Blockade of Interleukin 27 Signaling Attenuates Graft Versus Host Disease By Augmenting $CD4^+$ and $CD8^+$ Regulatory T Cell Reconstitution

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The interleukin-6 (IL-6) cytokine superfamily (i.e. IL-6, IL-12, and IL-23) plays a major role in the modulation of inflammatory and regulatory pathways during graft versus host disease (GVHD). IL-27, a recently discovered member of this family, is a heterodimeric cytokine that is composed of the p28 and EBI3 subunits and signals through a heterodimeric receptor composed of WSX-1 and gp130. Notably, IL-6 also uses gp130 as a signaling component which biologically links IL-27 and IL-6. IL-27 has been shown to have opposing proinflammatory and immunoregulatory effects, but its role in GVHD is not well understood. To define the functional significance of IL-27, lethally irradiated Balb/c ($H\text{-}2^d$) mice were transplanted with C57BL/6J ($H\text{-}2^b$) BM and spleen cells, and then treated with an anti-IL-27p28-specific antibody on days 0 and +6. p28 antibody-treated animals had significantly improved weight recovery and overall survival (47% versus 0% survival at day 60, p=0.002), as well as reduced numbers of proinflammatory $CD4^+$ and $CD8^+$ IFN-γ^+ T cells in GVHD target organs, when compared to isotype control antibody-treated mice. A similar outcome was observed in an MHC-matched, minor antigen disparate model (B6→Balb.B), indicating that this was not a strain-specific phenomenon. Given the similarities between IL-6 and IL-27, we examined whether blockade of IL-27 promoted regulatory T cell (Treg) reconstitution as has been observed with inhibition of IL-6 signaling. Recipients transplanted with BM grafts from B6 $Foxp3^{EGFP}$ reporter animals and treated with p28 antibody had a significant increase in the number of $CD4^+$nTregs, $CD4^+$ iTregs and $CD8^+$ iTregs in GVHD target organs, indicating that blockade of IL-27 augmented global Treg reconstitution. In fact, inhibition of IL-27 was more effective at augmenting Treg reconstitution than comparable antibody blockade of IL-6. To further elucidate the role of IL-27, we employed transgenic IL-$27^{-/-}$ and IL-$27R^{-/-}$ animals to dissect the relevant contributions of donor and recipient populations. Paradoxically, we observed that transplantation with IL-$27^{-/-}$donor grafts exacerbated GVHD mortality and augmented accumulation of proinflammatory T cells, whereas transplantation of recipient IL-$27^{-/-}$ mice with wild type grafts had no effect on transplant outcomes. This discordance between antibody-based and genetic studies was unexpected and led us to consider whether there were steady state alterations in T cells from IL-$27^{-/-}$ animals that biased these cells towards a proinflammatory phenotype. To that end, we observed that naive $CD8^+$ T cells from IL-$27^{-/-}$ mice had greater IFN-γ production than wild type cells after in vitro polyclonal stimulation and $CD4^+$ nTregs from these animals had diminished expression of CXCR3 which is critical for Treg trafficking into inflamed tissue sites. Thus, the lack of endogenous IL-27 resulted in intrinsic immune dysregulation which led to an exacerbation of GVHD after transfer of these T cells into recipients. To resolve this paradox, we employed IL-$27R^{-/-}$ (WSX-$1^{-/-}$) mice and demonstrated that mice transplanted with IL-$27R^{-/-}$ grafts had enhanced weight recovery and survival providing confirmation that blockade of IL-27 signaling reduced GVHD. In addition, using IL-$27R^{-/-}$ $Foxp3^{EGFP}$ reporter mice, we observed increased frequencies and numbers of $CD4^+$ and $CD8^+$ $Foxp3^+$ T cells in mice reconstituted with IL-$27R^{-/-}$ grafts, confirming results observed with p28 antibody blockade. Since IL-10 is a mechanism by which $CD4^+$ Tregs suppress GVHD and IL-27 has been shown to enhance T cell-derived IL-10 secretion in nontransplant models, we examined whether IL-27 blockade adversely affected IL-10 production by Tregs. Recipients transplanted with marrow grafts from IL-10.Bit$Foxp3^{EGFP}$ dual reporter animals and treated with p28 antibody had a significant reduction in the frequency of IL-10-producing conventional $CD4^+$ and $CD8^+$ T cells in GVHD target organs. Notably, however, there was no difference in the frequency of $CD4^+$ $Foxp3^+$IL-10^+ T cells, indicating that blockade of IL-27 signaling preferentially affected conventional T cells and had no adverse effect on $CD4^+$ $Foxp3^+$ T cell-derived IL-10 production. In summary, these studies demonstrate that blockade of IL-27 signaling potently augments Treg reconstitution leading to a reduction in the severity of GVHD and may therefore represent a novel strategy to reduce mortality from this disease in man.