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Interleukin 10 Receptor Signaling: Master Regulator of Intestinal Mucosal Homeostasis in Mice and Humans

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Abstract

Interleukin 10 (IL10) is a key anti-inflammatory cytokine that can inhibit proinflammatory responses of both innate and adaptive immune cells. An association between IL10 and intestinal mucosal homeostasis became clear with the discovery that IL10 and IL10 receptor (IL10R)-deficient mice develop spontaneous intestinal inflammation. Similarly, patients with deleterious mutations in *IL10*, *IL10RA*, or *IL10RB* present with severe enterocolitis within the first months of life. Here, we review recent findings on how IL10- and IL10R-dependent signaling modulates innate and adaptive immune responses in the murine gastrointestinal tract, with implications of their role in the prevention of inflammatory bowel disease (IBD). In addition, we discuss the impact of IL10 and IL10R signaling defects in humans and their relationship to very early-onset IBD (VEO-IBD).

1. INTRODUCTION

Interleukin-10 (IL10) is a key anti-inflammatory cytokine that is produced predominantly by leukocytes including T cells, B cells, monocytes, macrophages (Mφs), and dendritic cells (DCs), as well as by some epithelial cells (Medzhitov et al., 2011; Moore, de Waal Malefyt, Coffman, & O'Garra, 2001; Saraiva & O'Garra, 2010). In leukocytes, IL10 acts on both innate and adaptive immune cells and has a broad range of immunomodulatory activities that suppress proliferation, cytokine secretion, and costimulatory molecule expression of proinflammatory immune cells (Donnelly, Dickensheets, & Finbloom, 1999; Murray, 2006). A critical role for IL10 signaling in modulating intestinal mucosal homeostasis became

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evident with the description that IL10-deficient mice develop spontaneous enterocolitis (Kuhn, Lohler, Rennick, Rajewsky, & Muller, 1993). This was subsequently strengthened by the observation that interleukin 10 receptor (IL10R)-deficient mice also develop spontaneous colitis (Spencer et al., 1998). These findings led to an extensive research effort aiming to elucidate the role of IL10-dependent signaling in the regulation of intestinal immune function.

In humans, IL10 and IL10R play critical roles in controlling immune responses in the intestinal mucosa. Single-nucleotide polymorphisms (SNPs) in IL10 have been linked to inflammatory bowel disease (IBD) risk in genome-wide association studies (GWAS) (Franke et al., 2008; Franke, McGovern, et al., 2010; Jostins et al., 2012). In addition, patients with deleterious mutations in either IL10 or its receptor develop severe IBD, usually presenting within the first months of life (Glocker et al., 2009; Kotlarz et al., 2012, Moran et al., 2013). In this chapter, we will review recent findings of how IL10-dependent signaling modulates immune responses, focusing on the role of these signals in the regulation of mucosal homeostasis and prevention of IBD.

2. IL10 AND IL10 RECEPTOR EXPRESSION AND REGULATION

IL10 is the foremost member of the type-II cytokine family, comprising IL19, IL20, IL22, IL24, IL26, IL28, and IL29 (Commins, Steinke, & Borish, 2008). IL10 was first described by Fiorentino et al. as an inhibitor of cytokine synthesis and initially termed “cytokine synthesis inhibitory factor”, as it was released by Th2 cells and inhibited interferon-γ (IFNγ) production by Th1 cells (Fiorentino, Bond, & Mosmann, 1989). Subsequent studies revealed pleiotropic functions of IL10 on various adaptive and innate immune populations (Bhattacharyya et al., 2004; Bogdan, Vodovotz, & Nathan, 1991; de Waal Malefyt et al., 1991; Ding, Linsley, Huang, Germain, & Shevach, 1993; Ding & Shevach, 1992; Fiorentino, Zlotnik, Vieira, et al., 1991; Murphy et al., 1994; Ralph et al., 1992).

Human and mouse IL10 (hIL10 and mIL10, respectively) have roughly 73% sequence homology and are secreted as 178-amino acid proteins (Windsor et al., 1993). While both hIL10 and mIL10 are comprised of noncovalently linked homodimers, (Walter & Nagabhushan, 1995; Zdanov et al., 1995; Zdanov, Schalk-Hihi, Menon, Moore, & Wlodawer, 1997), mIL10 is glycosylated at the N-terminal region and does not activate human cells, whereas hIL10 is not N-glycosylated and can activate both human and mouse cells (Moore et al., 2001; Mosmann et al., 1990). Several innate and adaptive immune cells can secrete IL10 including monocytes, Mφs, DCs, natural killer (NK) cells, mast cells, neutrophils, CD4 and CD8 T cells, and B cells (Moore et al., 2001; O’Garra & Vieira, 2007; Saraiva & O’Gara 2010). For antigen-presenting cells (e.g., DCs, Mφs), production of IL10 is triggered by recognition of various bacterial or viral pathogen-associated molecular patterns by cell surface or cytoplasmic pattern recognition receptors (PRR) (Akbari, DeKruyff, & Umetsu, 2001; de Waal Malefyt et al., 1991; Fiorentino, Zlotnik, Vieira, et al., 1991; Siewe et al., 2006). Studies have shown that engagement of transmembrane PRRs, known as Toll-like receptors (TLRs) (i.e., TLR2, TLR3, TLR4 TLR9) lead to production of IL10 by Mφs and myeloid DCs (Agrawal et al., 2003; Boonstra et al., 2006; Dillon et al., 2004; Netea et al., 2004). Among the cytosolic PRRs, ligation of nucleotide-binding

oligomerization domain-containing protein 2 (NOD2) induces IL10 expression (Moreira et al., 2008). Interestingly, a nonfunctional frameshift mutation in NOD2 blocks IL10 transcription and is associated with Crohn's disease (CD) (Noguchi, Homma, Kang, Netea, & Ma, 2009). Other than TLRs and (NOD)-like receptors, stimulation of C-type lectins, DC-specific ICAM3-grabbing nonintegrin (DC-SIGN), and dectin 1 also induce IL10 production (Geijtenbeek et al., 2003; Rogers et al., 2005). In addition to PRRs, several cytokines such as IL21 produced by Th1 primed cells (Spolski, Kim, Zhu, Levy, & Leonard, 2009) and IL27 produced by Th1, Th2, and Th17 cells can increase IL10 expression via STAT1- and STAT3-dependent mechanisms (Batten et al., 2008; Fitzgerald et al., 2007; Pot et al., 2009; Stumhofer et al., 2007; Xu et al., 2009). In contrast, it has also been reported that IL27 can inhibit TLR-mediated IL10 production in human monocytes (Kalliolias & Ivashkiv, 2008).

