CD44 is required for the pathogenesis of experimental crescentic glomerulonephritis and collapsing focal segmental glomerulosclerosis

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A key feature of glomerular diseases such as crescentic glomerulonephritis and focal segmental glomerulosclerosis is the activation, migration and proliferation of parietal epithelial cells. CD44-positive activated parietal epithelial cells have been identified in proliferative cellular lesions in glomerular disease. However, it remains unknown whether CD44-positive parietal epithelial cells contribute to the pathogenesis of scarring glomerular diseases. Here, we evaluated this in experimental crescentic glomerulonephritis and the transgenic anti-*Thy1.1* model for collapsing focal segmental glomerulosclerosis in CD44-deficient (*cd44-/-*) and wild type mice. For both models albuminuria was significantly lower in *cd44-/-* compared to wild type mice. The number of glomerular Ki67-positive proliferating cells was significantly reduced in *cd44-/-* compared to wild type mice, which was associated with a reduced number of glomerular lesions in crescentic glomerulonephritis. In collapsing focal segmental glomerulosclerosis, the extracapillary proliferative cellular lesions were smaller in *cd44-/-* mice, but the number of glomerular lesions was not different compared to wild type mice. For crescentic glomerulonephritis the influx of granulocytes and macrophages into the glomerulus was similar. *In vitro*, the growth of CD44-deficient murine parietal epithelial cells was reduced compared to wild type parietal epithelial cells, and human parietal epithelial cell migration could be inhibited using antibodies directed against CD44. Thus, CD44-positive proliferating glomerular cells, most likely parietal epithelial cells, are essential in the pathogenesis of scarring glomerular disease.

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Parietal epithelial cells (PECs) line the Bowman capsule of a healthy glomerulus. Like podocytes, PECs have the same embryonic origin.[1,2] Unlike podocytes, PECs retain the ability to proliferate, which occurs under pathologic conditions.[3,4] In adult kidney, various subpopulations of PECs have been described, such as parietal podocytes,[5–7] adult parietal epithelial multipotent progenitors,[8,9] and activated PECs,[4,10,11] with some variation among species. Activated PECs are known mainly for their involvement in the development of hyperplastic lesions in focal and segmental glomerulosclerosis (FSGS) and crescentic glomerulonephritis.[10,12]

Extracapillary proliferative lesions or crescents are the hallmark of both inflammatory and noninflammatory glomerular diseases. In fact, lesion formation or glomerular scarring is responsible for the irreversible loss of renal function in most glomerular diseases. Evidence indicates that the extracapillary proliferative cellular lesions in (experimental) crescentic glomerulonephritis and collapsing focal and segmental glomerulosclerosis (FSGS) are composed mainly of activated PECs.[4,10] In experimental collapsing FSGS, induced damage to podocytes triggers the activation of PECs followed by the formation of adhesions and invasion by PECs of the glomerular tuft. The migrating and proliferating activated PECs deposit extracellular matrix material, which accumulates over time, resulting in segmental, and ultimately global, scarring of the glomerular tuft.[10,12] In experimental crescentic glomerulonephritis, anti-glomerular basement membrane

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(anti-GBM) antibodies, eventually in conjunction with toll-like-receptor (TLR) ligands, lead to activation of the complement system and glomerular influx of inflammatory cells, such as granulocytes and macrophages.[13–15] Simultaneously, PECs, and to a lesser extent podocytes, are activated, thereby generating proliferative cellular lesions and the typical crescents, which may block the tubular outlet and urinary flow.[4]

The proliferative cells within sclerotic lesions have been identified as activated PECs in human biopsies of a range of glomerular diseases as well.[11,16–21] Based on the aforementioned animal and human studies, activation of PECs has been proposed as a common mechanism in crescentic glomerulonephritis and primary and secondary FSGS.[12,22]

Activated PECs can be identified by an acquired expression of cluster of differentiation 44 (CD44), which is a cell surface glycoprotein that plays a key role in various cellular processes, such as cell differentiation, cell migration, cell-matrix binding, leukocyte trafficking, and scar formation.[23] Normally, CD44 is not expressed in a healthy glomerulus, but CD44 expression by proliferating PECs is higher in crescents and lesions in the presence of crescentic glomerulonephritis and FSGS.[11,24–26] The specific pattern of CD44 expression on activated PECs may suggest that CD44 expression by PECs contributes to the pathogenesis of scarring glomerular diseases. However, whether the acquired CD44 expression by activated PECs is a cause or consequence of glomerular scarring is not clear. In the current study, we evaluated the role of CD44 in experimental crescentic glomerulonephritis and FSGS, which revealed that CD44 is required for the pathogenesis of these scarring glomerular diseases.

