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Interleukin-10 Receptor Signaling in Innate Immune Cells Regulates Mucosal Immune Tolerance and Anti-Inflammatory Macrophage Function

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SUMMARY

Intact interleukin-10 receptor (IL-10R) signaling on effector and T regulatory (Treg) cells are each independently required to maintain immune tolerance. Here we show that IL-10 sensing by innate immune cells, independent of its effects on T cells, was critical for regulating mucosal homeostasis. Following wild-type (WT) $CD4^+$ T cell transfer, *Rag2*$^{-/-}$*Il10rb*$^{-/-}$ mice developed severe colitis in association with profound defects in generation and function of Treg cells. Moreover, loss of IL-10R signaling impaired the generation and function of anti-inflammatory intestinal and bone-marrow-derived macrophages and their ability to secrete IL-10. Importantly, transfer of WT but not *Il10rb*$^{-/-}$ anti-inflammatory macrophages ameliorated colitis induction by WT $CD4^+$ T cells in *Rag2*$^{-/-}$*Il10rb*$^{-/-}$ mice. Similar alterations in the generation and function of anti-inflammatory macrophages were observed in IL-10R-deficient patients with very early onset inflammatory bowel disease. Collectively, our studies define innate immune IL-10R signaling as a key factor regulating mucosal immune homeostasis in mice and humans.

INTRODUCTION

Interleukin-10 (IL-10) is a key immunosuppressive cytokine that is produced by a wide range of leukocytes, as well as nonhematopoietic cells (Shouval et al., 2014). Polymorphisms in the *IL10* locus confer risk for ulcerative colitis and Crohn's disease (Franke et al., 2008; Franke et al., 2010), and mice and humans deficient in either IL-10 or IL-10 receptor (IL-10R) exhibit severe intestinal inflammation and marked proinflammatory cytokines

secretion (Begue et al., 2011; Glocker et al., 2010; Glocker et al., 2009; Kotlarz et al., 2012; Kühn et al., 1993; Moran et al., 2013; Spencer et al., 1998). Thus, IL-10 has a central role in regulation of intestinal mucosal homeostasis and prevention of inflammatory bowel disease (IBD).

IL-10 mediates its anti-inflammatory effects through IL-10R-dependent signals emanating from the cell surface. The IL-10R is a heterotetramer that consists of two subunits of IL-10Rα and two subunits of IL-10Rβ (Moore et al., 2001). Whereas the IL-10Rα subunit is unique to IL-10 signaling, the IL-10Rβ subunit is shared by other cytokine receptors, including IL-22, IL-26, and interferon-λ (IFN-λ) (Moore et al., 2001). IL-10 downstream signaling through the IL-10R inhibits the induction of proinflammatory cytokines by blocking NF-κB-dependent signals (Saraiva and O'Garra, 2010).

Although the development of IBD is well established in mice and in humans with IL-10R deficiency, the precise mechanisms of IL-10R-dependent control of immune tolerance and intestinal mucosal homeostasis are not well defined. In mice, intact IL-10R signaling is important in T regulatory (Treg) cells for their suppressive function including prevention of colitis, and in T effector cells for preventing exaggerated T helper 17 (Th17) cell responses in mucosal compartments (Chaudhry et al., 2011; Huber et al., 2011; Kamanaka et al., 2011; Murai et al., 2009). While innate immune cell production of IL-10 is critical for maintaining mucosal homeostasis (Liu et al., 2011; Murai et al., 2009), a role for innate immune IL-10R signaling in the regulation of intestinal immune tolerance has not been explored. Several groups have demonstrated that IL-10 sensing by innate immune cells is required for suppression of proinflammatory cytokines secretion (Gu et al., 2008; Pils et al., 2010). Moreover, IL-10R-deficient dendritic cells (DCs) secrete high quantities of proinflammatory cytokines after LPS stimulation (Girard-Madoux et al., 2012). We hypothesized that innate immune IL-10R signaling is required for maintenance of intestinal immune tolerance and prevention of IBD.

Here we demonstrate that IL-10R signaling in innate immune cells was critical for regulating mucosal homeostasis and prevention of colitis. Loss of IL-10R-dependent signaling rendered wild-type (WT) $CD4^+$ T cells colitogenic and was associated with markedly aberrant Treg cells generation and function. Importantly, we show that IL-10R-dependent signals modulated the differentiation and function of bone-marrow-derived macrophages (BMDM) and intestinal macrophages into either proinflammatory macrophages or functionally competent anti-inflammatory macrophages. Similarly, monocyte-derived macrophages from very early onset IBD patients harboring loss of function mutations in *IL10RA* and *IL10RB* also exhibited impaired differentiation and function of pro- and anti-inflammatory macrophages. These results define a unique and nonredundant role for IL-10R signaling in innate immune cell control of intestinal mucosal homeostasis.

