http://www.kidney-international.org

© 2011 International Society of Nephrology

# Macrophages are essential contributors to kidney injury in murine cryoglobulinemic membranoproliferative glomerulonephritis

Shunhua Guo[1], Tomasz A. Wietecha[1], Kelly L. Hudkins[1], Yujiro Kida[2], Min W. Spencer[1], Warangkana Pichaiwong[1], Ichiro Kojima[1], Jeremy S. Duffield[2] and Charles E. Alpers[1]

[1]*Department of Pathology, University of Washington, Seattle, Washington, USA and* [2]*Division of Nephrology, Department of Medicine, University of Washington, Seattle, Washington, USA*

**Mice transgenic for thymic stromal lymphopoietin (TSLP), under regulation of the lymphocyte-specific promoter *Lck*, develop cryoglobulinemia and membranoproliferative glomerulonephritis (MPGN) similar to the disease in patients. To determine whether infiltrating macrophages, a hallmark of this disease, are deleterious or beneficial in the injury process, we developed *Lck-TSLP* transgenic mice expressing the human diphtheria toxin receptor (DTR) under control of the monocyte/macrophage-restricted *CD11b* promoter (*Lck-TSLP;CD11b-DTR*). Treatment with DT resulted in a marked reduction of monocytes/macrophages in the peritoneal cavity of both *CD11b-DTR* and *Lck-TSLP;CD11b-DTR* mice and marked reduction of macrophage infiltration in glomeruli of *Lck-TSLP;CD11b-DTR* mice. *Lck-TSLP;CD11b-DTR* mice, with or without toxin treatment, had similar levels of cryoglobulinemia and glomerular immunoglobulin deposition as *Lck-TSLP* mice. *Lck-TSLP;CD11b-DTR* mice, treated with toxin, had reduced mesangial matrix expansion, glomerular collagen IV accumulation, expression of the activation marker α-smooth muscle actin and transforming growth factor-β1 in mesangial cells, and proteinuria compared with control mice. Thus, macrophage ablation confers protection in this model and indicates a predominately deleterious role for macrophages in the progression of kidney injury in cryoglobulinemic MPGN.**

*Kidney International* (2011) **80,** 946–958; doi:10.1038/ki.2011.249; published online 3 August 2011

KEYWORDS: macrophages; membranoproliferative glomerulonephritis; pathophysiology of renal disease and progression

**Correspondence:** *Charles E. Alpers, Department of Pathology, University of Washington, 1959 NE Pacific Street, Box 357470, Seattle, Washington 98195, USA. E-mail: calp@u.washington.edu*

**Received 17 September 2010; revised 20 May 2011; accepted 31 May 2011; published online 3 August 2011**

Macrophages are key participants in inflammation, phagocytosis, tissue repair, and remodeling.[1–4] They are commonly present in glomerulonephritis (GN), and a large influx of monocytes/macrophages is a particular feature of human cryoglobulinemic membranoproliferative GN (MPGN). Although a characteristic feature of this injury, it is still unknown whether the infiltrating monocytes/macrophages fulfill a predominantly beneficial/reparative role in the evolution of this disease or whether they are predominately effectors or promoters of glomerular injury and sclerosis.

We have characterized a mouse model of cryoglobulinemia with consequent development of MPGN, the thymic stromal lymphopoietin (TSLP) transgenic mouse (*Lck-TSLP*), which overexpresses TSLP and has resultant abnormalities in B-cell development.[5–7] *Lck-TSLP* mice develop mixed cryoglobulinemia and a systemic inflammatory disease that involves kidney, lung, spleen, liver, and skin.[5–7] These mice develop a renal disease that closely resembles human cryoglobulinemia-associated MPGN. The glomerular injury is characterized by extensive subendothelial capillary and mesangial immune deposits, marked monocyte/macrophage influx, mesangial cell proliferation and matrix expansion, and capillary wall splitting. The renal lesion is evident from about age 30 days in female mice and rapidly progresses to fully developed MPGN by age 50 days. There are also significant lung,[5–7] liver,[5–7] and skin lesions.[8,9] Female mice usually die at 60 days to 70 days, presumably from severe lung disease. Male mice have a similar but more slowly evolving course, in which the disease is fully manifest by 120 days.[5–7]

A number of approaches to delete monocyte/macrophage populations, including the use of anti-macrophage serum and liposome-encapsulated clodronate, has been used to test the importance of monocytes/macrophages *in vivo* in renal disease processes.[10–12] Monocyte/macrophage depletion using liposomal clodronate reduced tubulointerstitial inflammation and fibrosis in ischemia/reperfusion injury in the rat and mouse.[13,14] A unique *CD11b-DTR* transgenic mouse, in which human diphtheria toxin receptor (DTR) is specifically expressed by CD11b-expressing cells, allows conditional

ablation of mouse monocytes/macrophages by administration of DT. These mice have been carefully characterized for specificity of ablation, and used to study peritoneal inflammation, kidney fibrosis, kidney repair and regeneration, lung, liver and pancreas injury, wound healing, and atherosclerosis.[15–18] In a model of rapidly progressive glomerular injury, injection of DT for 5 days, beginning at days 15–20 after a single administration of nephrotoxic serum in *CD11b-DTR* mice, resulted in markedly reduced glomerular crescents, attenuated tubular injury, and improved renal function.[18] The effect of monocyte/macrophage depletion has not been tested in a model of chronic GN with active and persistent immune complex deposition, such as that occurs in the cryoglobulinemic *Lck-TSLP* mice and in many patients with MPGN. In this study, we demonstrate that macrophages are essential contributors to the progression of GN and that their presence is a deleterious manifestation of injury.

