Inducible Co-Stimulatory Molecule Ligand Is Protective during the Induction and Effector Phases of Crescentic Glomerulonephritis

Dragana Odobasic, A. Richard Kitching, Timothy J. Semple, and Stephen R. Holdsworth
Centre for Inflammatory Diseases, Monash University, Department of Medicine, Monash Medical Centre, Clayton, Victoria, Australia

The inducible co-stimulatory molecule (ICOS)/ICOS ligand (ICOSL) co-stimulatory pathway is critical in T cell activation, differentiation, and effector function. Its role was investigated in a model of Th1-driven crescentic glomerulonephritis (GN). GN was induced by sensitizing mice to sheep globulin (day 0) and challenging them with sheep anti-mouse glomerular basement membrane antibody (Ab; day 10). Disease and immune responses were assessed on day 20. For testing the role of ICOSL in the induction of GN, control or anti-ICOSL mAb were administered from days 0 to 8. For examining the role of ICOSL in the effector phase of GN, treatment lasted from days 10 to 18. Blockade of ICOSL during the induction of GN increased glomerular accumulation of CD4+ T cells and macrophages and augmented renal injury. These results correlated with attenuated splenocyte production of protective Th2 cytokines IL-4 and IL-10 and decreased apoptosis of splenic CD4+ T cells. ICOSL was upregulated within glomeruli of mice with GN. Inhibition of ICOSL during the effector phase of GN enhanced glomerular T cell and macrophage accumulation and augmented disease, without affecting the systemic immune response (cytokine production, T cell apoptosis/proliferation, Ab levels). Increased presence of leukocytes in glomeruli of mice that received anti-ICOSL mAb was associated with enhanced cellular proliferation and upregulation of P-selectin and intercellular adhesion molecule-1 within glomeruli. These studies demonstrate that ICOSL is protective during the induction of GN by augmenting Th2 responses and CD4+ T cell apoptosis. They also show that ICOSL is upregulated in nephritic glomeruli, where it locally reduces accumulation of T cells and macrophages and attenuates renal injury.


Optimal activation of CD4+ T cells requires a co-stimulatory signal in addition to T cell receptor (TCR) engagement. Inducible co-stimulatory molecule (ICOS) and ICOS ligand (ICOSL) are a recently discovered receptor–ligand pair that belongs to the CD28/B7 family of co-stimulatory molecules (1–4). ICOS is induced on T cells after activation through the TCR and is present on effector and memory T cells (1,2). ICOSL mRNA is expressed constitutively in lymphoid and nonlymphoid tissues, including kidney, brain, and liver (3). The expression of ICOSL has been demonstrated on the surface of lymphoid cells, such as B cells and macrophages (2,3), and other cell types, such as endothelial and epithelial cells (5,6).

ICOS/ICOSL pathway plays an important role during the initiation of T cell–dependent immune responses. It has been demonstrated that ICOS ligation is critical for T cell activation and survival, as well as T cell–dependent humoral immunity (7–12). Although several experiments have provided evidence that ICOS co-stimulation is required for both Th1 and Th2 responses (13,14), the majority of studies have shown that ICOS engagement preferentially stimulates the production of Th2 cytokines IL-4 and IL-10 (9,15). Blockade of the ICOS/ICOSL pathway during the initiation of immune-mediated diseases has affected the outcome of both Th1- and Th2-mediated diseases. Inhibition of ICOS signaling during the induction of Th1-driven experimental autoimmune encephalomyelitis (EAE) exacerbated disease by enhancing IFN-γ production (16), whereas blockade of ICOSL during the initiation of collagen-induced arthritis (CIA) ameliorated injury by attenuating both Th1 and Th2 responses (13).

ICOS/ICOSL co-stimulation is particularly important in the efferent phase of immune-mediated diseases, when pathogenic T cells infiltrate target organs and mediate injury. ICOSL is upregulated at inflammatory sites in several models of immune-mediated diseases such as EAE, experimental autoimmune myocarditis, CIA, and lung inflammation, wherein it has been shown to affect local activation and effector function of Th1 and Th2 cells (13,16–19).

