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Interleukin 1 Beta Mediates Intestinal Inflammation in Mice and Patients with IL10 Receptor Deficiency

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Abstract

IL10 receptor (IL10R)-deficient mice develop spontaneous colitis and similarly, patients with loss-of-function mutations in *IL10R* develop severe infant-onset inflammatory bowel disease (IBD). Loss of IL10R signaling in mouse and human macrophages is associated with increased production of interleukin 1 beta (IL1B). We demonstrated that innate immune production of IL1B mediates colitis in IL10R-deficient mice. Transfer of *Il1r1*^{-/-} CD4⁺ T cells into *Rag1*^{-/-}/*Il10rb*^{-/-} mice reduced the severity of their colitis (compared to mice that received CD4⁺ T cells that express IL1R), accompanied by decreased production of interferon gamma, tumor necrosis factor, and IL17A. In macrophages from mice without disruption of IL10R signaling or from healthy humans (controls), incubation with IL10 reduced canonical activation of the inflammasome and production of IL1B through transcriptional and post-translational regulation of *NLRP3*. Lipopolysaccharide (LPS) and adenosine triphosphate stimulation of macrophages from *Il10rb*^{-/-} mice or IL10R-deficient patients increased production of IL1B. Moreover, in human IL10R-deficient macrophages, LPS stimulation alone increased IL1B secretion via non-canonical, caspase 8-dependent activation of the inflammasome. We treated 2 IL10-receptor deficient patients with severe and treatment-refractory infant-onset IBD with the IL1 receptor antagonist anakinra. Both patients had marked clinical, endoscopic, and histologic responses after 4–7 weeks. This treatment served as successful bridge to allogeneic hematopoietic stem cell transplantation in 1 patient. Our findings indicate that loss of IL10 signaling leads to intestinal inflammation, at least in part, through increased production of IL1 by innate immune cells, leading to activation of CD4⁺ T cells. Agents that block IL1 signaling might be used to treat patients with IBD resulting from IL10R deficiency.

Keywords

VEOIBD; Treg cell; ubiquitination; caspase-1

Interleukin-10 (IL10) is a key immunoregulatory cytokine¹. Patients with loss-of-function mutations in *IL10R* genes develop severe IBD in the first months of life^{2, 3} and similarly, *Il10rb*^{-/-} mice develop spontaneous colitis⁴. We and others have recently reported that IL10R deficiency in innate immune cells results in severe colitis and an intrinsic defect in generation and function of anti-inflammatory macrophages⁴⁻⁷. The mechanisms driving this hyperinflammatory state are unclear.

IL1B is a potent inflammatory cytokine that is produced in a two-step process⁸: induction of pro-IL1B, and activation of the inflammasome, which is necessary for conversion of pro-caspase-1 to caspase-1 that cleaves pro-IL1B into its mature form⁹. Recent work implicates IL1 in the development of colitis and T_H17-associated responses in the gut¹⁰. We hypothesized that IL10R deficiency results in dysregulated inflammasome activation leading to excessive IL1 secretion and subsequent mucosal inflammation.

Rag1^{-/-}*Il10rb*^{-/-} mice develop severe colitis following transfer of unfractionated wild-type (WT) CD4⁺ T cells, associated with increased IL1B in the colon (**Figure S1**). To assess the role of innate immune IL1B production in mediating colitis in IL10R deficiency through modulation of effector CD4⁺ T cells, we compared transfer of WT and *Il1r*^{-/-} total CD4⁺ T cells into *Rag1*^{-/-}*Il10rb*^{-/-} mice. Mice adoptively transferred with *Il1r*^{-/-} cells displayed an attenuated course of colitis, accompanied by a significant reduction in the number of interferon-gamma (IFN γ -), tumor necrosis factor-alpha (TNF α -) and IL17A-producing CD4⁺ T cells and an increase in regulatory T cell (Treg) frequency in the lamina propria (**Figure 1A-D, Figure S2**). Overall, our data indicates that production of innate IL1 facilitates the ability of CD4⁺ T cells to induce colitis in *Rag1*^{-/-}*Il10rb*^{-/-} mice.

