The atypical chemokine receptor 2 limits renal inflammation and fibrosis in murine progressive immune complex glomerulonephritis

Andrei Bideak[1], Alexander Blaut[1], John M. Hoppe[1], Martin B. Müller[1], Giuseppina Federico[2], Nuru Eltrich[1], Hermann-Josef Gröne[2], Massimo Locati[3,4] and Volker Vielhauer[1]

[1]*Nephrologisches Zentrum, Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, Ludwig-Maximilians-University Munich, Munich, Germany;* [2]*Department of Cellular and Molecular Pathology, German Cancer Research Center, Heidelberg, Germany;* [3]*Humanitas Clinical and Research Center, Rozzano, Italy; and* [4]*Department of Medical Biotechnologies and Translational Medicine, Università degli Studi di Milano, Milan, Italy*

The atypical chemokine receptor 2 (ACKR2), also named D6, regulates local levels of inflammatory chemokines by internalization and degradation. To explore potential anti-inflammatory functions of ACKR2 in glomerulonephritis, we induced autologous nephrotoxic nephritis in C57/BL6 wild-type and *Ackr2*-deficient mice. Renal ACKR2 expression increased and localized to interstitial lymphatic endothelium during nephritis. At two weeks Ackr2$^{-/-}$mice developed increased albuminuria and urea levels compared to wild-type mice. Histological analysis revealed increased structural damage in the glomerular and tubulointerstitial compartments within Ackr2$^{-/-}$ kidneys. This correlated with excessive renal leukocyte infiltration of CD4^{+} T cells and mononuclear phagocytes with increased numbers in the tubulointerstitium but not glomeruli in knockout mice. Expression of inflammatory mediators and especially markers of fibrotic tissue remodeling were increased along with higher levels of ACKR2 inflammatory chemokine ligands like CCL2 in nephritic Ackr2$^{-/-}$ kidneys. *In vitro*, *Ackr2* deficiency in TNF-stimulated tubulointerstitial tissue but not glomeruli increased chemokine levels. These results are in line with ACKR2 expression in interstitial lymphatic endothelial cells, which also assures efflux of activated leukocytes into regional lymph nodes. Consistently, nephritic Ackr2$^{-/-}$ mice showed reduced adaptive cellular immune responses indicated by decreased regional T-cell activation. However, this did not prevent aggravated injury in the kidneys of Ackr2$^{-/-}$ mice with nephrotoxic nephritis due to simultaneously increased tubulointerstitial chemokine levels, leukocyte infiltration and fibrosis. Thus, ACKR2 is important in limiting renal inflammation and fibrotic remodeling in progressive nephrotoxic nephritis. Hence, ACKR2 may be a potential target for therapeutic interventions in immune complex glomerulonephritis.

Correspondence: *Volker Vielhauer, Nephrologisches Zentrum, Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, Ludwig-Maximilians-University Munich, Ziemssenstrasse 1, 80336 Munich, Germany. E-mail: Volker.Vielhauer@med.uni-muenchen.de*

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Glomerulonephritis (GN) is a major cause of renal failure worldwide leading to end-stage renal disease.[1] It is often caused by glomerular deposition or *in situ* formation of immune complexes, which trigger adaptive humoral and cellular immune responses toward intrinsic or planted glomerular antigens, renal leukocyte infiltration, and cell-mediated renal damage.[2,3]

Chemokines and their receptors orchestrate leukocyte migration to sites of inflammation.[4,5] The atypical chemokine receptor 2 (ACKR2), previously named D6, belongs to the subfamily of atypical chemokine receptors, which are characterized by promiscuous binding of proinflammatory chemokines and play important roles in the resolution of inflammatory responses.[6–8] Unlike typical chemokine receptors, ACKR2 is unable to induce cell signaling in response to ligand binding, but it regulates local chemokine levels by scavenging, internalizing, and degrading these molecules. ACKR2 is expressed in many parenchymal organs, including barrier tissues such as the skin, gut, lung, and placenta.[9,10] The primary site of ACKR2 expression in resting tissues is lymphatic endothelium.[11] In addition, ACKR2 is expressed in leukocyte subsets including neutrophils and dendritic cells.[7,12] In several disease models, it was shown that ACKR2 limits *in vivo* inflammation, including skin inflammation,[13] myocardial infarction,[14] and systemic infection with *Mycobacterium tuberculosis*.[15] In addition, ACKR2 inhibits tumorigenesis in inflammation-dependent cancer models.[16,17] In contrast, in experimental autoimmune encephalitis and after subcutaneous challenge of ovalbumin-specific T cells, inflammatory activity was reduced in *Ackr2*-deficient mice due to reduced T-cell priming in draining lymph nodes.[18,19] Together, these studies demonstrated that ACKR2 not only plays a central role in the resolution of inflammation due to reduction of local chemokine

concentrations, but by scavenging inflammatory chemokines on lymphatic endothelial surfaces, ACKR2 also facilitates migration of antigen-presenting cells to lymph nodes, ensuring an efficient generation of adaptive immune responses.[20] Currently, there is no published evidence whether ACKR2 controls chemokine-dependent recruitment and activation of macrophages, dendritic cells, and T cells in an inflammatory renal disease such as GN. However, as these cell types contribute to the initiation and progression of GN, ACKR2 may also be a potential regulator limiting inflammatory responses in the kidney.

