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Lutheran/basal cell adhesion molecule accelerates progression of crescentic glomerulonephritis in mice

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Migration of circulating leukocytes from the vasculature into the surrounding tissue is an important component of the inflammatory response. Among the cell surface molecules identified as contributing to leukocyte extravasation is VCAM-1, expressed on activated vascular endothelium, which participates in all stages of leukocyte–endothelial interaction by binding to leukocyte surface expressed integrin VLA-4. However, not all VLA-4-mediated events can be linked to VCAM-1. A novel interaction between VLA-4 and endothelial Lutheran (Lu) blood group antigens and basal cell adhesion molecule (BCAM) proteins has been recently shown, suggesting that Lu/BCAM may have a role in leukocyte recruitments in inflamed tissues. Here, we assessed the participation of Lu/BCAM in the immunopathogenesis of crescentic glomerulonephritis. High expression of Lu/BCAM in glomeruli of mice with rapidly progressive glomerulonephritis suggests a potential role for the local expression of Lu/BCAM in nephritogenic recruitment of leukocytes. Genetic deficiency of Lu/BCAM attenuated glomerular accumulation of T cells and macrophages, crescent formation, and proteinuria, correlating with reduced fibrin and platelet deposition in glomeruli. Furthermore, we found a pro-adhesive interaction between human monocyte α4β1 integrin and Lu/BCAM proteins. Thus, Lu/BCAM may have a critical role in facilitating the accumulation of monocytes and macrophages, thereby exacerbating renal injury.

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Necrotizing crescentic rapidly progressive glomerulonephritis (RPGN) is a class of acquired renal disease that remains one of few human autoimmune diseases that represent an acute threat to survival.[1] This has stimulated investigation into the immunobiology of the condition in the hope of understanding the pathogenesis not only of anti-glomerular basement membrane disease, but also of other forms of glomerulonephritis (GN) in which the aggravating antigen(s) is as yet unknown. Focal necrotizing crescentic GN is the renal lesion typically associated with the clinical syndrome of RPGN and is a medical emergency that requires side effect–prone immunosuppressive therapies. Untreated RPGN progresses rapidly to renal insufficiency. This process is almost always associated with severe interstitial and periglomerular inflammation. The inflammatory infiltrate gives way to a progressive fibrotic process involving the crescents and the periglomerular and peritubular interstitium, accompanied by tubular atrophy and progressive renal failure. Although the pathogenesis of crescentic RPGN is incompletely understood and likely involves several convergent pathways, there is general agreement that circulating mononuclear phagocytes have a central role. Administration of nephrotoxic serum to rodents or rabbits results in a severe proliferative and necrotizing GN that is characterized by glomerular crescent formation and accumulation of leukocytes.[2–4] These infiltrating cells may then release inflammatory mediators that influence the behavior of glomerular, tubular, and interstitial cells. This interaction between infiltrating and resident cells leads to cellular proliferation, matrix expansion, and may ultimately lead to glomerular sclerosis and interstitial fibrosis. Monocytes and macrophages have a critical role as shown by ablation of macrophages in murine crescentic GN that reduced renal injury and improved renal function.[5] Part of the deleterious action of glomerular macrophages could be directly linked to the augmented glomerular procoagulant activity as a result of their expression of surface membrane procoagulant activity

and by their potential to indirectly augment glomerular procoagulant activity[6] by the production of cytokines capable of enhancing endothelial cell procoagulant activity.[7] In addition, infiltrating glomerular macrophages are the major source of IL-1[8] and tumor necrosis factor (TNF).[9] TNF-alpha was shown to promote VCAM-1 and ICAM-1 glomerular expression and the recruitment of PMNs and lymphocytes that were markedly reduced in TNF-deficient mice in experimental RPGN induced by anti-glomerular basement membrane (GBM) antibody.[10] Thus, strategies that reduce monocyte infiltrates could be a promising avenue for complementary therapy of RPGN. For example, neutralization of the chemokine monocyte chemoattractant protein-1 (MCP-1) resulted in a marked decrease in both glomerular crescent formation and deposition of type I collagen.[11] Another strategy to prevent leukocyte infiltration in the kidney could be to target endothelial molecules involved in cell adhesion. Among the candidates, Lutheran (Lu) blood group antigens and basal cell adhesion molecule (BCAM) antigen in endothelial cells are carried by Lu/BCAM (CD239) glycoproteins of the immunoglobulin superfamily. Lu/BCAM glycoproteins are receptors of laminin alpha-5 chains, a major component of the extracellular matrix.[12] Notably, the second ligand of Lu/BCAM is integrin α4β1. The α4β1 integrin or very late antigen (VLA-4) or CD49d/CD29 is expressed mainly on monocytes, lymphocytes, eosinophils, and immature circulating sickle red cells.[13] A novel interaction of α4β1 integrin in sickle red cells with endothelial Lu/BCAM proteins has been recently shown to mediate sickle cell adhesion to the endothelium.[13] The expression of Lu/BCAM in the mouse kidney has been localized in the glomerulus, distal tubule, collecting duct, and in blood vessels. Within glomeruli, Lu/BCAM is expressed at cell contact with GBM, in particular, at glomerular endothelium.[14] Thus, we hypothesized that an interaction between integrin α4β1 and Lu/BCAM could be considered to promote endothelial inflammation through α4β1-mediated adhesion of leukocytes in experimental RPGN. We tested the effect of the genetically determined Lu/BCAM deficiency on crescentic RPGN induced by the infusion of nephrotoxic serum (NTS) in mice.[15]

