Glomerular common gamma chain confers B- and T-cell–independent protection against glomerulonephritis

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Crescentic glomerulonephritis is a life-threatening renal disease that has been extensively studied by the experimental anti–glomerular basement membrane glomerulonephritis (anti-GBM-GN) model. Although T cells have a significant role in this model, athymic/nude mice and rats still develop severe renal disease. Here we further explored the contribution of intrinsic renal cells in the development of T-cell–independent GN lesions. Anti-GBM-GN was induced in three strains of immune-deficient mice (*Rag2*$^{-/-}$, *Rag2*$^{-/-}$*Il2rg*$^{-/-}$, and *Rag2*$^{-/-}$*Il2rb*$^{-/-}$) that are devoid of either T/B cells or T/B/NK cells. The *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ or *Rag2*$^{-/-}$*Il2rb*$^{-/-}$ mice harbor an additional deletion of either the common gamma chain (γC) or the interleukin-2 receptor β subunit (IL-2Rβ), respectively, impairing IL-15 signaling in particular. As expected, all these strains developed severe anti-GBM-GN. Additionally, bone marrow replenishment experiments allowed us to deduce a protective role for the glomerular-expressed γC during anti-GBM-GN. Given that IL-15 has been found highly expressed in nephritic kidneys despite the absence of lymphocytes, we then studied this cytokine *in vitro* on primary cultured podocytes from immune-deficient mice (*Rag2*$^{-/-}$*Il2rg*$^{-/-}$ and *Rag2*$^{-/-}$*Il2rb*$^{-/-}$) compared to controls. IL-15 induced downstream activation of JAK1/3 and SYK in primary cultured podocytes. IL-15–dependent JAK/SYK induction was impaired in the absence of γC or IL-2Rβ. We found γC largely induced on podocytes during human glomerulonephritis. Thus, renal lesions are indeed modulated by intrinsic glomerular cells through the γC/IL-2Rβ receptor response, to date classically described only in immune cells.

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Human crescentic glomerulonephritis (CG) and rapidly progressive glomerulonephritis (GN) are life-threatening immune-mediated diseases.[1] For many years, the pathogenesis of human rapidly progressive GN has been studied using the experimental model of passive anti–glomerular basement membrane GN (anti-GBM-GN), whereby heterologous anti-GBM serum is injected into rats or mice.[2] Following injection, there is a strong inflammatory response, which in a few days leads to severe glomerular and tubulointerstitial injuries[3,4]: renal cells become surrounded by lymphocytes, a feature reminiscent of human CG.[5–10] The development of anti-GBM-GN lesions in mice has been shown to involve a $CD4^+$ T-cell response through either Th1[4,11–14] or Th17[15–17] pathways, whereas the T-cell subset of invariant natural killer (NK) T cells exerts a protective role independently of T-cell polarization.[18]

Interestingly, other studies have reported that mice or rats born athymic or "nude" (i.e., constitutively lacking T cells) still develop classical features of GN after anti-GBM serum injection, which is hypothesis generating and provides an opportunity to explore the particular role potentially played by renal cells, independently from T cells[19,20] and by intra-renal signaling pathways or local factors normally used by lymphocytes. We thus induced an experimental GN in several immunodeficient mice strains: *Rag2*$^{-/-}$, *Rag2*$^{-/-}$*Il2rg*$^{-/-}$, and *Rag2*$^{-/-}$*Il2rb*$^{-/-}$ that are devoid of either T/B cells or T/B/NK

cells. Compared with *Rag2*$^{-/-}$ mice, *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ and *Rag2*$^{-/-}$*Il2rb*$^{-/-}$ strains carry the additional deletion of either the interleukin (IL)-2 receptor common gamma chain (γC) gene (*Rag2*$^{-/-}$*Il2rg*$^{-/-}$) or the IL-2 receptor β subunit (IL-2Rβ) gene (*Rag2*$^{-/-}$*Il2rb*$^{-/-}$). Several IL receptor complexes share the γC subunit (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21).[21] Importantly, IL-15 and IL-2, together with an IL-15Rα or IL-2Rα coreceptor, respectively, have their action further restricted to the β subunit. The trimeric IL-15 receptor (IL-15Rα, IL-2Rβ, γC) is necessary for NK cell ontogeny and explains the absence of NK cells in *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ or *Rag2*$^{-/-}$*Il2rb*$^{-/-}$ mice. Here we show that γC/ IL-2Rβ complexes expressed at the surface of intrinsic renal cells play an important role in the organization of renal lesions during severe immune-mediated GN.