Glomerulonephritis (GN) is characterized by intraglomerular inflammation and is a major cause of end-stage renal failure. The most severe form of GN is crescentic nephritis. The best characterized and most widely used model of crescentic GN is nephrotoxic serum nephritis (NTN), wherein foreign anti–glo-
merular basement membrane (anti-GBM) globulin acts as a planted antigen in glomeruli. The development of crescentic GN in this model occurs in two phases. During the induction phase, animals are sensitized to a foreign Ig. The effector phase is initiated in the kidney by challenging sensitized animals with foreign anti-mouse GBM globulin. It is mediated by infiltrating CD4+ Th1 cells that direct recruitment of macrophages (20–22), attenuated by Th2 cytokines (23–25), and independent of CD8+ T cells (26) and autologous antibodies (Ab) (27). Similarly, the development of human crescentic GN is thought to be mediated by CD4+ Th1 cells and macrophages, which invariably are present in glomeruli of patients with the disease (28).

The role of the ICOS/ICOSL pathway has been investigated in several models of GN. In lupus nephritis, both early and late blockade of ICOSL ameliorated clinical signs of disease (29). Inhibition of ICOS attenuated the development of GN in Th2-driven and exacerbated disease in Th1-mediated graft-versus-host disease (GVHD) (30). In contrast, recent studies have shown that ICOSL that is expressed by renal tubular epithelial cells (TEC) negatively regulates T cell activation and cytokine production in the kidney (6,31). The role of the ICOS/ICOSL co-stimulatory pathway in crescentic GN has not been defined clearly. The purpose of these studies was to investigate the role of ICOSL during the induction and effector phases of Th1-mediated crescentic GN using a model of NTN.

Materials and Methods

Experimental Design

Animals. Eight- to 10-wk-old male C57BL/6 mice that were obtained from Monash University Animal Services (Melbourne, Australia) were used for experiments.

Induction of Crescentic GN. Mice were sensitized by a subcutaneous injection of 0.5 mg of normal sheep globulin in complete Freund’s adjuvant (day 0). Sheep anti-mouse GBM globulin was given intravenously at a dose of 14 mg/mouse (day 10). Unless otherwise stated, immune responses and renal injury were assessed on day 20.

Monoclonal Ab Treatment. Each mouse received 250 μg of protein G-purified inhibitory rat anti-mouse ICOSL (HK5.3; hybridoma provided by Prof. Hideo Yagita, Juntendo University School of Medicine, Tokyo, Japan) mAb, which has been characterized previously (13), or control rat IgG intraperitoneally. For testing the role of ICOSL in the induction phase of GN, mice received anti-ICOSL mAb (n = 6) or rat IgG (n = 6) on days 0, 2, 4, 6, and 8. To investigate the role of ICOSL in the effector phase of GN, mice received anti-ICOSL mAb (n = 7) or rat IgG (n = 7) on days 10, 12, 14, 16, and 18. Nonimmunized mice (n = 8) provided normal controls without GN.

Renal Injury

Renal formation was assessed on periodic acid-Schiff–stained, Bouin-fixed 3-μm-thick paraffin sections. Glomeruli were considered to be crescentic when two or more cell layers were observed in Bowman’s space. At least 50 glomeruli/animal were counted to determine the percentage of crescent formation. Proteinuria (mg/24 h) was measured by a modified Bradford method (21) on urine that was collected during the final 24 h of experiments.

Glomerular Macrophages and CD4+ T Cells

Macrophages and CD4+ T cells were demonstrated by immunoperoxidase staining of periodate-lysine paraformaldehyde–fixed frozen 6-μm-thick kidney sections, as described previously (22,32), using FA/11 (rat anti-mouse CD68; ATCC, Manassas, VA) for macrophages and GK1.5 (rat anti-mouse CD4; ATCC) for CD4+ T cells. A minimum of 20 glomeruli/animal were assessed, and results are expressed as cells per glomerular cross-section.

Circulating Ab Levels

Serum Ag-specific Ab levels were assessed by ELISA on serum that was collected at the end of experiments, as described previously (33), using horseradish peroxidase–sheep anti-mouse Ig (Amersham, Little Chalfont, UK). Results are expressed as the mean OD405 ± SEM. Sera from nonimmunized mice provided baseline Ab levels.