The receptor for IL10 is a heterotetramer complex comprising two IL10Rα (also referred to as IL10R1) molecules (encoded by the *Il10ra* gene) and two IL10Rβ (also referred to as IL10R2) molecules (encoded by the *Il10rb* gene) (Kotenko et al., 1997; Moore et al., 2001). All IL10-responsive cells express IL10Rα, with antibody-mediated blockade of the surface receptor inhibiting its responsiveness (Ho et al., 1993; Liu et al., 1997; Liu, Wei, Ho, de Waal Malefyt, & Moore, 1994). IL10Rα is expressed on most hematopoietic cells at a basal level but is upregulated by various cells upon activation, suggesting its importance in inhibitory pathways. For example, at steady state, naïve $CD4^+$ T cells have low IL10Rα expression, but *in vivo* anti-CD3 treatment induces IL10Rα expression on Th17 cells in the small intestine (Huber et al., 2011). In addition, *in vitro* stimulation of naïve $CD45RB^{high}$ T cells, memory/effector T cells, and regulatory T cells (Tregs) leads to upregulation of IL10Rα expression (Kamanaka et al., 2011). Similarly, under basal conditions, human neutrophils express low levels of IL10R1; however, following lipopolysaccharide (LPS) or IL4 stimulation IL10R1 expression is upregulated (Crepaldi et al., 2001). Corinti and colleagues have shown that human DCs become unresponsive to IL10 after maturation by downregulating IL10R1 surface expression enabling them to produce higher levels of proinflammatory mediators and to prime T cells (Corinti, Albanesi, la Sala, Pastore, & Girolomoni, 2001). IL10Rα can also be induced in some nonhematopoietic cells such as fibroblasts upon activation with LPS (Weber-Nordt, Meraz, & Schreiber, 1994) as well as being constitutively expressed in colonic epithelial cells (Bourreille et al., 1999; Denning et al., 2000).

3. DOWN-STREAM SIGNALING THROUGH THE IL10 RECEPTOR

IL10Rα is 90–120 kDa and serves as the ligand binding subunit of the receptor complex (Liu et al., 1997, 1994; Tan, Indelicato, Narula, Zavodny, & Chou, 1993). IL10Rβ is the signaling subunit of the IL10R complex and is constitutively expressed in most cell types (Gibbs & Pennica, 1997; Kotenko et al., 1997; Moore et al., 2001). Earlier studies have suggested that IL10Rβ has almost no role in IL10-binding; its main role is to recruit the downstream signaling kinases (Kotenko et al., 1997; Spencer et al., 1998). More recent studies have found that upon binding to IL10, IL10Rα induces a conformational change in IL10Rβ, permitting IL10Rβ to also bind IL10 (Yoon, Logsdon, Sheikh, Donnelly, & Walter, 2006). Unlike IL10Rα, which is unique to IL10, the IL10Rβ-subunit is shared by receptors for other type-II cytokines including IL22, IL26, and INFλ.

The sequence of receptor assembly is initiated by IL10 binding to IL10Rα (Fig. 5.1). This complex then binds IL10Rβ forming a heterotetramer, permitting the assembly of the signaling complex (Yoon et al., 2006). Once the complex is assembled, tyrosine kinases Jak1 and Tyk2 that are constitutively associated with IL10Rα and IL10Rβ, respectively, are activated and phosphorylate specific tyrosine residues in the intracellular domain of IL10Rα. Phosphorylation of the receptor leads to the recruitment of signal transducer and activator of transcription 3 (STAT3) (Kotenko et al., 1997; Liu et al., 1994; Murray, 2007). Following their recruitment, JAK1 and TYK2 phosphorylate STAT3, leading to its homodimerization and subsequent translocation to the nucleus, where it binds to STAT3-binding elements of IL10-responsive genes (Finbloom & Winestock, 1995; Murray, 2007; Rodig et al., 1998; Williams, Ricchetti, Sarma, Smallie, & Foxwell, 2004). STAT3 also induces the expression of suppressor of cytokine signaling 3 (SOCS3), which inhibits PRR-induced expression of various inflammatory cytokines including TNF, IL6, and IL1β (Berlato et al., 2002; Murray, 2006; Williams, Bradley, Smith, & Foxwell, 2004). While both IL10 and IL6 highly induce STAT3-dependent SOCS3 expression in Mφs, the inhibitory role of SOCS3 appears to be restricted to IL6 (Croker et al., 2003; Lang et al., 2003; Murray, 2006; Nicholson et al., 2000; Schmitz, Weissenbach, Haan, Heinrich, & Schaper, 2000; Williams, Ricchetti, et al., 2004; Yasukawa et al., 2003). In this scenario, a proinflammatory cytokine (IL6) and an anti-inflammatory cytokine (IL10) signal through a shared transcription factor. While SOCS3 may play a role in driving-specific outputs, a detailed molecular understanding responsible for these differences remains unknown (Murray, 2007). In addition to STAT3, IL10-receptor activation of STAT1 and STAT5 have been reported (Finbloom & Winestock, 1995; Lai et al., 1996; Moore et al., 2001; Weber-Nordt et al., 1996). While much knowledge has been gained in recent years, the broad effects of IL10-mediated activation of STAT1 and STAT5 remain unclear (Miura et al., 2006; Williams, Ricchetti, et al., 2004).

4. REGULATION OF INTESTINAL IMMUNE RESPONSES BY IL10 IN MURINE MODELS

Intestinal homeostasis is a highly dynamic process requiring sensitivity to mount appropriate immune responses toward microbial or food antigens, yet necessitating the regulation of these responses in order to prevent chronic inflammation. A critical role for IL10 in maintaining gut homeostasis was first evident in the original description of IL10-deficient mice that developed spontaneous enterocolitis due to immune hyperactivation elicited by intestinal microbial antigens (Kuhn et al., 1993). Similar implications for IL10 and intestinal homeostasis were also demonstrated using a T-cell transfer model of colitis. In this early work by Powrie and colleagues, wild-type (WT) $CD4^+CD45RB^{high}$ T cells were transferred into lymphopenic severe combined immunodeficiency (SCID) mice to induce colitis, but the disease was abrogated in a cohort of mice-administered exogenous recombinant IL10 (Powrie et al., 1994). This effect was recapitulated in a transfer setting where the $CD4^+CD45RB^{high}$ T cells containing an IL10 transgene driven by an IL2 promoter prevented the development of colitis (Hagenbaugh et al., 1997). While this shows that IL10 production by an immune cell population is sufficient to prevent colitis, it does not exclude a role for IL10 production in nonleukocyte populations in mucosal tissues. In this regard,

transgenic expression of IL10 in intestinal epithelial cells protects mice from colitis induced by either dextran sodium sulfate (DSS) or $CD4^+CD45RB^{high}$ T cells transfer (De Winter et al., 2002). Intestinal epithelial cells were also reported to produce IL10 following antibody-mediated CD1d cross-linking (Colgan, Hershberg, Furuta, & Blumberg, 1999). A more recent study has shown that depletion of IL10 in mucosal explants leads to downregulation of IL10 inducible genes and upregulation of IFNγ, TNF, and IL17 (Jarry et al., 2008). Finally, intragastric administration of IL10-producing *Lactococcus lactis* also protects mice from DSS-induced colitis and can prevent development of spontaneous colitis in IL10-deficient mice (Steidler et al., 2000). Together these studies delineate the significance of IL10 in the regulation of intestinal homeostasis. The role of IL10 in regulating innate and adaptive immune response is discussed in the next sections.