RESULTS

Attenuated RPGN and no renal failure in *Lu*−/− mice

A total of 12 *Lu*−/− and 14 wild-type (WT) male littermates of mixed 129/Ola-C57BL/6J genetic background had similar renal histology and functional parameters (albuminuria to creatinine ratio, serum creatinine, and blood urea nitrogen) at baseline (Figure 1). Injection of anti-glomerular basement membrane (anti-GBM) nephrotoxic serum (NTS) induced nephrotic syndrome in WT animals. Nephrotic syndrome is caused by hypoproteinemia due to massive urinary loss of large proteins, particularly albumin, leading to hypoalbuminemia and ascites. Lu/BCAM deficiency significantly prevented both the incidence and severity of ascites (not shown) as well as the renal dysfunction reflected by albuminuria (Figure 1a) and blood urea nitrogen and serum creatinine concentrations (Figure 1b and c), which were normal in *Lu*−/− mice.

As an index of early renal microvascular damage, we measured renal blood flow velocity in the renal artery before and on day 4 of NTS-induced RPGN. Whereas renal blood flow velocity remained normal in NTS-challenged *Lu*−/− animals, *Lu*+/+ animals displayed a significantly more profound diminution of mean renal blood flow on day 4 than that measured in *Lu*−/− counterparts (Figure 2). More severe alteration of renal blood flow in *Lu*+/+ animals was concomitant to equal, and later significantly higher, systolic blood pressure levels compared with those measured in Lu−/− mice (Figure 2). This suggests that Lu/BCAM deficiency limited the early rise in renal vascular resistance.

We histologically examined WT mice injected with NTS, and found severe GN by day 21 (Figures 3a–f), whereas *Lu*−/− littermates had significantly less renal damage (Figure 3g–l). Overall, *Lu*−/− mice displayed significantly fewer (3.5-fold less) crescentic glomeruli (Figure 3m). *Lu*−/− mice also exhibited fewer fibrocellular crescents (Figure 3n), no increases in glomerular diameter, and virtually no rupture of Bowman's capsule (Supplementary Figure S1A and B online).

Reduced ultrastructural alterations in *Lu*−/− glomeruli

Because glomerular expression of Lu/BCAM is constitutive and differential loss of glomerular permselectivity with heavy albuminuria preceded the development of the crescents already on day 7, we evaluated the morphological features of podocytes in *Lu*+/+ and *Lu*−/− mice on day 4 after an injection of NTS. Notably, podocyte ultrastructure was normal and identical in *Lu*+/+ and *Lu*−/− mice under control conditions with focal thickening of the external lamina of the GBM in *Lu*−/− condition as previously described.[14] In response to NTS, WT *Lu*+/+ mice displayed mild-to-severe effacement of the foot processes of podocytes (Figure 3o). These ultrastructural alterations were markedly attenuated in *Lu*−/− animals (Figure 3p). Consistently Lu/BCAM deficiency was associated with fewer loss of differentiated podocytes than in *Lu*+/+ mice after NTS challenge, as assessed by WT-1 immunostaining on day 21 (Figure 3q and r).

Role of Lu/BCAM in the immuno-inflammatory response associated with RPGN

Although T cells and macrophages are central players both in our mouse model of NTS-induced GN and in human crescentic RPGN,[4,11,16] antibody deposition may also have a pathophysiological role during the early stages of the disease, promoting activation of complement.[16,17] Therefore, we assessed the humoral response of *Lu*−/− and *Lu*+/+ mice to sheep IgG. Sheep IgG deposition in glomerular basement membranes in the kidneys of both groups after NTS injection displayed similar intensity and pattern (Figure 4a). Glomerular deposition of mouse IgG was also similar in both NTS-injected groups (Figure 4b). Serial