Splenocyte Production of IFN-γ, IL-4, and IL-10

Spleens were removed from mice, and single-cell suspensions were obtained. Splenocytes (4 × 10^6 cells/ml per well) were cultured in DMEM (10% FCS) in the presence of 10 μg/ml sheep globulin for 72 h. Concentrations of IFN-γ, IL-4, and IL-10 in splenocyte supernatants were measured by ELISA, as described previously (23), using rat anti-mouse IFN-γ (Pharmingen, San Diego, CA), biotinylated rat anti-mouse IFN-γ (Pharmingen), rat anti-mouse IL-4 (ATCC), biotinylated rat anti-mouse IL-4 (BVD6; DNAX, Palo Alto, CA), rat anti-mouse IL-10 (Pharmingen), and biotinylated rat anti-mouse IL-10 (Pharmingen).

Expression of CD44 on CD4+ T Cells

Expression of CD44 on CD4+ T cells was determined by flow cytometry. Spleens were obtained from mice, and single-cell suspensions were prepared. Splenocytes were labeled with allophycocyanin-conjugated anti-mouse CD44 (Pharmingen) and phycoerythrin-conjugated rat anti-mouse CD4 (Pharmingen). Results are expressed as percentage of CD4+ CD4 cells.

Apoptosis of CD4+ T Cells

Apoptosis of CD4+ cells was assayed by flow cytometric analysis of Annexin-V staining according to the manufacturer’s instructions (Roche Diagnostics, Penzberg, Germany). Briefly, splenocytes that were isolated from mice on days 5, 10, and 20 were stained with phycoerythrin-conjugated anti-mouse CD4 Ab (Pharmingen), Annexin-V-Fluos (Roche Diagnostics), and propidium iodide. Results are expressed as percentage of Annexin-V+ propidium iodide− CD4+ cells.

Proliferation of CD4+ T Cells

Mice received intraperitoneal injections of 1 mg of 5-bromo-2’-deoxyuridine (BrDU; Sigma, St. Louis, MO) in saline 48, 36, 24, and 12 h before the end of experiments. As a negative control, one mouse received an injection of saline only. Proliferation of CD4+ cells from isolated splenocytes was assessed by flow cytometric analysis of intracellular BrDU incorporation, as described previously (34). Results are expressed as percentage of BrDU+ CD4+ cells.

Renal Expression of P-Selectin and Intercellular Adhesion Molecule-1

P-selectin and intercellular adhesion molecule-1 (ICAM-1) were identified in 6-μm-thick frozen kidney sections by immunofluorescence, as described previously (35). Intraglomerular P-selectin and ICAM-1 expression was scored from 0 to 3 as follows: 0, background staining; 1, low positive staining; 2, moderate positive staining; and 3, very strong positive staining. A minimum of 20 glomeruli/animal were assessed to determine the average score.
Renal mRNA Expression of Chemokines and Chemokine Receptors

Renal mRNA expression of chemokines (regulated upon activation, normal T cell expressed and secreted [RANTES], macrophage inflammatory protein-1α [MIP-1α], interferon γ-inducible protein 10 [IP-10], monocyte chemoattractant protein-1, and TCA-3; template set mCK-5c) and chemokine receptors (CCR1, CCR2, CCR5, and CXCR3; template set mCR-5 including template for CXCR3) was measured by RNase protection assay as described previously (36) using Pharmingen’s RibobQuant system. The results (mRNA levels) are expressed as arbitrary units relative to the housekeeping gene L32.

Intraglomerular Proliferation

Proliferating cells were identified in 6-μm-thick frozen kidney sections by immunohistochemistry, as described previously (35), using rat anti-mouse Ki-67 mAb (DakoCytomation, Glostrup, Denmark). A minimum of 20 glomeruli/animal were examined to determine the number of Ki-67+ cells per glomerular cross-section.

Renal Expression of ICOSL

ICOSL was detected in 6-μm-thick frozen kidney sections by immunofluorescence. Sections were blocked with 10% normal sheep serum in 5% BSA/PBS for 30 min and then incubated with rat anti-mouse ICOSL mAb (HK5.3) overnight at 4°C. Isotype-matched rat IgG2a (Pharmin-gen) was used as a negative control. Sections were washed, incubated with FITC-conjugated sheep anti-rat IgG (DakoCytomation) for 1 h, and analyzed by fluorescence microscopy.