4.1. IL10-dependent regulation by innate immune cells

Innate immune responses in the intestine are largely directed toward microbes or virions, which trigger activation of antigen-presenting cells (e.g., DCs, Mφ) via PRRs. Signaling through PRRs is a critical first line of defense against potential pathogens. However, PPR signaling may result in aberrant activation of antigen presenting cells (APCs) and the development of chronic intestinal inflammation in the setting of specific genetic susceptibilities. PRR-mediated inflammatory responses can be regulated by IL10 (Boonstra et al., 2006; Chang, Guo, Doyle, & Cheng, 2007; Fiorentino, Zlotnik, Mosmann, Howard, & O'Garra, 1991). In the absence of IL10, PRR-induced proinflammatory expression is markedly increased in innate immune cells. One specific IL10-mediated regulatory mechanism occurs through abrogating MyD88-dependent responses by inducing proteosomal degradation of downstream signaling molecules IL1 receptor-associated kinase 4 (IRAK4) and TNF-receptor-associated factor 6 (TRAF6) (Chang, Kunkel, & Chang, 2009). Though microbial communities are required for driving intestinal inflammation in IL10-deficient mice, the essential role for APCs in this process was only recently investigated. The best evidence to date came when deletion of MyD88 in either $LysM^+$ or $CD11c^+$ mononuclear phagocytes in IL10-deficient mice proved to be protective against colitis (Hoshi et al., 2012). These data suggest that upon PRR triggering, defective IL10 signaling in APCs results in the acquisition of a proinflammatory state and/or loss of tolerance. In contrast, deletion of TLR4 in combination with IL10 exaggerates intestinal inflammation due to dysregulation of epithelial turnover leading to accumulation of apoptotic cells in the lamina propria implicating a protective role in TLR4 signaling (Matharu et al., 2009).

A direct role for innate immune cell dependent secretion of IL10 in regulating mucosal homeostasis was first demonstrated by Murai et al. who showed that IL10-deficient $Rag1^{-/-}$ failed to permit suppression of $CD4^+CD45RB^{high}$ T cells by $Foxp3^+$ Tregs (Murai et al., 2009). Similar studies were reported by Liu et al. who showed that IL10-deficient $Rag2^{-/-}$ mice are more susceptible to $CD4^+$ T-cell transfer-induced colitis than IL10-suficient $Rag2^{-/-}$ mice (Liu, Tonkonogy, & Sartor, 2011). A $CD11b^+$ myeloid cell population in the lamina propria, found to secrete large amounts of IL10, was suggested as a critical cell population in the intestine-regulating immune homeostasis (Murai et al., 2009). Intestinal Mφs constitutively produce IL10 and are generally hypo-responsive to TLR-mediated

stimulation in terms of inflammatory cytokine production (Kamada et al., 2005). The precise role of specific IL10-secreting Mφs in regulating mucosal homeostasis is not known. Work by Takeda's group recently showed that intestinal $CX3CR1^{high}CD11b^{+}CD11c^{+}$ cell populations suppress intestinal inflammation by inhibiting T-cell responses. This process may be mediated by IL10 signaling since protection was abrogated when these cells were isolated from myeloid cell-specific STAT3-deficient mice (Kayama et al., 2012).

Even when IL10 is absent from innate immune cells, T cells are required for colitis induction (Liu et al., 2011; Murai et al., 2009). Increasing data suggest that innate immune cell-derived IL10 is required for the maintenance and/or function of Tregs (Liu et al., 2011; Murai et al., 2009). One study demonstrated that the $CD11b^{+}$ myeloid cell-derived IL10 can regulate Foxp3 expression in Tregs *in vitro* (Murai et al., 2009). These authors also showed that myeloid cell-specific deletion of IL10 leads to reduced T reg numbers after *Citrobacter rodentium* infection compared to controls (Murai et al., 2013). These data point to a critical role for myeloid cell-derived IL10 in the generation and/or maintenance of Tregs and bridge a critical gap in the cross talk between the innate and adaptive immune systems in mucosal homeostasis. Several groups have suggested that intestinal $CX3CR1^{high}F4/80^{+}$ myeloid cell population secretes IL10 at steady state (Bain et al., 2013; Rivollier, He, Kole, Valatas, & Kelsall, 2012; Zigmond et al., 2012). These cells continue to produce high levels of IL10 in the presence of inflammation, both *in vivo* during DSS challenge (Zigmond et al., 2012) and *in vitro* following LPS stimulation (Bain et al., 2013). $CX3CR1^{high}$ cells have also been shown to express high levels of several IL10-inducible genes, such as CD163 and CD209, and Kayama et al. have recently reported that their transfer to SCID recipient can prevent $CD45RB^{high}$-associated colitis (Kayama et al., 2012).

4.2. IL10-dependent regulation by adaptive immune cells

Chronic inflammation of the gut is often associated with expansion of Th1, Th2, or Th17 cells. IL10 signaling can inhibit the expansion of these pathological helper-T-cell populations to promote immune homeostasis. Many effector helper-T-cells can also produce IL10 under specific stimulating conditions. For instance, Th1 cells can produce IL10 in the presence of a strong T-cell receptor signal and APC-derived IL12 (Saraiva et al., 2009). Th2 cells produce IL10 in the presence of IL4-dependent STAT6 activation, and Th17 cells produce IL10 downstream of STAT3 activation (Fitzgerald et al., 2007; McGeachy et al., 2007; Stumhofer et al., 2007). Although effector T cells can produce IL10 in certain conditions, $CD4^{+}$ T-cell activation and proliferation are typically globally inhibited by IL10-dependent suppression of APCs (de Waal Malefyt et al., 1991; Fiorentino, Zlotnik, Vieira, et al., 1991). While this seems to be redundant, IL10 expression by activated T cells may ensure another negative feedback loop to avoid pathogenic T-cell expansion.

Treg-derived IL10 is critical for the maintenance of immunological tolerance in the intestinal mucosa (Rubtsov et al., 2008). Under steady state, Tregs in the spleen and mesenteric lymph nodes (MLNs) express very little IL10. However, lamina propria Tregs express high amounts of IL10 accounting for nearly 30% of the IL10-producing $CD4^{+}$ T cells in the small intestine (Maloy et al., 2003; Tiittanen, Westerholm-Ormio, Verkasalo, Savilahti, & Vaarala, 2008; Uhlig et al., 2006). Loss of IL10 in $Foxp3^{+}$ Tregs is sufficient to

promote the development of colitis in mice, albeit to a lesser extent than global IL10 deficiency when mice are colonized with *Helicobacter* spp. (Roers et al., 2004; Rubtsov et al., 2008). However, these mice do not develop systemic autoimmunity, which further indicates the regulatory specificity of Treg-derived IL10 in the intestinal mucosa (Rubtsov et al., 2008). In contrast, some, but not all studies, have suggested that IL10-deficient $CD4^+CD45RB^{low}$ Tregs can function normally in the suppression of $CD4^+CD45RB^{high}$ T-cell-induced colitis in SCID mice (Asseman, Mauze, Leach, Coffman, & Powrie, 1999; Murai et al., 2009). These studies suggest unique characteristics of $Foxp3^+$ and $CD4^+CD45RB^{low}$ regulatory T-cell subsets.