RESULTS

Rag2$^{-/-}$*Il2rg*$^{-/-}$ mice are more susceptible than *Rag2*$^{-/-}$ mice to anti-GBM serum

Experiments were first carried out to test the effect of anti-GBM serum in *Rag2*$^{-/-}$ mice devoid of T/B cells, *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice devoid of T/B/NK cells, and wild-type (WT) mice from the same C57BL/6J background. Administration of anti-GBM serum impaired renal function parameters and induced lesions typical of GN in WT, *Rag2*$^{-/-}$, and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice (Figure 1a) evaluated at day 9. No marked differences were observed in the levels of blood urea nitrogen and percentage of pathologic glomeruli between anti-GBM–treated WT mice and *Rag2*$^{-/-}$ mice (Figures 1b and c). However, the score of interstitial lesions, assessed by tubular dilations and epithelial atrophy, was lower in anti-GBM–treated *Rag2*$^{-/-}$ mice than in anti-GBM-GN–treated WT mice (Figure 1d). The levels of plasma urea, proteinuria, and the severity of renal histological lesions were even greater in anti-GBM–treated *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice than in anti-GBM–treated *Rag2*$^{-/-}$ mice (Figure 1b–e). Anti-GBM-GN–treated *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice consistently exhibited more significant tubulointerstitial lesions, CD44 *de novo* staining (Supplementary Figure S1), a higher percentage of pathologic glomeruli (Figures 1c and d) and a more important decrease in the podocytic marker podocalyxin (Figure 1f) than anti-GBM–treated *Rag2*$^{-/-}$ mice. Therefore, *Rag2*$^{-/-}$ mice appeared only partially protected against anti-GBM-GN, whereas *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice were found to be more susceptible to anti-GBM serum than WT or *Rag2*$^{-/-}$ mice were.

NK cells have no protective role against renal lesions induced by anti-GBM serum

Rag2$^{-/-}$ mice have been shown to have a significant proportion of circulating NK cells (around 25% vs. 5% in WT mice), whereas *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice totally lack NK cells.[22] To determine the role of NK cells in the results shown, *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice were replenished by NK cells (1×10^6 cells/mouse), isolated from the spleen of WT C57BL/6J mice, and injected together with anti-GBM serum. At day 7, flow cytometry analysis showed that ~2% of circulating cells in *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice were NK cells (Figure 2a). At day 9, NK cells were also detected in the mice kidneys (Figure 2b), where an increase in mRNA expression of the *Nkg2b*/*Cd159a* NK cell receptor (Figure 2c) was detectable. Despite that, plasma urea levels and renal lesions were similar to nonreplenished *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice (Figures 2d to 2f). These findings suggest that NK cells do not account for the severity of GN in *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice.

The lack of γC expression in renal cells worsens renal lesions induced by anti-GBM serum

Using bone marrow graft experiments, we then analyzed the role of kidney-expressed γC in the development of renal lesions in anti-GBM-GN. Bone marrow cells isolated from WT or *Rag2*$^{-/-}$ CD45.1$^+$ mice were grafted into irradiated *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ CD45.2$^+$ (referred to as ep.γc$^{-/-}$) mice, in order to obtain chimeric mice knockout only for the γC chain in nonimmune cells. Six weeks after bone marrow transplantation, at least 90% of circulating cells expressed the CD45.1$^+$ marker in *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ CD45.2$^+$ recipients (Supplementary Figure S2A). NK cell counts measured after bone marrow transplantation are represented in Supplementary Figure S2B. In all anti-GBM-GN–induced ep.γc$^{-/-}$ chimeric mice, plasma urea levels were consistently higher than in mice with WT epithelia (Figure 3a). All ep.γc$^{-/-}$ chimeric mice exhibited significantly severe glomerular lesions with numerous extracapillary crescents (Figures 3b and e) and proteinuria (Figure 3d). In addition, the tubulointerstitial lesions were more severe in ep.γc$^{-/-}$ mice than in mice with WT epithelia, independent of the origin of the bone marrow (WT or *Rag2*$^{-/-}$) (Figures 3c and e). These findings suggest that γC expressed on renal cells plays a protective role during anti-GBM-GN.

Despite the absence of lymphocytes, IL-15 appears as the main effective ligand of γC in the renal cortex during GN

We next analyzed relative expression levels of cytokines (e.g., IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21) acting through receptor complexes containing the common γC subunit in the renal cortex of WT, *Rag2*$^{-/-}$, or *Rag2*$^{-/-}$γc$^{-/-}$ mice (Figure 4a) after anti-GBM-GN. In all cases, IL-21 and IL-4 mRNA were undetectable. The level of IL-2 mRNA remained very low in all mouse strains (Figure 4a). IL-7 and IL-9 mRNA were detectable in the renal cortex, but no difference was observed among WT, *Rag2*$^{-/-}$, or *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice. Interestingly, IL-15 mRNA was found to be highly expressed in the renal cortex after anti-GBM-GN in the absence of T/B lymphocytes (*Rag2*$^{-/-}$ mice) or even in the absence of T/B/NK cells in *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ animals (Figure 4a). Thus, and as described by others,[23] IL-15 is abundantly produced by intrinsic renal cells, which we confirmed by immunohistochemistry of the renal cortex (Figure 4b). We also showed that from cytokines classically associated with the γC response, only IL-15 plasma levels were found to be higher in *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice when compared with WT animals