## RESULTS

### *Lck-TSLP; CD11b-DTR* and *Lck-TSLP* mice develop equivalent spontaneous glomerulonephritis

*Lck-TSLP* mice and *CD11b-DTR* mice, both on C57BL/6 background, were bred to obtain double-transgenic mice (*Lck-TSLP; CD11b-DTR*). Cohorts of C57BL6 wild-type (WT), *CD11b-DTR*, *Lck-TSLP,* and *Lck-TSLP; CD11b-DTR* mice were studied with intraperitoneal administration of 20 ng/g DT body weight or similar volume of phosphate-buffered saline (PBS) from age days 30–50.

*CD11b-DTR* mice are similar to WT mice with no detectable kidney pathology. The *Lck-TSLP; CD11b-DTR* mice have similar levels of serum cryoglobulinemia, measured by cryocrits, as to *Lck-TSLP* mice (data not shown). Both *Lck-TSLP* and *Lck-TSLP; CD11b-DTR* mice developed typical features of cryoglobulin-associated MPGN as described previously.[5] Mice showed progressive kidney injury from age 30 days (*Lck-TSLP; CD11b-DTR*-30D) to age 50 days, with extensive mesangial cell proliferation and mesangial matrix expansion as demonstrated by increased glomerular tuft area, glomerular hypercellularity, and increased silver methanemine-stained extracellular matrix (Figures 1 and 2). Mesangial cell activation, assessed by α-smooth muscle actin (α-SMA) expression, was also markedly increased.

### Glomerular macrophages in *Lck-TSLP* mice show marked heterogeneity

Day 50 *Lck-TSLP* mice show a fivefold increase in glomerular Mϕs compared with WT mice: $2.32 \pm 0.2$ CD68+ cells per glomerular tuft area versus $0.64 \pm 0.05$ cells in WT mice.

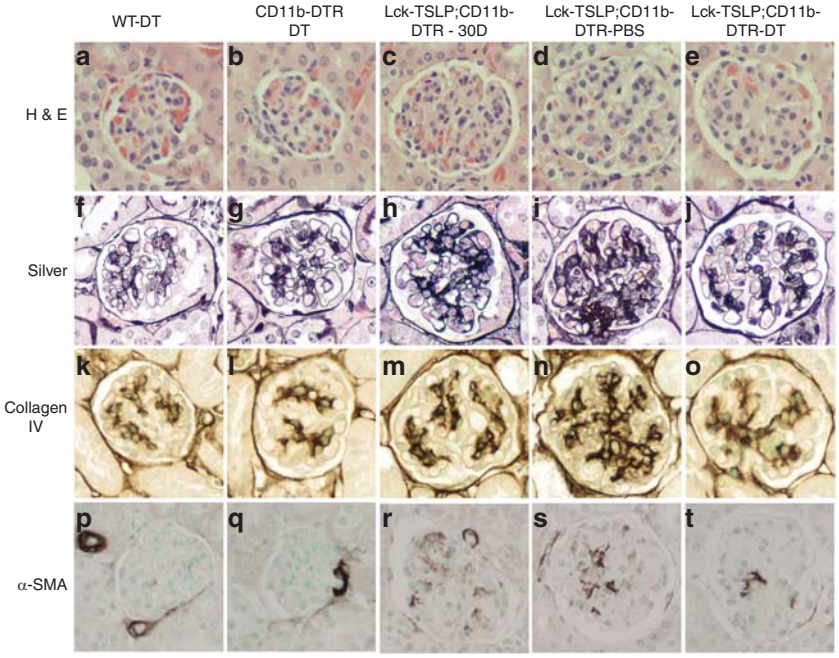


**Figure 1 | Macrophage ablation results in amelioration of glomerulonephritis.** Histological appearance of glomeruli stained with hematoxylin and eosin (H&E) (**a–e**), silver methenamine (**f–j**), and immunohistochemical (IHC) staining for type IV collagen (**k–o**), and α-smooth muscle actin (α-SMA) (**p–t**) in wild-type (WT) and *CD11b-DTR* mice of 50 days of age after 20 days diphtheria toxin (DT) treatment (WT-DT and *CD11b-DTR*-DT, respectively), *Lck-TSLP; CD11b-DTR* mice at 30 days of age (*Lck-TSLP; CD11b-DTR*-30D), and *Lck-TSLP; CD11b-DTR* mice at 50 days of age after 20 days of phosphate-buffered saline (PBS) or DT intraperitoneal administration (*Lck-TSLP; CD11b-DTR*-PBS and *Lck-TSLP; CD11b-DTR*-DT, respectively). *Lck-TSLP; CD11b-DTR* mice with PBS treatment show increased glomerular cellularity, mesangial matrix accumulation (black silver staining area increase and type IV collagen expression increase), and mesangial α-SMA expression as compared with WT and *CD11b-DTR* mice, whereas *Lck-TSLP; CD11b-DTR* mice with DT treatment demonstrate less hypercellularity, significantly reduced mesangial matrix expansion, and reduced α-SMA expression in glomeruli. Original magnification: × 400.

Download English Version:

https://daneshyari.com/en/article/6162295

Download Persian Version:

https://daneshyari.com/article/6162295

Daneshyari.com