Suppression of Experimental Glomerulonephritis by the Interleukin-1 Receptor Antagonist: Inhibition of Intercellular Adhesion Molecule-1 Expression


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Abstract

Interleukin-1 is a proinflammatory cytokine produced in glomerulonephritis. Blocking the action of interleukin-1 by the administration of the interleukin-1 receptor antagonist (IL-1ra) has been shown to prevent renal function impairment, reduce glomerular injury, inhibit leukocyte infiltration, and suppress tubulointerstitial damage in experimental antiglomerular basement membrane disease. A key mechanism in the entry of leukocytes into the kidney is the interaction between the interleukin-1 inducible intercellular adhesion molecule-1 (ICAM-1, CD54) and lymphocyte function-associated antigen-1 (CD11a/CD18). Therefore, this study investigated whether the inhibition of this mechanism was the means by which IL-1ra suppressed leukocyte infiltration in rat accelerated antiglomerular basement membrane glomerulonephritis. Disease was induced in two groups of six rats; animals were treated by constant sc infusion of recombinant human IL-1ra or saline from the initiation of disease until being euthanized 14 days later. In saline-treated animals, there was marked up-regulation of ICAM-1 in the glomerulus and interstitium, which was associated with leukocyte infiltration. In particular, focal accumulation of CD11a+ and CD18+ cells was apparent in areas of tubulointerstitial damage exhibiting intense ICAM-1 expression. IL-1ra treatment partially reduced glomerular ICAM-1 expression and leukocyte infiltration. However, IL-1ra treatment resulted in a dramatic inhibition of interstitial ICAM-1 expression, interstitial leukocyte infiltration, and tubulointerstitial damage. In conclusion, this study has shown that interleukin-1 is a major inducer of ICAM-1 expression within the renal tubulointerstitium—a process associated with focal leukocyte infiltration and tubulointerstitial damage. This is the first demonstration of a specific mechanism by which interleukin-1 participates in the pathogenesis of renal injury.

Key Words: Interleukin-1, adhesion molecules, lymphocyte function-associated antigen-1, leukocytes, immunohistochemistry

Interleukin-1 (IL-1) is a cytokine that elicits a wide range of proinflammatory and immunologic effects, including activation of endothelium, stimulation of T and B cell activation, and the induction of a number of other cytokines including interleukins-1, 2, 6, and 8 and tumor necrosis factor-α (TNF-α) (1). Glomerular IL-1 production has been demonstrated in human and experimental glomerulonephritis, including rat antiglomerular basement membrane (GBM) disease (2–9). In addition, the administration of IL-1 to rats at the time of the induction of anti-GBM disease exacerbates glomerular injury (10), whereas the administration of anti-IL-1β antibodies suppresses injury (11). Thus, it has been postulated that IL-1 participates in renal injury.

One approach to determine whether IL-1 production plays a pathogenic role in the development of renal injury is to block IL-1 activity in a disease model. This has been made possible by the recent characterization of the IL-1 receptor antagonist (IL-1ra) (12). The IL-1ra is structurally related to both IL-1α and IL-1β and competes equally with both forms of IL-1 for binding to IL-1 cell surface receptors (13). However, the binding of the IL-1ra to the IL-1 receptor does not trigger a cellular response, and therefore, the IL-1ra can be used to block the function of IL-1 both in vitro and in vivo (12–14).

IL-1ra treatment of rat accelerated anti-GBM disease reduced glomerular injury, prevented renal function impairment, inhibited leukocyte infiltration, and suppressed tubulointerstitial damage—thus demonstrating a pathogenic role for IL-1 in the development of renal injury in this model (15). A key
action whereby IL-1ra treatment suppressed disease development was the inhibition of leukocyte infiltration. Recent studies in a number of experimental models of glomerulonephritis have provided strong evidence that the interaction between intercellular adhesion molecule-1 (ICAM-1; CD54) and its counter-receptor lymphocyte function-associated antigen-1 (LFA-1; CD11a/CD18) plays a pivotal role in glomerular leukocyte infiltration (16-20). The importance of leukocyte adhesion molecules in the immune system and renal disease has been recently reviewed (21,22). IL-1 is known to induce ICAM-1 expression by a number of cell types in vitro; however, it is unknown whether IL-1 is involved in the up-regulation of ICAM-1 expression, which is apparent in many types of human glomerulonephritis (23,24). Therefore, the aim of this study was to determine whether the suppression of leukocyte infiltration in anti-GBM disease produced by IL-1ra treatment was due to the inhibition of ICAM-1 up-regulation.