We hypothesized that *Ackr2* deficiency aggravates renal inflammation and injury in chronic immune complex GN. We tested this hypothesis by inducing autologous nephrotoxic nephritis (NTN), a rodent model of immune complex-mediated, T-cell–dependent progressive GN, in *Ackr2*-deficient mice and characterized their phenotype compared with the phenotype of wild-type control mice. Here, we show for the first time that ACKR2 plays an important role in limiting renal inflammation, injury, and fibrosis during GN by reducing proinflammatory chemokine levels in inflamed kidneys and subsequent leukocyte infiltration into the tubulointerstitial compartment. In addition, our data demonstrate that ACKR2 facilitates adaptive T-cell responses in regional lymph nodes during autologous NTN. Despite its opposing effects on tubulointerstitial inflammation and adaptive local immune responses in the autologous NTN model, we identified ACKR2 as an endogenous regulator primarily limiting renal injury and fibrosis in chronic GN.

RESULTS

ACKR2 expression in nephritic kidneys with autologous NTN

Analysis of mRNA expression levels in normal and nephritic wild-type kidneys at day 14 of NTN revealed induced expression of ACKR2 in inflamed kidneys (Figure 1a). *In situ* hybridization of Ackr2 mRNA combined with immunofluorescence detection of lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1)-positive lymphatic endothelial cells localized renal ACRK2 expression to the interstitial lymphatic endothelium in nephritic wild-type kidneys, whereas no mRNA transcripts could be detected in *Ackr2*-deficient ($Ackr2^{-/-}$) mice (Figure 1b). These results show that ACKR2 is prominently expressed in lymphatic endothelial cells of nephritic kidneys with NTN.

Ackr2 deficiency deteriorates renal functional parameters in autologous NTN

To investigate the potential role of ACKR2 in controlling renal inflammation in GN, we induced autologous NTN in wild-type and $Ackr2^{-/-}$ mice. At day 14 of NTN, $Ackr2^{-/-}$ mice, compared with wild-type mice, developed significantly increased albuminuria (Figure 2). Consistently, renal function deteriorated more significantly in $Ackr2^{-/-}$ mice, compared with wild-type mice, indicated by higher serum urea levels (Figure 2). Thus, *Ackr2* deficiency significantly aggravated functional parameters of renal injury in autologous NTN.

Ackr2 deficiency increases glomerular and tubulointerstitial injury in autologous NTN

Increased albuminuria suggested more severe glomerular injury in $Ackr2^{-/-}$ mice compared with wild-type mice. This was confirmed by the presence of more severe glomerulosclerosis, more glomerular fibrinoid necrosis, and a reduced number of preserved podocytes in glomeruli of *Ackr2*-deficient mice (Figure 3a to c). Increased podocyte damage was also indicated by reduced glomerular staining of nephrin and reduced renal nephrin mRNA expression in $Ackr2^{-/-}$ kidneys (Figure 3d).

Moreover, tubulointerstitial injury substantially increased in $Ackr2^{-/-}$ mice compared with wild-type mice at day 14 of NTN, with more tubular casts, tubular dilation, and interstitial volume expansion in $Ackr2^{-/-}$ kidneys (Figure 4a to c). This correlated with higher protein expression of the tubular damage marker kidney injury molecule 1 (KIM-1) in $Ackr2^{-/-}$ mice (Figure 4d). Together, *Ackr2* deficiency leads

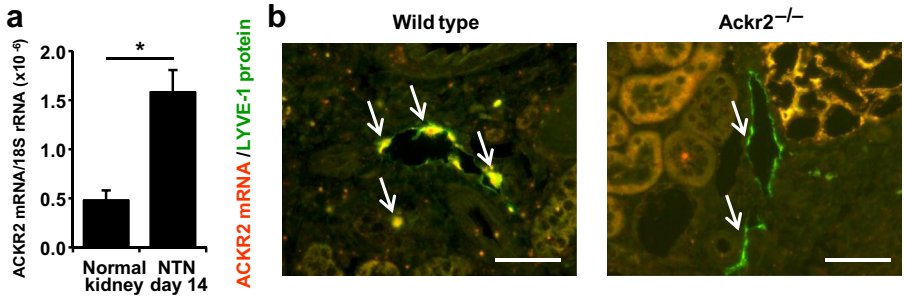

Figure 1 | Renal expression of atypical chemokine receptor 2 (ACKR2) is induced in mice with autologous serum nephrotoxic nephritis (NTN) and localizes to lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1)-positive lymphatic endothelial cells. (**a**) Ackr2 mRNA levels, normalized to 18S ribosomal RNA (rRNA), in normal murine kidney and in nephritic kidneys at day 14 of NTN. Data represent mean ± SEM; *$P < 0.05$. (**b**) Fluorescence-based *in situ* hybridization of ACKR2 (red) combined with immunofluorescence localization of LYVE-1 (green) demonstrates Ackr2 mRNA expression in LYVE-1–positive interstitial lymphatic endothelial cells (merged yellow signal, indicated by arrows) in nephritic wild-type kidneys at day 14 of NTN (left panel). Ackr2 mRNA expression was not detectable in nephritic kidneys of *Ackr2*-deficient ($Ackr2^{-/-}$) mice (right panel). Images show representative cortical tissue from each group; original magnification ×400, bars = 50 μm. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.