We next sought to determine whether neutralizing IL1 may be beneficial in controlling inflammation in patients with IL10R deficiency. Two patients aged 2 and 28 years with history of severe infant-onset medical-refractory colitis and fistulizing disease due to loss-of-function mutations in *IL10RA* were treated with anakinra, an IL1 receptor antagonist. Both patients displayed marked clinical, endoscopic and histological improvement in response to therapy after 4-7 weeks (**Figure 1E; Supplemental Document 1**). Analysis of intestinal biopsies in both patients showed a substantial reduction in inflammatory transcripts (**Figure 1F**). The clinical improvement in the first patient permitted a reduction in her steroid requirement and a successful bridge to allogeneic hematopoietic stem cell transplantation (HSCT). The second patient is currently being evaluated for transplant. This treatment strategy, to our knowledge, is the first therapeutic approach leading to reduced intestinal inflammation in the setting of IL10R deficiency.

To assess the role of IL10 in regulating IL1B production, bone-marrow derived macrophages (BMDM) from *WT* and *Il10rb*^{-/-} mice and monocyte-derived macrophages from ten control subjects and five IL10R-deficient patients (patients 1-5 in **Table S1**) were stimulated with LPS. ATP was used as a secondary signal for inflammasome activation. In both murine and human IL10R-deficient macrophages, LPS+ATP triggered increased IL1B production that was not suppressed by IL10 pre-treatment, compared to WT/control macrophages (**Figure 2A-D**). Similarly, stimulation of human control macrophages with LPS+ATP in the presence of a blocking IL10R1 antibody also resulted in increased production of IL1B (**Figure 2C-**

D). Western blot analyses demonstrated that IL10 suppresses production of pro-IL1B and conversion of pro-caspase-1 to its mature form in both mouse and human macrophages (**Figure 2B+2D**). In contrast to murine macrophages, in human IL10R-deficient macrophages LPS stimulation alone, in the absence of ATP, resulted in IL1B production (**Figure 2C-D**), suggesting that addition of a secondary inflammasome activation signal is not required in the setting of human IL10R deficiency. Overall, these data indicate that IL10R signaling regulates inflammasome-dependent IL1 β production in murine and human macrophages.

To understand how IL10 regulates IL1B production we examined the effect of IL10 on the expression of NLRP3 and ASC, two important components of the inflammasome complex. While LPS stimulation upregulated the expression of murine NLRP3 in WT macrophages, NLRP3 mRNA and protein levels were inhibited by IL10 (**Figure S3**). This inhibition of NLRP3 expression was IL10-dependent as it was abrogated in murine IL10R-deficient macrophages and in human macrophages in the presence of blocking IL10R1 antibodies (**Figure S3**). ASC expression was unchanged following LPS or IL10 stimulation (**Figure S3**). Interestingly, treatment with MG132, an inhibitor of proteasome- and autophagy-mediated proteolysis, partially blocked IL10-dependent down-regulation of NLRP3 in murine and human WT/control macrophages (**Figure 2E**), indicating that IL10 modulates NLRP3 fate by enhancing proteasomal degradation. We demonstrated that IL10 promotes K48-linked polyubiquitination of the NLRP3 complex further supporting a role for IL10 in regulating inflammasome protein degradation (**Figure S3**). Together these data identify IL10 as a critical transcriptional and post-translational regulator of NLRP3-mediated inflammasome activation.

One of the main differences between human and murine IL10R-deficient macrophages was the ability of the former to produce IL1B following LPS activation, without a secondary inflammasome activation trigger (such as ATP). Recently it has been shown that human monocytes can produce IL1B in response to LPS alone through a TLR4-TRIF-RIPK1-FADD-CASP8 mediated non-canonical (alternative) activation of the inflammasome¹¹. To examine whether IL10 regulates alternative inflammasome signaling we used Z-IETD-FMK, a caspase-8 small-molecule inhibitor that selectively blocks non-canonical signaling, Z-YVAD-FMK, a caspase-1 inhibitor that blocks both canonical and non-canonical signaling, and KN-62, a P2RX7 inhibitor that blocks only canonical, ATP-induced, inflammasome signaling. Blocking non-canonical inflammasome activation with either Z-IETD-FMK or Z-YVAD-FMK completely abrogated LPS-induced IL1B production in both monocyte-derived macrophages from an IL10RA-deficient patient and anti-IL10R1 treated control macrophages (**Figure 2F**). In contrast, LPS-induced IL1B production in these cells was not abrogated with KN-62, which blocks only canonical inflammasome activation (**Figure 2F**). These findings suggest that in human macrophages IL10 can suppress production of IL1B through non-canonical activation of the inflammasome, and that in the absence of this inhibitory signal macrophages can produce IL1B in response to LPS alone.