Finally, although much of the attention regarding IL10 secretion has focused primarily on myeloid cells and T lymphocytes, B cells can also produce IL10 and possess regulatory functions. A special subset of B cells, termed B10 regulatory B cells (B10 cells), secrete IL10 and upregulate CD1d under chronic inflammatory conditions, which serves to suppress intestinal inflammation (DiLillo, Matsushita, & Tedder, 2010; Mauri & Bosma, 2012; Mizoguchi, Mizoguchi, Takedatsu, Blumberg, & Bhan, 2002; Yanaba et al., 2008).

5. REGULATION OF MUCOSAL HOMEOSTASIS BY IL10 RECEPTOR SIGNALING IN MICE

A critical role for IL10R signaling in the regulation of mucosal homeostasis was demonstrated over 15 years ago with the report that mice on a mixed 129SvEv/C57BL/6 background devoid of *Il10rb* develop colonic, but not small bowel, inflammation (Spencer et al., 1998). More recent studies have shown that IL10Rβ-deficient mice on the C57BL/6 background develop mild disease at 3–4 months of age, which becomes pronounced when TGFβ signaling in T cells is also impaired (Kang et al., 2008). Our own unpublished studies indicate that $Il10rb^{-/-}$ mice on the 129SvEv background develop moderate to severe colitis by 2–4 months of age, characterized by marked crypt hyperplasia, lamina propria infiltration, presence of crypt abscesses (Fig. 5.2). Since IL10Rβ is shared by several other important cytokine receptors (IL22R, IL26R, IFNλR) (Pestka et al., 2004), the mucosal inflammation observed in these aforementioned studies may result, at least in part, by loss or impairment of cytokine signaling by these additional members. In support of this hypothesis, IL10Rα-deficient mice generated on the C57BL/6 background do not develop colitis spontaneously (Pils et al., 2010), similar to IL10-deficient mice which are also resistant to colitis on this strain (Bristol et al., 2000; Farmer et al., 2001). Somewhat paradoxically, IL10Rα deletion limited to Tregs was associated with the development of severe spontaneous colitis (Chaudhry et al., 2011). While these results are rather perplexing, the intestinal flora can differ among animal facilities, altering microbial exposure and the development of intestinal inflammation in mice lacking IL10 or IL10R (see discussion below). Thus, phenotypic disparities between strains and studies may be impacted most from variations in microbial communities across institutions. Taken together, these data, complemented by similar clinical presentation and sequelae in *IL10RA*- or *IL10RB*-deficient patients, solidify the integral role IL10R-signaling plays in maintaining immunological tolerance in the intestinal mucosa (Begue et al., 2011; Engelhardt et al., 2013; Glocker et al.,

2009; Kotlarz et al., 2012; Moran et al., 2013). Studies investigating the specific cell types requiring IL10R signaling are addressed in the following sections.

5.1. IL10R signaling regulates T-cell function

IL10R signaling on Tregs has been shown by several groups to be critical for the regulation of intestinal immune responses. Murai and colleagues cotransferred *Il10rb*$^{-/-}$ Tregs with WT CD4^{+}CD45RB^{high} T cells and showed that these Tregs failed to suppress colitis (Murai et al., 2009). In these experiments, Tregs lost Foxp3 expression over time but not when transferred in the absence of CD4^{+}CD45RB^{high} cells, suggesting that IL10 signaling in Tregs is important for maintenance of Foxp3 expression under inflammatory conditions. Similarly, Chaudhry et al. demonstrated that mice with a targeted deletion of *Il10ra* in Foxp3^{+} Tregs develop severe spontaneous colitis, associated with a marked increase in memory T cells and a selective impairment in suppression of Th17^{+} cells (Chaudhry et al., 2011). Interestingly, Foxp3 expression was maintained in these *Il10ra*-deficient Tregs, even in the presence of inflammation. These differences may be explained by inherent limitations associated with the CD45RB transfer model and/or the effect of different microbiota on the intestinal immune system. Likewise, we reported the presence of Foxp3^{+} Tregs in the blood of a patient harboring an *IL10RA* null mutation, suggesting that IL10R signaling is dispensable for the development of Foxp3^{+} Tregs (Moran et al., 2013). However, further studies are needed to assess the function of Tregs in the blood and tissue of IL10R-deficient patients.

IL10 sensing by effector T cells is also important for intestinal mucosal homeostasis. Asseman and colleagues were the first to show that IL10R-blocking antibodies administered to SCID mice previously transferred with CD4^{+}CD45RB^{low} cells results in intestinal inflammation (Asseman et al., 1999), suggesting that within this fraction of T cells, now known to contain Foxp3^{+} regulatory cells, are pathogenic cells responsive to IL10. This finding was corroborated when Kamanaka et al. transferred IL10R-deficient CD4^{+}CD45RB^{low}Foxp3-delpeted T cells, which escaped control by Tregs and elicited a Th17-mediated colitis in lymphopenic hosts (Kamanaka et al., 2011). Furthermore, colitis induction by the transfer of Th17 cells *Rag1*$^{-/-}$ recipient mice is prevented by the cotransfer of Foxp3^{+} Tregs or IL10^{+}Foxp3^{-} Tr1 cells (Huber et al., 2011). However, such suppression requires functional expression of IL10R on Th17 cells, since abrogation of IL10R function on T cells is associated with expansion of both IL17A^{+}IFNγ^{-} and IL17A^{+}IFNγ^{+} subsets (Huber et al., 2011). These studies underscore the importance of IL10R-dependent signals on restricting Th17 responses. The role of IL10R signaling on Th1 cells is less clear. Kamanaka and colleagues showed that among CD45RB^{high} cells, which are known to trigger a Th1 response when adoptively transferred into lymphopenic hosts, IL10R signaling was not required for the T-effector cells to receive suppressive signals by WT Tregs (Kamanaka et al., 2011). However, we have observed in our *l10rb*$^{-/-}$ mouse colony expansion of both Th1^{+} and Th17^{+} CD4^{+} T cells in the lamina propria (Fig. 5.3), suggesting that IL10R might regulate proliferation of both Th1 and Th17 subsets.

