http://www.kidney-international.org

Decreased renal ischemia–reperfusion injury by IL-16 inactivation

S Wang[1], H Diao[1], Q Guan[1,2], WW Cruikshank[3], TL Delovitch[4,5], AM Jevnikar[1,4,5,6] and C Du[1,2,4,6]

[1]*Department of Medicine, The University of Western Ontario, London, Ontario, Canada;* [2]*Department of Urologic Sciences, The University of British Columbia, Vancouver, British Columbia, Canada;* [3]*Department of Pulmonary, Allergy, and Critical Care Medicine, School of Medicine, Boston University, Boston, Massachusetts, USA;* [4]*Department of Microbiology and Immunology, The University of Western Ontario, London, Ontario, Canada;* [5]*The Robarts Research Institute, London, Ontario, Canada and* [6]*The Multi-Organ Transplantation Program, London Health Sciences Center, London, Ontario, Canada*

T-cell-mediated renal injury is a major cause of kidney transplant rejection and renal failure; hence, understanding T-cell migration within the kidney is important for preventing renal injury. Interleukin (IL)-16 is a T-cell chemoattractant produced by leukocytes. Here we measured IL-16 expression in the kidney and its role in renal ischemia–reperfusion injury induced by different conditions in several strains of mice. IL-16 was strongly expressed in distal and proximal straight tubules of the kidney. The IL-16 precursor protein was cleaved to a chemotactic form in cultured tubular epithelial cells. Inactivation of IL-16 by antibody therapy or IL-16 deficiency prevented ischemia–reperfusion injury as shown by reduced levels of serum creatinine or blood urea nitrogen compared to control mice. Further studies indicated that fewer CD4-cells infiltrated the post-ischemic kidneys of IL-16-deficient mice and that the protective effect of IL-16 antibody treatment was lymphocyte-dependent. Our results suggest that IL-16 is a critical factor in the development of inflammation-mediated renal injury and may be a therapeutic target for prevention of ischemia–reperfusion injury of the kidney.

Kidney International (2008) **73,** 318–326; doi:10.1038/sj.ki.5002692; published online 14 November 2007

KEYWORDS: interleukin-16; renal ischemia; T cell; chemokine

Correspondence: *C Du, Department of Urologic Sciences, The University of British Columbia, VGH-Jack Bell Research Centre, 2660 Oak St, Vancouver, British Columbia, Canada V6H 3Z6. E-mail: caigan@interchange.ubc.ca*

Received 23 March 2007; revised 12 September 2007; accepted 19 September 2007; published online 14 November 2007

Renal ischemia–reperfusion injury (IRI) contributes to function loss and long-term changes of kidney transplants, and also is a common cause for acute renal failure or acute kidney injury in native kidneys.[1–3] To date, the pathogenesis of renal IRI is not fully understood, but leukocyte infiltration, particularly T cells, is currently considered as a crucial pathogenic factor for renal tubular epithelial cell (TEC) and endothelial cell death (apoptosis and necrosis).[4–6] Indeed, T-cell deficiency results in resistance to renal IRI, which could be restored by the adoptive transfer of naive T cells in an animal model.[7] Targeting T cells, such as blocking T-cell costimulating pathway CD28-B7, has been found to be effective in the prevention of renal IRI.[8–10] Therefore, understanding of the molecular mechanisms by which T cells are recruited and migrate to the post-ischemic renal tissue is critical for the development of new therapeutic strategy for patients with acute kidney injury and kidney transplant rejection.

Interleukin (IL)-16 was first identified as a $CD8^+$ lymphocyte-producing T-cell chemoattractant factor.[11–13] So far it has been found in a variety of leukocytes and in some non-immune cells (that is, fibroblasts, lung epithelial cells, and brain cells).[14,15] The secreted mature form of IL-16 is released from pre-formed IL-16 protein (pro-IL-16) by cleavage of caspase-3.[16,17] Although the biological functions of IL-16 are not completely known, its chemotactic activity seems the most prominent, and more importantly it does not require earlier activation of target cells (for example, T cell and monocyte)[18–21] or connective tissue matrices.[11] Thus, IL-16 may act as an initial chemokine for recruitment of leukocytes in the development of local inflammation. Indeed, a recent study indicates that in the animal model of peripheral ischemia, the contribution of $CD8^+$ infiltrate to the early phase of collateral developments depends on its IL-16 production, which recruits $CD4^+$ mononuclear cells.[22] In this study, we have examined the expression of IL-16 in the nephron and TEC cultures. Our results demonstrate that constitutive expression of pro-IL-16 is strongly detected in various segments of the nephron, including distal tubules, glomerula, and proximal straight tubules in the medulla and

chemotactic form of IL-16 in TEC cultures. Neutralization of IL-16 by monoclonal antibody treatment results in the reduction of renal IRI in a murine model.

RESULTS

Expression of IL-16 mRNA and protein in renal tissue and TEC culture

The presence of IL-16 (mRNA and protein) was first examined by reverse transcription-PCR and western blot. As shown in Figure 1a, IL-16 mRNA was detected in RNA samples from primary and cloned NG1.1 TECs as well as naive kidney sections. The expression of IL-16 in the kidney and TEC cultures was further confirmed by the presence of its protein, indicated in western blot. As demonstrated in Figure 1b, there was a prominent anti-IL-16 antibody-bound protein in the protein extracts of TEC and kidney tissue, which likely was pro-IL-16 protein. The specificity of this antibody in the detection of IL-16 protein was confirmed by western blot using protein extract from IL-16-deficient kidneys, in which no pro-IL-16 protein was shown (Figure 1c). Furthermore, it was noted that the position of pro-IL-16 protein in TEC was different from that in kidney tissue in the blot, indicating that the modification occurred to this protein when the kidney cells were grown in a culture system *in vitro*. In agreement with that, a small size of protein (~19 kDa) was uniquely recognized by anti-IL-16 antibody in the protein extracts of TEC cultures, indicating the presence of a mature form of IL-16 from these TEC cultures, respectively.