METHODS

Animals

Inbred male Sprague-Dawley rats (150 g) were obtained from the Monash University Animal House.

Nephrotoxic Serum

Rabbit anti-rat GBM nephrotoxic serum was raised by repeated immunization of a New Zealand White rabbit with particulate rat GBM, as previously described (25). The anti-GBM nephrotoxic serum was decomplemented and adsorbed extensively against normal rat erythrocytes.

Experimental Design

Passive accelerated anti-GBM disease was induced in 12 rats by sc immunization with 5 mg of normal rabbit immunoglobulin G in Freund’s incomplete adjuvant followed 5 days later (Day 0) by the iv injection of 10 mL/kg body wt rabbit anti-rat GBM serum (nephrotoxic serum). One group of six rats received a constant infusion of human recombinant IL-1ra (Synergen) from Day -1 until being euthanized at Day 14 by means of an Alzet 2002 miniosmotic pump (Alza Corp., Palo Alto, CA) implanted under the skin of the back that delivered 0.5 \( \mu \)L/h of 50 mg/mL IL-1ra in saline. The second group of six rats received saline only via an implanted pump over the same time period. In addition, one group of normal rats was examined.

Antibody Labeling of Tissue Sections

Tissues were fixed in 2% paraformaldehyde-lysine-periodate, cryoprotected in 7% sucrose, and frozen in OCT (Miles, Elkart, IN). Serial cryostat sections (6 \( \mu \)m) were adhered to gelatin-coated slides and labeled with monoclonal antibodies (mAb) by the immunoperoxidase staining technique as previously described (26). The mAb used in this study were as follows:OX-1, rat leukocyte common antigen (27); 1A29, anti-rat ICAM-1 (CD54) (28); WT1, anti-rat CD11a (29); WT3, anti-rat CD18 (29).

Quantitation of ICAM-1 Expression

Anti-ICAM-1 mAb labeling of kidney tissue sections was scored as follows—Glomerulus: the approximate number of positive cells was semi-quantitatively assessed per glomerular cross-section as follows: (0.5) <25% labeled cells; (1) 25 to 50% labeled cells; (2) 50 to 75% labeled cells; and (3) >75% labeled cells. Tubular epithelium—300 to 400 tubular cross-sections were scored in high-power fields (X400), moving consecutively from the outer to inner cortex, and data were expressed as the percentage of positive tubules. A positive tubule was defined as a tubular cross-section containing two or more labeled cells. Interstitial cells: the number of labeled nucleated cells located in between tubules, excluding clearly identified peritubular capillary endothelial cells, were counted in 20 high-power fields (X400), moving consecutively from the outer to inner cortex by means of a 0.02-mm\(^2\) graticule. Data were expressed as cells per square millimeter of tissue. The number of glomerular and interstitial cells labeled by the ox-1, anti-CD11a, and anti-CD18 mAb were quantitated as previously described (30).

Statistical Analysis

Data from scoring ICAM-1 expression in the glomerulus were compared by use of the nonparametric Wilcoxon-Rank test. Data obtained from counting the percentage of ICAM-1-positive tubules, the numbers of ICAM-1 interstitial cells, and the numbers of ox-1-, CD11a-, and CD18-positive cells were compared by use of an unpaired t test from the Complete Statistical System (CSS; Statsoft, Tulsa, OK) programme.

RESULTS

Normal Kidney

ICAM-1 was constitutively expressed in normal rat kidney (Figure 1a). In the glomerulus, ICAM-1 was weakly expressed by endothelial cells, parietal epithelial cells, and some mesangial cells. In the interstitium, there was patchy ICAM-1 expression involving weak luminal expression by approximately 9% of tubules (Table 1; Figure 1a). Blood vessel endothelium, including peritubular capillaries, expressed ICAM-1. There was also a population of ICAM-1++
interstitial cells, which most likely comprised resident leukocytes (macrophages and dendritic cells) and fibroblasts. Only rare CD11a+ and CD18+ cells were seen in normal glomeruli, whereas a resident population of CD11a+ and CD18+ cells was apparent within the interstitium (Figure 1b; Table 1).

**Anti-GBM Disease**

On Day 14 of saline-treated, accelerated anti-GBM disease, there was a marked up-regulation of ICAM-1 expression. Most cells within the glomerular tuft strongly expressed ICAM-1 (Figure 2a; Table 1). There was a significant glomerular leukocyte infiltrate (OX-1+ cells; Table 1) that consisted almost entirely of macrophages. Only a small proportion of the glomerular leukocyte infiltrate was labeled with anti-CD11a or anti-CD18 mAb (Figure 2b; Table 1).