5.2. IL10R signaling regulates innate immune cell function

Recent data presented above describe an important role of IL10R signaling in maintaining intestinal mucosal homeostasis in various T-cell subsets. For many years, it has been known that IL10 decreases the expression of MHCII and costimulatory molecules on DCs and Mφ, as well as their proinflammatory cytokine secretion (Bhattacharyya et al., 2004; Corinti et al., 2001; Fiorentino, Zlotnik, Mosmann, et al., 1991; Steinbrink, Wolfl, Jonuleit, Knop, & Enk, 1997). In addition, pretreating immature DCs *in vitro* with IL10 decreases their capacity to stimulate $CD4^+$ T cells in a dose-dependent manner. However, the role of IL10R-dependent signaling in innate immune cells at mucosal surfaces such as the gastrointestinal tract has not been well defined. Mellilo and colleagues reported that DC-specific loss of STAT3 signaling, which is downstream of the IL10R, leads to mild small- and large-intestine inflammation (Melillo et al., 2010). However, STAT3 is also a component of the signaling cascade of other cytokines, such as IL6, and therefore the phenotype observed in these mice might not be solely due to loss of IL10R signaling. Nevertheless, evidence from other immune compartments suggests that IL10R signaling plays an important role in differentiation and function of various innate immune cells. *Il10ra*-targeted deletion in monocytes/Mφs (LysM-Cre^+-*Il10ra*$^{flox/flox}$ mice) leads to enhanced susceptibility to an LPS-induced model of endotoxemia, with elevated serum levels of IL17, TNF, IL1, and IL12 (Pils et al., 2010). In addition, employing CD11c-Cre^+-*Il10ra*$^{flox/flox}$ mice, Girard-Madoux et al. assessed the role of IL10Rα in DCs (Girard-Madoux, Kel, Reizis, & Clausen, 2012). At steady state, IL10Rα-deficient DCs retain an immature phenotype and express similar levels of costimulatory molecules compared to nontransgenic mice. However, LPS or sCD40L stimulation results in enhanced DC secretion of IL6 and TNF. In both transgenic mouse models, secretion of IL10 by stimulated DCs or Mφs was enhanced, implying that there might be an autocrine feedback regulating IL10 production in these cells. These investigators also assessed the role of IL10R signaling on DCs in different phases of a contact hypersensitivity model. They demonstrate that priming of T cells by DCs in the skin draining lymph node does not require IL10R signaling in DCs, as CD11c-Cre^+-*Il10ra*$^{flox/flox}$ mice have similar ear swelling 24 h following hapten challenge. However, T-effector cell responses in the skin of mice 48 h after challenge were enhanced with exaggerated ear swelling. These data suggest that autocrine or paracrine sensing of IL10 by DCs is critical to limiting T-effector-induced inflammation.

IL10R signaling has also been shown to orchestrate the development of monocytes into different subsets of peritoneal Mφs (Nguyen, Tran, Muller, & Jack, 2012). During early phases of peritonitis, IL10 expression is increased, and monocytes that migrate into the peritoneum develop into $MHCII^{low}$ Mφs, while in the later stages when IL10 levels are decreased, monocytes differentiate into $MHCII^{high}$ Mφ. WT monocytes transferred into *Il10ra*-deficient mice develop into both MHC^{low} and MHC^{high} Mφ, while host monocytes develop almost exclusively into MHC^{high} Mφ. This is supported by lack of $MHCII^{low}$ monocytes in the peritoneum of IL10-deficient mice. It remains to be determined whether IL10R-dependent signals regulate myeloid cell differentiation in other immune compartments, especially at mucosal surfaces.

IL10 can also modulate responses of neutrophils and NK cells. In non-inflammatory environments, neutrophils do not express IL10Rα and are therefore unresponsive to IL10 (Crepaldi et al., 2001). However, following *in vitro* stimulation of human neutrophils with LPS or IL4, or in neutrophils isolated from septic patients (Crepaldi et al., 2001; Tamassia et al., 2008), IL10Rα is synthesized, allowing for modulation of cytokine production by IL10 and enhanced expression of IL1RA, a soluble factor that competes with cognate receptors for proinflammatory mediators IL1α and IL1β (Crepaldi et al., 2001). In NK cells, IL10 has a unique role in which it delivers a stimulatory signal rather than a suppressive one. IL10, possibly in combination with IL18, has been shown to enhance cell proliferation and cytotoxic activity (Cai, Kastelein, & Hunter, 1999; Mocellin et al., 2004). The role of IL10 in modulating intestinal neutrophils and NK cell function at baseline and in the setting of inflammation at the present time remains unknown.

6. IL10-DEPENDENT SIGNALING SHAPES THE INTESTINAL MICROBIOME

The initial characterization of IL10-deficient mice pointed to a critical role for the intestinal flora in regulating disease severity and location (Kuhn et al., 1993). Conventionally, housed IL10-deficient mice develop severe enterocolitis while disease in specific pathogen free (SPF) conditions is restricted to the colon. Interestingly, SPF IL10-deficient mice free of *Helicobacter* species do not develop colitis (Burich et al., 2001). Moreover, germfree IL10-deficient mice are also free of intestinal inflammation (Sellon et al., 1998). Subsequently, considerable effort has been focused on identifying single bacterial strains sufficient to drive disease initiation in IL10-deficient mice. Extensive work from the Sartor laboratory characterized germ-free IL10-deficient mice mono-colonized with various bacterial strains. While most individual strains failed to induce intestinal inflammation including *Helicobacter hepaticus*, *Pseudomonas fluorescens*, *Candida albicans*, *or L. lactis*, monocolonization with *Enterococcus faecalis*, *E. coli*, or *Bifidobacterium animalis* is each sufficient to induce intestinal inflammation, albeit with different kinetics and anatomical locations (Dieleman et al., 2000; Kim, Tonkonogy, Karrasch, Jobin, & Sartor, 2007; Moran, Walter, Tannock, Tonkonogy, & Sartor, 2009). This is not an artifact of mono-association since colonization of germfree WT mice with any of these strains does not induce intestinal inflammation. Further support for microbial stimulation influencing IL10-dependent disease initiation comes from more recent work by Stappenbeck and colleagues using a non-germ-free approach which demonstrated that *IL10rb*$^{-/-}$ mice defective in T-cell TGFβ signaling (CD4-dn*Tgfbr2 Il10rb*$^{-/-}$), following a course of antibiotic treatment, developed severe colitis upon colonization with commensal *Bacteroides* species (*B. thetaiotaomicron* and *B. vulgatus*) (Bloom et al., 2011). It is noteworthy that although *Helicobacter* spp. is required for colitis development in *IL10*$^{-/-}$ mice, *Helicobacter* spp. alone is insufficient to cause disease (Dieleman et al., 2000). This suggests that the role of *Helicobacter* spp. in colitis associated with IL10 deficiency may be attributed to alterations in the microbial community or changes in bacterial metabolism that, in turn, impact immunological tolerance. Interestingly, although *Enterobacteriaceae* species are elevated in colitis, colonization of CD4-dn*Tgfbr2.Il10rb*$^{-/-}$ mice with these organisms following antibiotic treatment does not induce disease, highlighting a possible disconnect between abundance and consequence in IBD (Bloom et al., 2011).