Release of chemotactic IL-16 in TEC culture

To further evaluate whether the mature form of IL-16 in TEC culture had chemotactic activity, the leukocyte chemotaxis to protein extracts of TEC (primary and NG1.1 cells) vs naive kidney tissue was examined by chemotaxis assay. As shown in Figure 2, protein extracts from both primary and cloned NG1.1 TECs had a significant chemotactic activity of IL-16 to

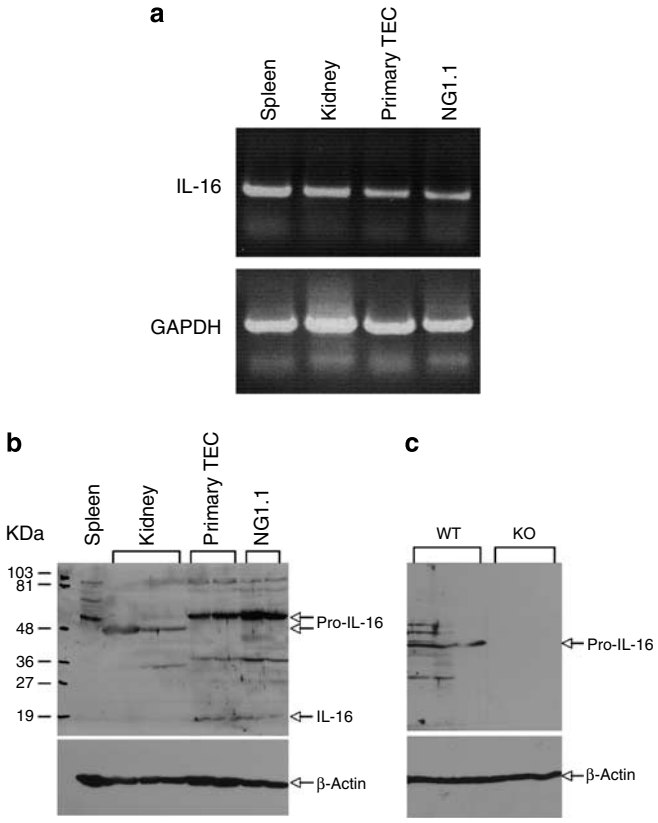

Figure 1 | Expression of IL-16 mRNA and protein in renal tissue and TEC culture. Total RNA or protein extract was prepared from primary and cloned NG1.1 TEC cultures as well as spleens and PBS-perfused kidneys of naive C57BL/6 (WT) or IL-16 knockout (KO) mice. (**a**) The presence of IL-16 mRNA was determined by RT-PCR with appropriate PCR cycle numbers. The GAPDH mRNA was included as an internal control for amount of total RNA in each sample. (**b**) IL-16 protein was detected by western blot with anti-IL-16 antibody, and β-actin was reprobed in the same blot with anti-β-actin IgG antibody. (**c**) The specificity of anti-IL-16 antibody in the detection of IL-16 protein was confirmed using IL-16-deficient kidney tissue (KO) vs wild-type (WT) kidney tissue in western blot analysis. The data are representative of three independent experiments.

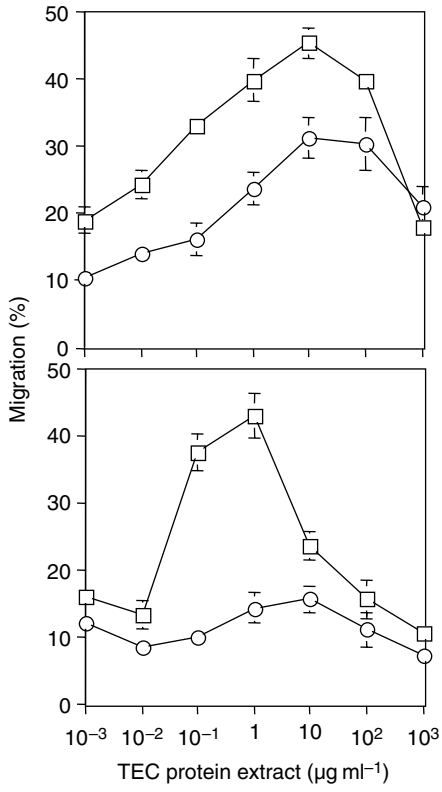

Figure 2 | Presence of chemotactic IL-16 in TEC cultures. The leukocyte chemotaxis assay of IL-16 was determined by the fluorescence-based method with the ChemoTx Disposable Chemotaxis System. Protein extracts from cloned TEC NG1.1 TEC (upper graph) or primary TEC (lower graph) from C57BL/B6 mice were incubated with dye-labeled B6 splenocytes in the presence of control IgG (open square) or anti-IL-16 IgG 2 μl (open circle) for 4 h. The migration was calculated as described in the Materials and Methods section. The data are presented as mean ± s.d. of triplicate determinants in a typical experiment, which was repeated twice with consistent results.