Our results highlight the central role of IL10 in regulating inflammasome-mediated IL1B production. We demonstrate that IL1 signaling in T cells drives colitis in the absence of IL10R. Mechanistically, we show that IL10 regulates NLRP3 both through transcriptional

regulation as well as proteasomal degradation, at least in part through K48-linked polyubiquitination. The murine data is consistent with recent reports that normal IL10R signaling is required to suppress NLRP3-dependent inflammasome activation and pro-IL1B production, potentially through autocrine IL10 production^{7, 12-15}. We further demonstrate that intact IL10R signaling is required to suppress IL1B production in human macrophages, but in contrast to mice, loss of signaling is sufficient to trigger release of IL1B even in the absence of a secondary inflammasome activation signal. This is likely mediated through non-canonical inflammasome activation of caspase-8.

IL10- and IL10R-deficient patients fail to respond to immunosuppressive medications^{2, 3}. Our data suggests that blocking IL1 in these patients may be beneficial as a bridge to an allogeneic HSCT or potentially longer if a suitable donor is unavailable. In both patients treatment was well tolerated without side effects. Though not thoroughly studied, neutralizing IL1 therapies in IBD have been shown to be effective in selected cases¹⁶. While we demonstrated successful outcome of anakinra therapy in two patients, a prior report of anakinra treatment in an IL10R-deficient patient suggested it was ineffective¹⁷. Given the central role of IL10 in suppressing inflammasome-mediated IL1B production, additional clinical studies are needed to assess whether IL1 blockade can be used effectively in IL10/IL10R deficiency or more broadly in IBD patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. IL1 is a key factor driving colitis in IL10R deficiency

Rag1^{-/-}*Il10rb*^{-/-} mice were transferred with WT or *Il1r*^{-/-} total CD4⁺ T cells. (A) Mean percent initial body weights ± SEM following transfer (n>20 per group). (B-C) Representative macroscopic and H&E images (10X) and histological scores ± SEM. Scale bar represents 200μm. (D) Absolute numbers of effector CD4⁺ T cells in lamina propria of *Rag1*^{-/-}*Il10rb*^{-/-} mice post transfer. (E) Representative endoscopic and histologic images (100X) of descending colon of an IL10RA-deficient toddler pre- and post-anakinra therapy. (F) Transcript analysis by RT-PCR of colonic biopsies of an IL10RA-deficient toddler (top) and by RNA *in situ* hybridization (bottom image, 40X magnification on left side; dashed square indicates the magnified area depicted in the images on the right) of ileal biopsies of an IL10RA-deficient adult patient, before and after anakinra therapy. * p < 0.05; *** p < 0.001.

Figure 2. IL10 has a central role in suppressing inflammasome-mediated IL1B production in macrophages

Caspase-1 and IL1B production in (A-B) WT and *Il10rb*^{-/-} BMDM, and (C-D) monocyte-derived macrophages from control subjects (n=10) and IL10R-deficient patients (n=5 except samples LPS→ATP and IL10→LPS+ATP n=2) following different stimuli as indicated, determined by ELISA and western blot. Blocking IL10Ra and IL10R1 antibodies were used in murine WT macrophages and in human control macrophages, respectively, to mimic IL10R deficiency. (E) Murine (top) and human (bottom) macrophages were stimulated with LPS and IL10 in the absence or presence of MG132 proteasomal inhibitor. Figure displays NLRP3 expression. (F) Non-canonical activation of the inflammasome in human monocyte-derived macrophages was assessed by stimulating cells with LPS in the presence of different small molecules, as indicated. Figure shows IL1B production determined by ELISA. Western blot images and ELISA experiments are representative of 2-3 independent experiments. ** p < 0.01; *** p < 0.001.