What is the mechanism by which certain bacterial species drive intestinal inflammation? *IL10*$^{-/-}$ in hematopoietic lineages is linked to colitis development since transplantation of WT bone marrow into *IL10*$^{-/-}$ mice prevents diseases (Bamba et al., 2006). Using a more focused approach, experiments from the Rennick laboratory dissected the role of CD4^{+} T cells in colitis induction in *IL10*$^{-/-}$ mice. CD4^{+} T cells isolated from the lamina propria of colitic *IL10*$^{-/-}$ mice that were injected into *Rag2*$^{-/-}$ mice transferred colitis (Davidson et al., 1996). Later, it was shown that *H. hepaticus* Ag-specific IL10-deficient CD4^{+} T-cell clones elicit colitis in *Rag2*$^{-/-}$ mice only when they are colonized with *Helicobacter* spp. (Kullberg et al., 2002). Since in the transfer studies with IL10-deficient colitogenic T cells from the lamina propria, the CD4^{+} cell population was unfractionated and also contained regulatory CD4^{+} T cells (Davidson et al., 1996); it is possible that the phenotype may have been driven by microbial-induced defects in the development or function Tregs. Evidence supporting this hypothesis came from additional transfer experiments in which cotransfer of WT CD4^{+}CD45RB^{low} Tregs prevented induction of colitis by *IL10*$^{-/-}$ CD4^{+} T cells into *Rag1*$^{-/-}$ mice, only when the WT Tregs were obtained from *Helicobacter*-infected donor mice (Kullberg et al., 2002).

One of the primary functions of Tregs in the intestine is to induce/maintain immunological tolerance to luminal antigens by suppressing helper-T-cell responses, which requires interactions with APCs in the draining MLNs. Altered sensing of microbial signals by APCs in *IL10*$^{-/-}$ animals may be critical to the disruption of mucosal homeostasis. The Medzhitov laboratory recently explored this hypothesis employing *IL10*$^{-/-}$ mice also deficient in MyD88, an essential signaling adapter molecule downstream of many bacterial sensing TLRs, in selective myeloid cell subsets. They demonstrated that MyD88-dependent microbial sensing in CD11c^{+} or LysM^{+} cells, but not epithelial cells, was required to cause colitis in *IL10*$^{-/-}$ mice (Hoshi et al., 2012). What is striking about these results is that earlier work showed that *Il10*$^{-/-}$ mice deficient for TLR4 developed worse disease (Matharu et al., 2009). Thus, it seems that microbial sensing through TLRs can promote mucosal immune homeostasis, but these regulatory mechanisms are compromised in a setting of defective IL10 signaling.

Finally, a link between chronic inflammation and cancer development has been appreciated for more than a century by initial observation made by Rudolf Virchow in 1863 (Balkwill & Mantovani, 2001). However, the contribution of the microbiota in colitis-associated cancer has only recently been explored. Jobin and colleagues demonstrated that monoassociating germ-free *IL10*$^{-/-}$ mice with *E. coli*, but not *E. coli* lacking the *polyketide synthases (pks)* pathogenicity island, in combination with the mutagen azoxymethane, possess the capacity to drive dysplastic lesions at sites of inflammation (Arthur et al., 2012). This highlights the need to consider microbial metagenomics when investigating the role of inflammation and intestinal cancer development in genetically susceptible hosts, such as those with IL10 deficiency.

Collectively, these data suggest that enteric microbial antigens drive intestinal inflammation in genetically susceptible IL10- and IL10R-deficient mice. In the absence of immunomodulatory IL10-dependent signals, upon recognition of commensal bacteria, myeloid cells adopt a proinflammatory phenotype driving antigenic Th1/Th17 CD4^{+} T-cell

responses. Regulatory circuits may be further compromised in the setting of IL10 deficiency due to the decreased suppressive capacity of induced Tregs (Schmitt et al., 2012). The resulting crescendo is a breakdown in immunological tolerance to specific enteric commensal bacteria leading to chronic intestinal inflammation.

7. IMPACT OF IL10 AND IL10R SIGNALING DEFECTS IN HUMANS

Recent studies have additionally confirmed a role for IL10-dependent signals regulating mucosal homeostasis in humans, with particular relevance to the development of IBD. Large-scale GWAS that have focused on adult-onset IBD identified *IL10* as an IBD risk allele, along with several down-stream IL10/IL10R signaling components, including *TYK2* and *STAT3* (Franke et al., 2008; Franke, McGovern, et al., 2010; Jostins et al., 2012). However, while large-scale adolescent and adult GWAS have not identified *IL10RA* or *IL10RB* to be associated with IBD risk (Imielinski et al., 2009; Kugathasan et al., 2008), numerous studies focusing on infantile-onset IBD (IO-IBD) have captured the importance of IL10R variants in this regard (Begue et al., 2011; Engelhardt et al., 2013; Glocker et al., 2009, 2010; Glocker, Kotlarz, Klein, Shah, & Grimbacher, 2011; Kotlarz et al., 2012; Mao et al., 2012). Furthermore, a recent study focusing on very early-onset IBD (VEO-IBD), defined as disease onset before 6 years of age, has further expanded the importance of *IL10RA* variants in IBD risk in infants and young children with ulcerative colitis (UC) (Moran et al., 2013). This section will review the current data of the various mutations identified in IL10 and the IL10R genes and their relationship to the development of IO- and VEO-IBD.

The study of infants presenting with IBD has led to monumental advances in our understanding of the pivotal role of IL10/IL10R signaling in maintaining human gut homeostasis. In 2009, Klein, Grimbacher, and colleagues were the first to identify causative loss of function variants in *IL10RA* and *IL10RB* in patients with severe infantile-IBD (Glocker et al., 2009). These patients typically present within the first few months of life with severe enterocolitis, perianal abscesses, enterocutaneous fistulas, and chronic folliculitis (Begue et al., 2011; Glocker et al., 2009, 2011; Kotlarz et al., 2012). One of the first illustrative examples includes a family with two affected infants, harboring a causal homozygous nonsense mutation in *IL10RB* (p.W159Stop) that led to loss of surface expression of IL10R2 and aberrant IL10R-dependent signaling characterized by defective IL10-induced STAT3 phosphorylation (Glocker et al., 2009). This group concurrently described two distinct homozygous missense mutations in *IL10RA* identified in two unrelated patients presenting with infantile colitis: (p.T84I and p.G141R), each leading to abrogated IL10-mediated signaling (Glocker et al., 2009).

While some reported cases of infantile colitis with variant IL10R result in loss of IL10R surface expression, a novel compound heterozygote mutation in *IL10RA* (p.T84I; p.R101W) was recently identified, resulting in normal IL10R1 expression, normal binding to IL10, but lack of IL10R1 phosphorylation following IL10 binding, ultimately resulting in defective IL10-mediated signaling (Mao et al., 2012). In many cases, the functional significance of the identified variant was confirmed by demonstrating that the patient cell lines (or the expressed variant in cell lines) could not induce IL10-mediated STAT3 phosphorylation or

inhibition of TNF-induced proinflammatory cytokine secretion in mononuclear cells (Glocker et al., 2009; Kotlarz et al., 2012).

Subsequently, numerous additional causative mutations have been identified in patients with infantile-IBD in *IL10RA*, *IL10RB* as well as in *IL10* itself (Table 5.1). These include homozygous mutations including nonsense mutations, missense mutations, large deletions of exons, non-protein coding single nucleotide mutations (splice site and 3′UTR mutations), as well as compound heterozygote mutations (Begue et al., 2011; Engelhardt et al., 2013; Glocker et al., 2009; Kotlarz et al., 2012; Mao et al., 2012; Moran et al., 2013). Our review of the literature to date indicates that there are at least 24 distinct variants identified in *IL10R* in patients presenting with IO- and VEO-IBD. Of interest, all but one of these variants was identified in IO-IBD patients (Engelhardt et al., 2013).