Statistical Analyses

Results are expressed as the mean ± SEM. Unpaired t test was used for statistical analysis (GraphPad Prism; GraphPad Software Inc., San Diego, CA). Differences were considered to be statistically significant at P < 0.05.

Results

Effect of ICOSL Blockade during the Initiation Phase of GN on Disease Outcome and Immune Responses

Renal Injury and Glomerular Accumulation of Effector Leukocytes. Mice that were treated with control rat IgG developed proliferative and crescentic GN (Figures 1A and 2A) with significant proteinuria (Figure 1B) and CD4+ T cells (Figure 1C) and macrophages (Figure 1D) present in their glomeruli. The development of GN was exacerbated significantly after ICOSL blockade during the induction phase of the disease. Crescent formation (Figures 1A and 2) and proteinuria (Figure 1B) were enhanced by inhibition of the ICOS/ICOSL pathway. Exacerbated renal injury was associated with increased accumulation of CD4+ T cells (Figure 1C) and macrophages (Figure 1D) in glomeruli of mice that were treated with anti-ICOSL Ab compared with controls.

Th1/Th2 Balance. Production of IL-4 (Figure 3A) and IL-10 (Figure 3B) by splenocytes that were cultured ex vivo in the presence of antigen was significantly reduced in mice that were treated with anti-ICOSL Ab compared with controls. In contrast, splenocyte IFN-γ (Figure 3C) production was not affected by ICOSL inhibition.

CD4+ T Cell Apoptosis, Proliferation, and Activation. Apoptosis of splenic CD4+ T cells that were collected at the end of the experiment (day 20) was significantly reduced by ICOSL blockade (Figure 4A) compared with controls. Similarly, there was a trend toward reduced apoptosis of splenic CD4+ cells on day 5 (3.9 ± 0.3 versus 3.3 ± 0.1%; P = 0.07) and day 10 (12.4 ± 0.8 versus 10.1 ± 1.1%; P = 0.098) in mice that received anti-ICOSL Ab. Inhibition of ICOSL did not significantly affect proliferation of splenic CD4+ T cells (Figure 4B) or their activation (Figure 4C).
Ab Production. Control Ab-treated mice developed significant humoral immunity against the immunizing antigen as assessed by circulating Ab levels (Figure 5). ICOSL blockade during the induction phase of GN significantly reduced IL-4 (A) and IL-10 (B) production while having no effect on IFN-γ (C) levels. *P < 0.05.

ICOSL Expression in Glomeruli of Mice with Crescentic GN

Immunofluorescent staining demonstrated that ICOSL was absent from kidneys of normal nonimmunized mice (Figure 6A) but was strongly upregulated within glomeruli of mice with crescentic GN (Figure 6B). No staining was observed when isotype control Ab was used (Figure 6C).

Figure 3. Effect of ICOSL blockade during the induction phase of GN on splenocyte production of IL-4, IL-10, and IFN-γ. Cytokine levels were measured by ELISA on supernatants that were collected from splenocytes that were cultured in the presence of Ag (sheep globulin) for 72 h. Inhibition of ICOSL significantly reduced IL-4 (A) and IL-10 (B) production while having no effect on IFN-γ (C) levels. *P < 0.05.

Figure 4. ICOSL blockade during the induction of GN decreases apoptosis of splenic CD4+ T cells that were isolated on day 20 while having no effect on their proliferation or activation. CD4+ T cell apoptosis (A), proliferation (B), and activation (C) were assessed by flow cytometric analysis of Annexin-V staining, in vivo 5-bromo-2'-deoxyuridine (BrdU) incorporation, and CD44 expression, respectively. *P = 0.01.

Figure 5. Circulating antigen-specific Ig levels in mice that received control rat IgG or anti-ICOSL mAb during the induction of GN. Ab levels were measured by ELISA on serum that was collected at the end of experiments. Baseline represents Ab levels from nondiseased mice. Blockade of ICOSL during the initiation of GN reduced circulating Ig levels. *P < 0.05.