RESULTS

CD44 deficiency reduces glomerular fibrinogen deposition and albuminuria in crescentic glomerulonephritis

To evaluate the role of CD44 in scarring glomerular diseases, we induced crescentic glomerulonephritis in wild-type (WT) and CD44-deficient (*cd44–/–*) mice and analyzed these mice at day 14. WT mice injected with saline showed no renal CD44 expression (Figure 1a), whereas nephrotoxic serum (NTS)–injected WT mice acquired glomerular, peri-glomerular, and tubular CD44 expression (Figure 1b and e). As expected, NTS-injected *cd44–/–* mice displayed no renal CD44 expression (Figure 1c, d, and f). To assess whether CD44 deficiency results in a kidney phenotype, we examined the histology and presence of granulocytes, macrophages, B cells, and T cells in normal, i.e., non-treated, WT and *cd44–/–* mice. Both types of mice had a normal renal histology (Supplementary Figure S1), and no differences were observed in the number of immune cells within the kidney (data not shown).

To exclude the possibility that CD44 deficiency affects the binding of the sheep anti-GBM serum, we stained for sheep Ig and found a similar appearance for WT and *cd44–/–* mice

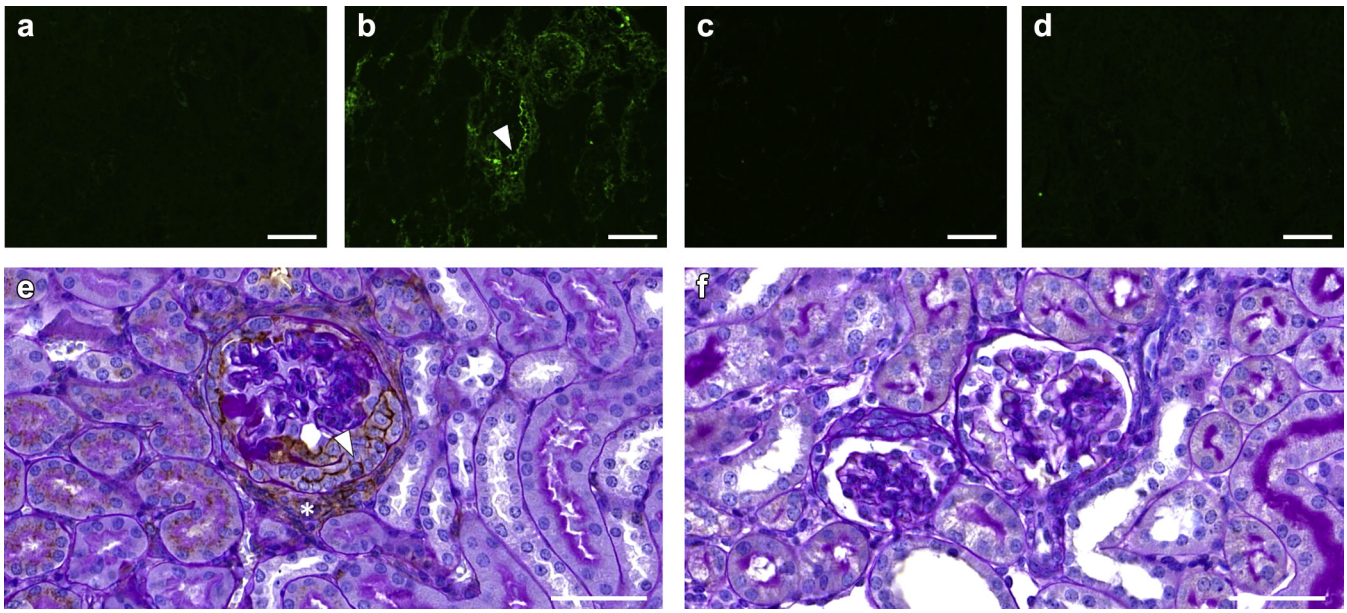

Figure 1 | Confirmation of CD44 deficiency in cd44–/– mice. To confirm cluster of differentiation 44 (CD44) deficiency in *cd44–/–* mice, renal cryosections were stained with anti-CD44 antibody 14 days after nephrotoxic serum (NTS) injection, versus controls. Shown are the following: wild-type (WT) mouse injected with saline only (**a**); WT mouse injected with NTS (**b**); *cd44–/–* mouse after NTS injection (**c**); and secondary antibody control (**d**). Only in the WT mouse injected with NTS was a *de novo* expression of CD44 detected (**b**, arrowhead). Bar = 100 μm. Immunostaining for CD44 on a kidney section stained with periodic acid–Schiff of an NTS-injected WT (**e**) and a *cd44–/–* mouse (**f**), respectively. Only in the WT mouse CD44 immunostaining was detected in the epithelial cells of affected glomeruli and in the peri-glomerular area (asterisks). Bar = 50 μm. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.