There was also marked up-regulation of ICAM-1 expression in the interstitium on Day 14 of saline-treated, accelerated anti-GBM disease. There was a marked increase in endothelial cell ICAM-1 expression in both vessels and peritubular capillaries. In addition, a dramatic increase in both the intensity and number of tubules expressing ICAM-1 was evident (Figure 2a; Table 1). Indeed, strong tubular ICAM-1 expression was apparent in areas of focal leukocyte accumulation and tubulointerstitial damage (Figure 2a). Anti-ICAM-1 mAb staining was most prominent in the luminal surface of tubular cells, but a diffuse cellular staining pattern was also apparent, indicating possible weak basolateral ICAM-1 expression. In contrast, areas of weak tubular ICAM-

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**Figure 1.** Immunoperoxidase staining of cryostat sections showing constitutive expression of ICAM-1 and CD18 antigens in normal rat kidney. (a) ICAM-1 is constitutively expressed by some glomerular cells and interstitial peritubular capillaries and weakly by some tubules (arrows); (b) few CD18-positive cells are present in normal kidney (arrows). Original magnification, ×250.

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<table>
<thead>
<tr>
<th>TABLE 1. ICAM-1 expression and leukocyte infiltration in accelerated anti-GBM disease</th>
<th>untreated</th>
<th>Saline</th>
<th>IL-1α Treated</th>
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<td><strong>Glomerulus</strong></td>
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<td>ICAM-1 (cells/μm²)</td>
<td>6.8 ± 0.8</td>
<td>1.6 ± 0.8</td>
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<td>OX-1 (cells/tub)</td>
<td>1.0 ± 1.0</td>
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<td>0.1 ± 0.1</td>
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<td>CD18 (cells/tub)</td>
<td>3.0 ± 0.3</td>
<td>2.0 ± 0.2</td>
<td>0.3 ± 0.3</td>
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<td><strong>Intersstitium</strong></td>
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<td>ICAM-1 (cells/mm²)</td>
<td>270 ± 0.3</td>
<td>250 ± 0.3</td>
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<td>CD11a (cells/mm²)</td>
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<tr>
<td>CD18 (cells/mm²)</td>
<td>34.5 ± 13.4</td>
<td>34.5 ± 13.4</td>
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- *Details of the scoring of immunoperoxidase-stained sections and the statistical analysis are given in the Methods section, gj1, glomerular cross-section. Results are shown as mean ± SD for groups of six animals.*
- *P < 0.01 versus normal animals.*
- *P < 0.001 versus saline-treated animals.*
IL-1Ra Inhibits ICAM-1 Expression

Treatment with the IL-1Ra caused a marked suppression of accelerated anti-GBM disease in terms of reducing proteinuria, preventing renal function impairment, and preventing tubulointerstitial damage (15). IL-1Ra treatment produced a significant reduction in the intensity of glomerular ICAM-1 expression and a partial reduction in the number of ICAM-1+ cells within the glomerulus (Figure 2c; Table 1). There was also a partial reduction in the glomerular leukocyte infiltrate (OX-1+ cells), although the small number of CD11a+ and CD18+ cells was unchanged (Table 1).

It was in the interstitium where IL-1Ra treatment produced the most dramatic inhibition of ICAM-1 expression. The intensity of peritubular capillary ICAM-1 expression was reduced. There was also a marked reduction in both the intensity of tubular ICAM-1 expression and the number of tubules expressing ICAM-1 (Figure 2c; Table 1). The reduction in tubular ICAM-1 expression was associated with a 70% reduction in interstitial leukocyte (OX-1+ cells) infiltration and a virtual abrogation of tubulointerstitial damage. Numbers of interstitial CD11a+ and CD18+ cells were significantly reduced by IL-1Ra treatment (Figure 2d; Table 1). In addition, there was a significant reduction in the number of interstitial ICAM-1+ cells.

DISCUSSION

In this experimental model of glomerulonephritis, there was marked up-regulation of ICAM-1 expression within both the glomerulus and the tubulointerstitium that was associated with leukocyte infiltration and tissue injury. IL-1Ra treatment of this disease inhibited the up-regulation of ICAM-1 expression, suppressed leukocyte infiltration and leukocyte activation, and reduced renal injury. Therefore, it is postulated that IL-1 production contributes to leukocyte infiltration by up-regulating ICAM-1 expression. However, it is difficult to definitively establish a "cause and effect" relationship in vivo. For example, one alternative interpretation of the data is that leukocyte extravasation occurs via constitutive endothelial ICAM-1 expression or some other mechanism and that up-regulation of ICAM-1 expression on local parenchymal cells occurs as the result of IL-1 production by infiltrating leukocytes.