Effect of ICOSL Blockade during the Effector Phase of GN on Disease Outcome and Immune Responses

Renal Injury and Glomerular Accumulation of Effector Leukocytes. Blockade of the ICOS/ICOSL pathway during the effector phase of GN exacerbated the development of disease. Mice that were treated with anti-ICOSL Ab had increased numbers of CD4+ T cells (Figure 7A) and macrophages (Figure 7B) present in glomeruli compared with controls that received rat IgG. Increased accumulation of effector leukocytes in glomeruli of mice that were treated with anti-ICOSL Ab was associated with enhanced crescent formation (Figures 7C and 8). No difference in urinary protein excretion was detected between controls that received rat IgG and mice that were treated with anti-ICOSL Ab (12.4 ± 2.3 versus 11.0 ± 2.3 mg/24 h; P = 0.7).

Intraglomerular Proliferation. Cellular proliferation within glomeruli was significantly increased in controls with GN compared with nondiseased mice (Figure 9). Blockade of ICOSL during the effector phase of GN significantly enhanced intraglomerular proliferation compared with controls that received rat IgG (Figure 9).

Glomerular Expression of Adhesion Molecules. P-selectin was absent from kidneys of nondiseased mice but was upregulated within glomeruli of mice that had GN and received control Ab (12.4 ± 2.3 versus 11.0 ± 2.3 mg/24 h; P = 0.7). ICAM-1 was constitutively expressed in nondiseased mice in interstitial and glomerular areas and was upregulated in control Ab-treated mice with GN (Figures 10B and 11C). Administration of anti-ICOSL mAb during the effector phase of the disease significantly
increased intraglomerular expression of P-selectin (Figures 10A and 11B) and ICAM-1 (Figures 10B and 11D).

**Renal Chemokine and Chemokine Receptor Expression.** Several chemokines (IP-10 and MIP-2) and chemokine receptors (CCR1 and CCR2) were not affected by the blockade of ICOSL during the effector phase of GN (Table 1). However, there was a trend toward increased levels of some chemokines (monocyte chemoattractant protein-1, MIP-1α, RANTES, and TCA-3) and chemokine receptors (CCR5 and CXCR3) in the kidneys of mice that were treated with anti-ICOSL mAb compared with controls (Table 1).

**Systemic Immunity.** The systemic immune response was not affected by inhibition of ICOSL during the effector phase of GN. Production of cytokines, IFN-γ, IL-4, and IL-10 was not altered significantly in mice that were treated with anti-ICOSL Ab compared with controls (Table 2). ICOSL blockade did not result in statistically significant changes in splenic CD4+ T cell proliferation or apoptosis (Table 2). Circulating Ag-specific Ig levels also were not significantly affected by inhibition of ICOSL during the effector phase of the disease (Figure 12).

**Discussion**

These studies examined the role of the ICOS/ICOSL costimulatory pathway in the induction and effector phases of a
Th1-mediated model of crescentic GN. For testing the role of ICOSL during the induction of GN, ICOSL was blocked only during the initiation of the immune response (days 0 to 8). Inhibition of ICOSL augmented renal injury as assessed by crescent formation and proteinuria, which was associated with increased numbers of effector leukocytes, CD4+ T cells and macrophages, in glomeruli. These results suggest that ICOS-mediated co-stimulation plays a protective role during the initiation phase of crescentic GN.

The co-stimulatory signal through ICOS can affect the Th1/Th2 balance. Although several studies have shown that ICOS is important for the production of both Th1 and Th2 cytokines (13,14), the majority of evidence suggests that ICOS co-stimulation is more important for the development of Th2 than Th1 responses (37), most likely because of the higher expression levels of ICOS on differentiated Th2 than Th1 lymphocytes (1,38). For example, ICOS is critical for the production of IL-10 (11,15), and ICOS-deficient T cells produce reduced amounts of IL-4 but are fully competent to produce IFN-γ (9). The development of crescentic GN in NTN is exacerbated by Th1 cytokines such as IFN-γ (39) and attenuated by Th2 cytokines,

Table 1. Effect of ICOSL blockade during the effector phase of GN on the renal mRNA expression of chemokines and chemokine receptors