We have yet to determine the significance of more subtle deficits in the IL10/IL10R pathway on patients with less extreme presentations of IBD, including older children and adults. It is possible that altered but not complete loss of function of IL10/IL10R signaling results in less severe presentations of IBD, either in childhood or later in life. There is some evidence that such a scenario might hold true. As noted earlier, GWAS have identified SNPs in *IL10* and are associated with increased risk of both UC and CD (Franke et al., 2008; Franke, Balschun, et al., 2010; Jostins et al., 2012). Moreover, Sanchez et al. linked SNPs within *IL10* to disease location of childhood IBD (Sanchez, Levy, Costea, & Sinnett, 2009). While large-scale GWAS have not identified IL10R as a risk allele in adolescent- and adult-onset IBD, we have identified two *IL10RA* polymorphisms (rs2228054 and rs2228055) associated with increased risk of UC presenting in young children prior to 6 years of age (Moran et al., 2013). Even so, missense mutations in *IL10RA* have been studied for their role in predisposition to adult-onset IBD. Within *IL10RA*, a polymorphism resulting in a single-amino acid change, S138G, results in loss of function with defective IL10-mediated STAT1- or STAT3-dependent signaling (Grundtner et al., 2008). However, the relevance of this mutation with respect to overall IBD predisposition remains unclear as, rather paradoxically, it was found to be *protective* for the risk of UC in some, but not all, populations (Grundtner et al., 2008). In addition, another SNP in the *IL10RA* gene, G330R, in strong linkage disequilibrium with the S138G variant appears to affect the kinetics of STAT-phosphorylation (Finsterbusch, Khare, Campregher, Evstatiev, & Gasche, 2011). Finally, a variant identified in the *IL10* gene in the proximal end of the 3′UTR has been found to be associated with predisposition to both UC and CD in several populations due to its impact on IL10 expression (Andersen et al., 2010; Doecke et al., 2013). Further studies are needed to define the functional significance of these *IL10* and *IL10R* SNPs in regulating anti-inflammatory circuits in IBD.

Most patients with deleterious mutations in *IL10* and/or *IL10R* exhibit severe and extensive intestinal inflammation that is resistant to immunosuppressive medications including steroids, anti-TNF antibodies, methotrexate, and thalidomide (Begue et al., 2011; Engelhardt et al., 2013; Glocker et al., 2010, 2009; Kotlarz et al., 2012). The understanding, that loss of IL10R signaling on hematopoietic cells is a key factor driving the inflammation, has put forth an alternate means of treatment: allogeneic hematopoietic stem cell transplantation (HSCT). Glocker and colleagues described the first case of HSCT in their patient with a

nonsense mutation in *IL10RB* (W159X) (Glocker et al., 2009). While grade III graft versus host disease involving the skin developed, this was treated with prednisone and no further complications ensued. Most remarkable is that within a year this patient experienced resolution of cutaneous folliculitis, perianal fistulas, and intestinal inflammation (Glocker et al., 2009). To date, HSCT has been reported successfully in at least seven patients with mutations in *IL10RA* and *IL10RB* (five for mutations in *IL10RA*; two for *IL10RB*) (Engelhardt et al., 2013; Glocker et al., 2009; Kotlarz et al., 2012; Mao et al., 2012; Dinwiddie et al., 2013). In addition, another patient underwent successful HSCT for a functional variant in *IL10* (Glocker et al., 2010). Strikingly, many of these patients have since been able to discontinue immunosuppressive therapy and continue to do well clinically. Interestingly, no obvious differences in the outcome of transplantation have been reported for patients transplanted for either IL10R1 or IL10R2 deficiency (Engelhardt et al., 2013; Glocker et al., 2009; Kotlarz et al., 2012; Moran et al., 2013). This is noteworthy since IL10R2 is also expressed on nonhematopoietic cells and is utilized as the signaling component in concert with other cytokine receptors (IL22R, IFNλR) known to regulate intestinal epithelial function. To date, HSCT is the only available curative intervention for patients with such severe clinical phenotype resulting from *IL10/IL10R* mutations with aberrant signaling. These patients should be strongly considered for transplant, as they tend to remain refractory to the most optimized medical and surgical interventions.

Given the critical role of IL10/IL10R signaling in dampening the inflammatory response and its strong implication in IBD, this avenue has been explored for innovative therapeutic benefits. Several human clinical trials have examined the role of IL10 for the treatment of adult CD, with disappointing results (Braat et al., 2006; Colombel et al., 2001; Fedorak et al., 2000; Schreiber et al., 2000; van Deventer, Elson, & Fedorak, 1997). The Cochrane review concluded that recombinant human IL10 did not show any benefit in the treatment of active CD, as compared to placebo (Buruiana, Sola, & Alonso-Coello, 2010). Several explanations have been proposed for lack of therapeutic benefit, including insufficient intestinal concentrations of IL10 to yield consistent therapeutic response, interpatient variability in response, short half-life of IL10, and concern that elevated levels of circulating IL10 may result in a proinflammatory state with associated increased IFNγ production (Marlow, van Gent, & Ferguson, 2013; Paul, Khare, & Gasche, 2012). Despite these results, novel approaches of delivering IL10 that permit increased stability or target to the intestinal mucosa are warranted. In addition, identifying patient cohorts predicted based on genetic and/or functional findings, to respond to IL10 merits further study.

8. CONCLUSIONS

In this chapter, we have reviewed recent data of how IL10 regulates different cell populations and modulates immune responses, especially in the intestine. Evidence from murine models lacking IL10/IL10R signaling as well as from patients harboring functional mutations in either IL10 or IL10R genes suggest that this cytokine and its receptor are key regulators of mucosal homeostasis. Despite tremendous progress in our understanding of the biology related to IL10-associated responses, several important questions remain unanswered: How do cells that express cytokine receptors that both signal through STAT3 (e.g., IL6R and IL10R) result in either a proinflammatory or anti-inflammatory signature?

What is the precise role of innate immune IL10R signaling in the gut? What are the sources of IL10 in the intestine that mediate tolerance? What are the functional consequences of different variants within *IL10* and *IL10RA/IL10RB* in humans that do not lead to complete loss of function? Further exploration of these processes will aid in developing new therapies for IBD and other inflammatory disorders—especially those that entail targeted IL10 delivery to the intestinal mucosa.

