD4 T cells are responsible, in part, for the increase in glomerular cells in IL-15−/− during NSN.

IL-15 inhibits intrarenal MCP-1 expression during NSN. We have previously reported that MCP-1, CSF-1, and RANTES recruit macrophages and T cells into the kidney (15, 18, 20, 21). In addition, we established that IFN-γ and IL-12 are nephritogenic (16, 22). During NSN, MCP-1, CSF-1 and RANTES transcripts were increased in the renal cortex in IL-15−/− and IL-15+/+ mice compared with normal mice (day 11; Figure 11). However, only MCP-1 was greater in the IL-15−/− kidneys compared with the IL-15+/+ kidneys. Thus, IL-15 inhibits intrarenal MCP-1, and in turn may be responsible for dampening the influx of macrophages and T cells during NSN.

IL-15−/− mice do not have an increase in circulating anti–sheep Ig isotypes, or IgG in the kidney during NSN. We evaluated serum Ig isotypes in IL-15−/− and wild-type mice during NSN on day 11 and day 14. Although there is a consistent trend to lower serum isotype titres on day 14 in IL-15−/− mice, these values were not statistically significant. The OD450 readings were: IgG, 1.0 ± 0.1 and 0.8 ± 0.1; IgG1, 1.1 ± 0.1 and 0.7 ± 0.1; IgG2a, 0.4 ± 0.1 and 0.2 ± 0.1, for IL-15−/− (n = 5) and wild type (n = 6), respectively. The serum isotype findings in IL-15−/− and wild-type mice at day 11 were similar to those of day 14 with the exception that total IgG levels were statistically decreased in the IL-15−/− mice (P < 0.03). Taken together, this data suggests that the increased renal disease in IL-15−/− mice was not a result of an increase in serum Ig’s or a result of immune deviation toward a Th1 phenotype. In fact, the tendency for the serum Ig isotypes to be decreased in IL-15−/− mice would suggest that renal disease should have been less, and not greater, in IL-15−/− mice during NSN.

To establish whether the deposition of IgG differed between IL-15−/− and wild-type mice, we quantitatively evaluated the deposition of host (mouse) IgG in the IL-15−/− kidneys on day 14 after the induction of NSN. The results revealed similar amounts of IgG in the IL-15−/− and wild-type kidneys (12.09 ± 1.18 and 11.39 ± 0.74 luminosity units, respectively; n = 5 per group; P < 0.6). Mouse IgG is located in the capillary walls of the glomeruli, and the distribution is similar in both groups. This indicates that the total amount of mouse anti–sheep IgG deposited in the glomeruli is not different in the two groups, and that the amount of antibody deposited in the kidney in IL-15−/− mice was not influenced by the lack of IL-15.

Discussion

We now report that IL-15 counteracts renal injury in a T cell–dependent disease akin to human kidney illnesses. This is the first report that: (a) IL-15 is a survival factor for kidney epithelial cells; (b) IL-15 is protective in a T cell–dependent disease; (c) IL-15 inhibits the induction of MCP-1 in vivo; and (d) IL-15 protects the kidney during nephritis. In addition, this data is particularly compelling since it was generated using a strain genetically deficient in IL-15.

Interstitial inflammation is a major determinant of the outcome of human renal disease (23, 24). Most
Cytokines are not expressed by normal kidneys, but are induced during inflammation (18, 25) and are largely expressed in TECs (15, 18, 26, 27). The interplay between the kidney-infiltrating leukocytes and the TECs determines the extent of interstitial inflammation (28, 29). An amplification cascade drives kidney disease: interstitial T cells release IFN-γ which activates adjacent TECs (15, 18, 30, 31), which in turn release chemokines (MCP-1) and cytokines (CSF-1) that are responsible for recruiting macrophages and T cells (32). Subsequently, upon activation, macrophages release molecules that induce apoptosis in TECs (15). Since TEC apoptosis correlates with tubulointerstitial fibrosis, protecting the TECs from injury is critical in combating kidney disease (33).

We now report that intrarenal IL-15 inhibits TEC apoptosis. Typically, cytokines are not constitutively expressed in TECs, but are induced during inflammation (18, 25) and are largely expressed in TECs (15, 18, 26, 27). The interplay between the kidney-infiltrating leukocytes and the TECs determines the extent of interstitial inflammation (28, 29). An amplification cascade drives kidney disease: interstitial T cells release IFN-γ which activates adjacent TECs (15, 18, 30, 31), which in turn release chemokines (MCP-1) and cytokines (CSF-1) that are responsible for recruiting macrophages and T cells (32). Subsequently, upon activation, macrophages release molecules that induce apoptosis in TECs (15). Since TEC apoptosis correlates with tubulointerstitial fibrosis, protecting the TECs from injury is critical in combating kidney disease (33).