<table>
<thead>
<tr>
<th>Chemokines</th>
<th>Control (mRNA, AU)</th>
<th>Anti-ICOSL (mRNA, AU)</th>
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<tbody>
<tr>
<td>RANTES</td>
<td>226.4 ± 13.8</td>
<td>275.6 ± 41.6</td>
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<tr>
<td>MIP-1α</td>
<td>10.0 ± 1.3</td>
<td>12.2 ± 2.7</td>
</tr>
<tr>
<td>MIP-2</td>
<td>39.6 ± 8.3</td>
<td>44.5 ± 4.0</td>
</tr>
<tr>
<td>MCP-1</td>
<td>102.3 ± 19.7</td>
<td>124.8 ± 17.90</td>
</tr>
<tr>
<td>IP-10</td>
<td>28.1 ± 3.5</td>
<td>29.5 ± 5.3</td>
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<tr>
<td>TCA-3</td>
<td>50.6 ± 8.5</td>
<td>63.8 ± 8.9</td>
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<tr>
<th>Chemokine receptors</th>
<th>Control (mRNA, AU)</th>
<th>Anti-ICOSL (mRNA, AU)</th>
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<tbody>
<tr>
<td>CCR1</td>
<td>6.1 ± 0.7</td>
<td>6.5 ± 1.0</td>
</tr>
<tr>
<td>CCR2</td>
<td>40.8 ± 5.7</td>
<td>45.0 ± 6.3</td>
</tr>
<tr>
<td>CCR5</td>
<td>25.9 ± 3.8</td>
<td>32.7 ± 3.9</td>
</tr>
<tr>
<td>CXCR3</td>
<td>2.6 ± 0.6</td>
<td>3.3 ± 0.5</td>
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*AU, arbitrary units; GN, glomerulonephritis; ICOSL, inducible co-stimulatory molecule ligand; IP-10, interferon γ-inducible protein 10; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; RANTES, regulated upon activation, normal T cell expressed and secreted.

Table 2. Effect of ICOSL inhibition during the effector phase of GN on splenic CD4+ cell apoptosis and proliferation and cytokine production by Ag-challenged splenocytes

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Anti-ICOSL</th>
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<tbody>
<tr>
<td>CD4+ cell apoptosis</td>
<td>6.1 ± 0.5</td>
<td>7.6 ± 0.7d</td>
</tr>
<tr>
<td>(% Annexin-V + PI–</td>
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<tr>
<td>CD4+ cells)</td>
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<tr>
<td>CD4+ cell proliferation</td>
<td>19.3 ± 1.2</td>
<td>19.2 ± 0.9</td>
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<tr>
<td>(% BrdU+ CD4+ cells)</td>
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<tr>
<td>IL-4 (pg/ml)</td>
<td>54.3 ± 7.8</td>
<td>56.6 ± 8.1</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>118.3 ± 28.8</td>
<td>126.0 ± 22.6</td>
</tr>
<tr>
<td>IFN-γ (pg/ml)</td>
<td>634.6 ± 176.1</td>
<td>528.0 ± 184.8</td>
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*Ag, antigen; PI, propidium iodide; BrdU, 5-bromo-2’-deoxyuridine.

bP = 0.12.

The co-stimulatory signal through ICOS can affect the Th1/Th2 balance. Although several studies have shown that ICOS is important for the production of both Th1 and Th2 cytokines (13,14), the majority of evidence suggests that ICOS co-stimulation is more important for the development of Th2 than Th1 responses (37), most likely because of the higher expression levels of ICOS on differentiated Th2 than Th1 lymphocytes (1,38). For example, ICOS is critical for the production of IL-10 (11,15), and ICOS-deficient T cells produce reduced amounts of IL-4 but are fully competent to produce IFN-γ (9). The development of crescentic GN in NTN is exacerbated by Th1 cytokines such as IFN-γ (39) and attenuated by Th2 cytokines,
cause these were not affected by the treatment.

attributed to changes in T cell activation or proliferation, be-
from our study indicate that exacerbation of GN after ICOSL
CD4 a recent immunization with antigen. Our results are also supported by
blockade at two earlier time points, on days 5 and 10 after
a trend toward reduced apoptosis of splenic T cells after ICOSL
shown that ICOS provides an antiapoptotic signal in CD4
immune response. The majority of previous evidence has
of CD4 ICOSL is protective in crescentic GN is by decreasing survival

tivation phase of GN, surprisingly, reduced apoptosis of splenic
(7,12,15,38). In this study, inhibition of ICOSL during the initi-
many between the two results may be explained by the obser-
reduced Ab levels and deposition in the kidney. The discrep-
role of ICOS during the effector phase of crescentic GN, which does not play a role in the development of severe cres-
centric GN observed in our study (27).