RESULTS

IL-10 Regulates Intestinal Inflammation Independent of T Cell-Specific IL-10R Signaling

We have recently reported that aberrant interactions between innate immune cells devoid of the cytoskeletal regulator Wiskott-Aldrich syndrome protein (WASP) and WT $CD4^+$ T cells lead to colitis development (Nguyen et al., 2012a). In this model, $Was^{-/-}Rag2^{-/-}$ mice develop severe intestinal inflammation following WT $CD4^+$ T cell transfer, characterized by reduced production of IL-10; colitis development can be prevented by exogenous administration of IL-10Ig. To elucidate whether IL-10 acts on innate or adaptive immune cells in this model, we transferred $Il10rb^{-/-}$ $CD4^+$ T cells into $Was^{-/-}Rag2^{-/-}$ mice, which resulted in severe colitis in less than 2 weeks. We then assessed the effects of exogenous IL-10 in preventing disease, and as depicted in Figure S1 available online, colitis was readily abrogated by exogenous IL-10Ig administration, indicating that IL-10 can prevent intestinal inflammation independent of its function on either regulatory or effector $CD4^+$ T cells. These data are consistent with aberrant function of IL-10R signaling in innate immune cells in the setting of WASP-deficiency.

Colitis Development in $Il10rb^{-/-}$ Mice Requires an Adaptive Immune System

To assess directly the role of IL-10R-dependent signals in innate immune cells in the control of mucosal homeostasis, we first analyzed $Il10rb^{-/-}$ mice. Consistent with prior observations (Spencer et al., 1998), $Il10rb^{-/-}$ mice (on the 129SvEv background) developed spontaneous colitis starting around 3 months of age, characterized by extensive bowel wall thickening, lamina propria (LP) lymphoid cell infiltration, and presence of crypt abscesses, in association with increased IFN-γ^+- and IL-17A$^+$-producing $CD4^+$ T cells in the LP and mesenteric lymph node (MLN) (Figure S2). In order to assess whether lymphocytes are required for colitis development in $Il10rb^{-/-}$ mice we generated $Rag2^{-/-}Il10rb^{-/-}$ mice, which lack mature B and T lymphocytes. Importantly, these mice are viable and do not develop clinical, endoscopic, or microscopic signs of colitis (data not shown). These data indicate that lymphocytes are essential for colitis development in $Il10rb^{-/-}$ mice.

$Il10rb^{-/-}$ Innate Immune Cells Render WT $CD4^+$ T Cells Colitogenic

We next hypothesized that colitis development in $Il10rb^{-/-}$ mice, although lymphocyte-dependent, is initiated by defects in the innate immune compartment. To assess whether $Il10rb^{-/-}$ deficient innate immune cells cause WT $CD4^+$ T cells to become colitogenic, we introduced unfractionated WT $CD4^+$ T cells by intraperitoneal (i.p.) injection into $Rag2^{-/-}$ and $Rag2^{-/-}Il10rb^{-/-}$ recipient mice. $Rag2^{-/-}Il10rb^{-/-}$ mice developed severe colitis following WT $CD4^+$ T cell transfer within 3–4 weeks (Figures 1A and 1B). Hematoxylin and eosin (H&E)-stained colonic sections demonstrated significant hyperplasia and immune cell infiltration of the LP, as well as occasional crypt abscesses (Figure 1C).

Because IL-10Rβ is also expressed on nonhematopoietic cells (Moore et al., 2001), we assessed whether loss of IL-10Rβ signaling in innate immune cells was sufficient to drive intestinal inflammation by generating bone-marrow (BM) chimeric animals. BM cells were isolated from either $Rag2^{-/-}$ or $Rag2^{-/-}Il10rb^{-/-}$ mice and transferred into lethally irradiated $Rag2^{-/-}$ or $Rag2^{-/-}Il10rb^{-/-}$ recipient mice, which after reconstitution received unfractionated WT $CD4^+$ T cells. Upon T cell transfer, $Rag2^{-/-}$ mice reconstituted with $Rag2^{-/-}Il10rb^{-/-}$ BM developed colitis within several weeks (Figures 1D and 1E). In contrast, transfer of WT T cells into $Rag2^{-/-}Il10rb^{-/-}$ mice reconstituted