There are two main points that support the concept that IL-1 production contributes to leukocyte recruitment by up-regulating ICAM-1 expression. First, we examined ICAM-1 and LFA-1 expression during the first 24 h of this disease model by immunoelectron microscopy (31). Within 3 h of nephrotoxic serum administration, there was a marked increase in
ICAM-1 expression on glomerular and peritubular capillary endothelium, on mesangial cells, and on the luminal surface of proximal tubules. ICAM-1 and LFA-1 proteins were observed at sites of adhesion between activated glomerular endothelium and polymorphonuclear cells and between activated peritubular endothelium and mononuclear cells. At this stage of the disease, there was little or no extravasation of leukocytes. Second, our results are consistent with experiments in which blocking the ICAM-1/LFA-1 interaction by antibody administration inhibits leukocyte infiltration and glomerular injury in a number of models of experimental glomerulonephritis (17–20).

Another possible interpretation of this study is that IL-1ra administration caused a general inhibition of T and B cell activation. However, this did not appear to be the case because IL-1ra treatment did not impair the host immunoglobulin response to rabbit immunoglobulin G, nor did it prevent the development of an immunoglobulin response to the administered human IL-1ra (15). This is consistent with other studies showing that the IL-1ra does not inhibit antigen-specific immune responses in vivo (32).

IL-1ra treatment provided a more dramatic suppression of tubulointerstitial injury than glomerular injury. This effect may be due to differences in the extent to which IL-1ra administration inhibited the up-regulation of ICAM-1 expression in the two compartments. IL-1ra treatment made no difference to the development of significant proteinuria during the first 24 h of the disease, suggesting that the neutrophil-mediated glomerular injury occurring within this period was unaffected (15). This is consistent with another study of rat anti-GBM disease in which IL-1ra administration also failed to inhibit neutrophil-mediated glomerular injury, even though this injury was shown to be dependent on the ICAM-1/LFA-1 pathway of cellular adhesion (20). Therefore, in our model, it appears that IL-1 is not important in the early up-regulation of glomerular ICAM-1 expression, which is involved in the initiation of glomerular injury—other factors, such as TNF-α, are likely to be involved in this process (20,33). However, IL-1ra treatment did partially suppress glomerular ICAM-1 expression and macrophage infiltration occurring later in the disease course, and this was associated with an inhibition of mesangial hypercellularity, glomerulosclerosis, crescent formation, and prevention of GFR decline.

In contrast to the glomerulus, there is no significant neutrophil-mediated tubulointerstitial injury in this disease model. In the tubulointerstitium, IL-1ra treatment had a profound inhibition of ICAM-1 expression and leukocyte infiltration and activation that was associated with marked suppression of tubulointerstitial injury. This suggests that IL-1 is the major inducer of ICAM-1 expression in this compartment, although a synergistic effect between IL-1 and TNFα cannot be excluded.

After the entry of leukocytes into the interstitium, the up-regulation of ICAM-1 expression by fibroblasts and tubular epithelium may play a role in the focal accumulation and activation of leukocytes in this compartment. This is suggested by the focal accumulation of CD11a+ and CD18+ cells at sites of strong ICAM-1 expression and the presence of IL-2 receptor-positive immune-activated mononuclear cells within these focal infiltrates—events abrogated by IL-1ra treatment. In support of this concept is a ultrastructural study of the initiation of this disease in which ICAM-1 and LFA-1 were shown to be present at sites of lymphocyte and macrophage attachment to fibroblast-like cells within the interstitium (31). In addition, ICAM-1 expression by antigen-presenting cells is known to costimulate T cell activation in vitro (34). It is attractive to speculate that tubular cell ICAM-1 expression may participate in T cell activation, but this remains to be established (35).

In summary, the use of the IL-1ra has shown that IL-1 production during experimental anti-GBM disease is a major inducer of ICAM-1 expression within the renal tubulointerstitium—a process associated with focal leukocyte infiltration and tubulointerstitial damage. This is the first demonstration of a specific mechanism by which IL-1 participates in the pathogenesis of renal injury in glomerulonephritis.

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REFERENCES