For investigation of the role of the ICOS/ICOSL co-stimula-
tory pathway during the effector phase of crescentic GN, ICOSL was blocked only during the effenter phase of the ne-
phritogenic immune response (days 10 to 18). We first demon-
strated that ICOSL is strongly upregulated in glomeruli of mice
with GN, suggesting that glomerular expression of ICOSL may
be important in local Th1 effector responses that induce cres-
centric GN. Inhibition of ICOSL during the effector phase of the
disease significantly enhanced glomerular accumulation of T
and macrophages, resulting in exacerbated renal injury as
indicated by crescent formation. No increase was observed in
urinary protein excretion, most likely as a result of the control
mice having very high proteinuria. The systemic immune re-
sponse was not significantly affected by ICOSL blockade, indi-
cating that the increased presence of effector leukocytes in
glomeruli and enhanced injury cannot be explained by changes
in systemic immunity but that ICOSL plays a protective role
during the effenter phase of crescentic GN by locally attenuating
accumulation of Th1 effectors and macrophages in glomeruli.

This is the first study to demonstrate a protective role of the
ICOS/ICOSL co-stimulatory pathway in the in vivo Th1 effector
responses. Previously, blockade of this pathway during the
effenter phase of Th1-mediated immune diseases such as EAE
(16) and experimental autoimmune myocarditis (19) attenuated
disease. Furthermore, late inhibition of ICOSL during the de-
velopment of lupus nephritis improved renal pathology (29);
hower, reduced disease in that study was due to decreased
Ab deposits in the kidney, which play a pathogenic role in
lupus nephritis (42) but are not required for the development of
crescentic GN in NTN (27). The results of these experiments,
nevertheless, are supported by previous evidence demonstrating that ICOSL that is expressed by renal TEC negatively regulates T cell activation and cytokine production in the kidney (6,31).

The mechanisms by which ICOSL may locally attenuate glomerular accumulation of Th1 effectors and macrophages are unknown, but several possibilities exist. P-selectin and ICAM-1 are upregulated in glomeruli of mice with crescentic GN, where they mediate glomerular infiltration of T cells and macrophages (30,32,35,43,44). In this study, inhibition of ICOSL during the effector phase of GN markedly increased intraglomerular expression of P-selectin and ICAM-1, suggesting that ICOSL might decrease initial glomerular infiltration of Th1 effectors and macrophages by downregulating local adhesion molecules that these cells need to migrate into the target tissue and mediate injury.

ICOSL-provided co-stimulation may alter renal expression of chemokines and/or chemokine receptors that might be important for Th1 effectors and macrophages to migrate into glomeruli and mediate disease (45–49). Previous experiments have demonstrated that blockade of the ICOS/ICOSL pathway in the effector phase of cell-mediated diseases, including EAE, acute cardiac allograft rejection, and lung inflammation, can affect expression of chemokines and chemokine receptors in the target organ (16,17,50). In our study, enhanced leukocyte accumulation as a result of ICOSL inhibition is not likely to be explained by changes in chemokine and/or chemokine receptor expression in the kidney because it was not associated with a significant increase in any chemokine or chemokine receptor.

In addition, increased glomerular accumulation of T cells and macrophages after blockade of ICOSL during the effector phase of the disease was associated with augmented intraglomerular proliferation, suggesting that another mechanism by which ICOSL locally attenuates glomerular leukocyte accumulation is by reducing cellular expansion within glomeruli. Similarly, ICOSL expression by renal TEC negatively regulates T cell activation in the kidney by reducing the production of an essential T cell growth factor, IL-2 (6).

**Conclusion**

Our studies demonstrate, for the first time, that the ICOS/ICOSL co-stimulatory pathway plays a protective role during both the induction and effector phases of Th1-mediated crescentic GN. During the induction phase, ICOSL enhances the production of Th2 cytokines and reduces survival of CD4+ T cells, resulting in decreased renal injury. In the kidney, during the effector phase of the disease, ICOSL is strongly upregulated within nephritic glomeruli, where it locally attenuates accumulation of effector leukocytes, CD4+ T cells and macrophages, and consequent development of GN, without affecting the systemic immune response.

**Acknowledgments**

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