We now report that intrarenal IL-15 inhibits TEC apoptosis. Typically, cytokines are not constitutively expressed in TECs, but rather are upregulated during renal disease (30, 31). IL-15 is atypical. It is constitutively and abundantly expressed in normal TECs and is reduced during renal injury, as shown in NSN and in spontaneous lupus nephritis in MRL-Fas<sup>−/−</sup> mice (data not shown). Thus, IL-15 in normal TECs is well positioned to guard against unwanted immunologic insults.

Initially, IL-15 was identified as a survival factor in T cells. It is now clear that IL-15 is a survival factor for a broader array of cell types. IL-15 promotes cell survival in hematopoietic cells, including T cells (34), B cells, mast cells (35), and neutrophils (36). The evidence that IL-15 is a survival factor for non-hematopoietic cells is far more limited. In vitro data supports the concept that IL-15 is anti-apoptotic in fibroblasts (37) and keratinocytes (38). Moreover, in vivo data is limited to suppression of apoptosis in hepatocytes using a long-acting IL-15 (IL-15–IgG<sub>b</sub> fusion protein) to inhibit anti-Fas-induced rapid hepatic failure (39). Thus, it is plausible that IL-15 is a general survival factor for epithelial cells.

The mechanism responsible for IL-15 counteracting TEC apoptosis is not known. We have established that TECs express all three chains of the IL-15R (α, β, and γ). In a murine fibroblast cell line (L929), the binding of IL-15 to IL-15R<sub>α</sub> competes with the TNFR1 complex for TRAF2 binding, impeding the assembly of key adapter proteins in the TNFR1 complex and in turn inducing IκBα phosphorylation (37). Thus, it is possible that IL-15R<sub>α</sub> signaling of TECs may induce similar anti-apoptotic mechanisms. In addition, members of the Bcl2 protein family have been implicated as regulators of TEC cell death during acute renal failure (40). Since IL-15 induces Bcl-x<sub>L</sub> expression and prevents apoptosis in a mouse mast cell line,
it is possible that IL-15 induces Bcl-xL in TECs (41). We are investigating the precise IL-15–mediated anti-apoptotic pathways in TECs.

Although there is a substantial increase in TEC apoptosis in IL-15−/− mice, many cells survive. Thus, IL-15 is not the only TEC survival factor. Osteopontin, a secreted phosphoprotein expressed by TECs, protects TECs from apoptosis (42). However, unlike IL-15, osteopontin is a macrophage chemoattractant, and therefore contributes to nephritis. We are probing for the other TEC survival factors that combat renal disease.

It is intriguing that despite the absence of the T cell growth factor IL-15, there is a marked increase in CD4 T cells and macrophages in the renal interstitium, and a subtler increase in these leukocytes in glomeruli during NSN. This suggests that the action of IL-15 as a survival factor for epithelial cells and an inhibitor of MCP-1 has a greater impact on the pathogenesis of renal disease than does IL-15’s role in promoting the expansion of T cells. Moreover, this data indicates that IL-15–deficient mice have a pronounced reduction in renal disease than does IL-15’s role in promoting the general Shwartzman reaction, and delayed-type hypersensitivity, we plan to determine whether blocking IL-15 during NSN enhances or protects against renal injury (10–12, 52).

There are several mechanisms that may be responsible for enhanced apoptosis in IL-15−/− TECs during NSN. MCP-1 plays a major pathogenic role in this model (15, 53). MCP-1 recruits macrophages into the interstitium, and activated macrophages induce TEC apoptosis (15). During NSN, MCP-1 is increased in IL-15−/− kidneys compared with IL-15+/+ kidneys. This finding is consistent with IL-15 downregulating MCP-1 in a colonic epithelial cell line (54). Therefore, it is possible that an IL-15 deficiency leads to exaggerated TEC apoptosis for two reasons: anti-apoptotic molecules are not induced, and MCP-1 expression (and in turn, the increased interstitial macrophage infiltration) is not inhibited.

In conclusion, we suggest that provision of IL-15 in some human kidney diseases may be therapeutic.

**Acknowledgments**

This work was supported in part by a National Kidney Foundation grant to J. Hirahashi, and by NIH grants to V.R. Kelley (DK-36149, DK-56848, and DK-52369) and D.J. Salant (DK-30932 and HL-63894). We wish to acknowledge Surya M. Nauli for his assistance in preparation of this manuscript.