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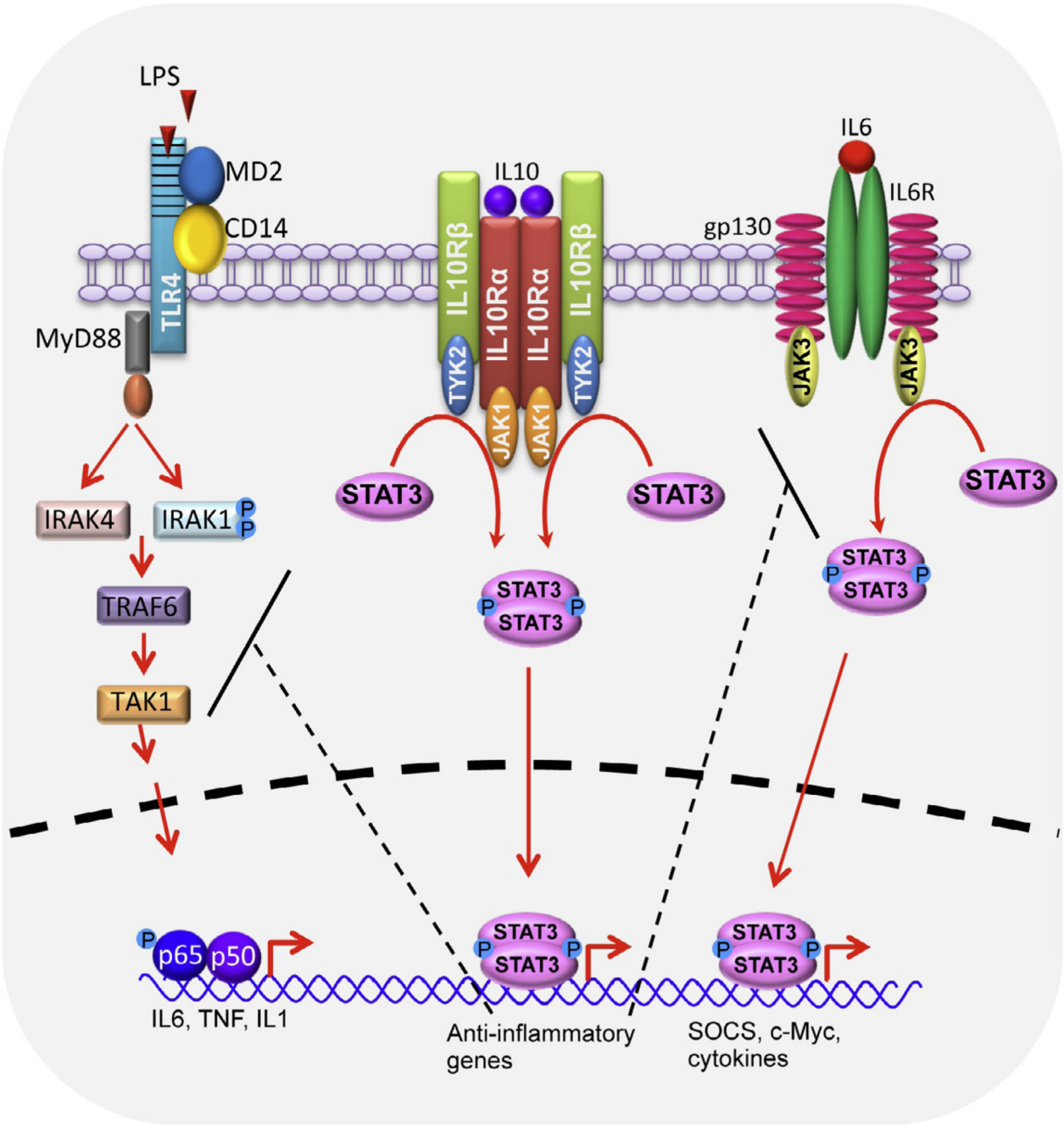

Figure 5.1.

The IL10/IL10R signaling pathway. IL10 signals through heterotetrameric IL10R complex comprising of IL10Rα (IL10R1 in humans) and IL10Rβ (IL10R2 in humans). Binding of IL10 to its receptor leads to JAK1- and TYK2-mediated phosphorylation of STAT3. Following phosphorylation, STAT3 forms a homodimer and undergoes nuclear translocation where it binds to STAT3-binding elements of IL10-responsive genes and drives expression of anti-inflammatory mediators that block various inflammatory pathways. IL10-responsive gene products inhibit TLR4 signaling at the level of IRAK and TRAF6 resulting in reduced

NF-κB-mediated expression of IL6, TNF, and IL1 and also inhibit IL6 signaling at the level of gp30 receptor subunit. MyD88, myeloid differentiation primary response gene (88); TLR, toll-like receptor; IRAK, interleukin-1 receptor-associated kinase; TRAF, TNF receptor-associated factor.

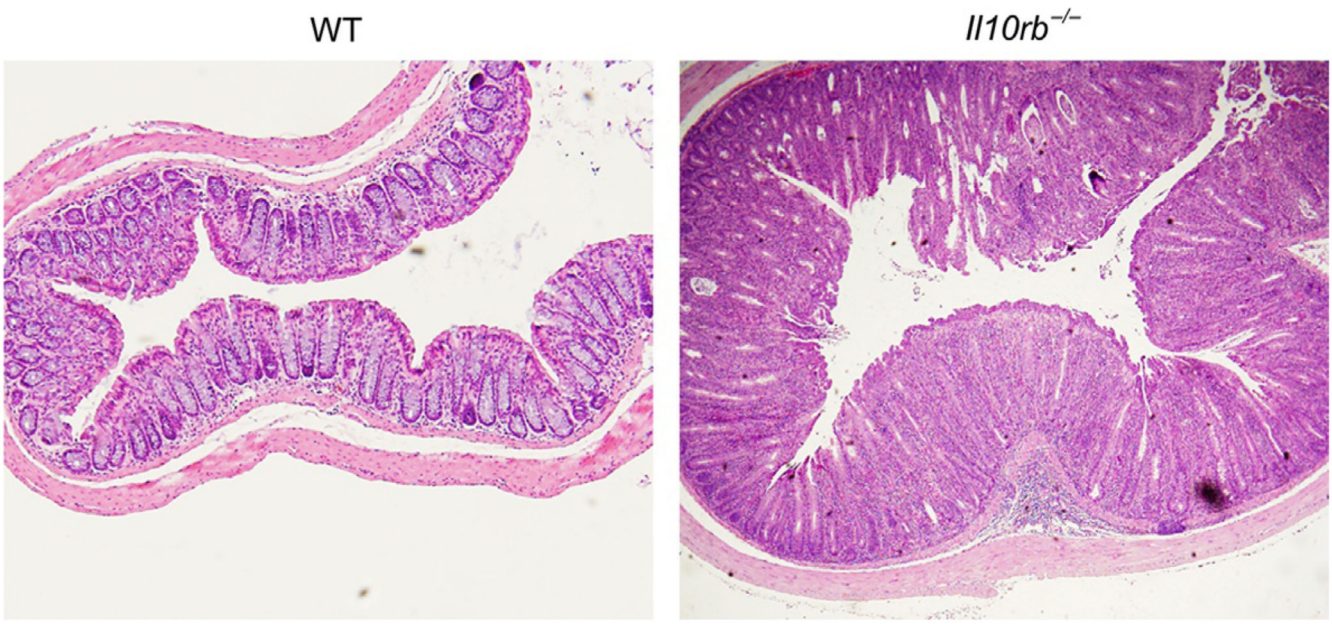

Figure 5.2.

Il10rb$^{-/-}$ mice develop spontaneous colitis. Histology images (4×) of distal colonic tissue obtained from 6-month-old wild type and *Il10rb*$^{-/-}$ mice.

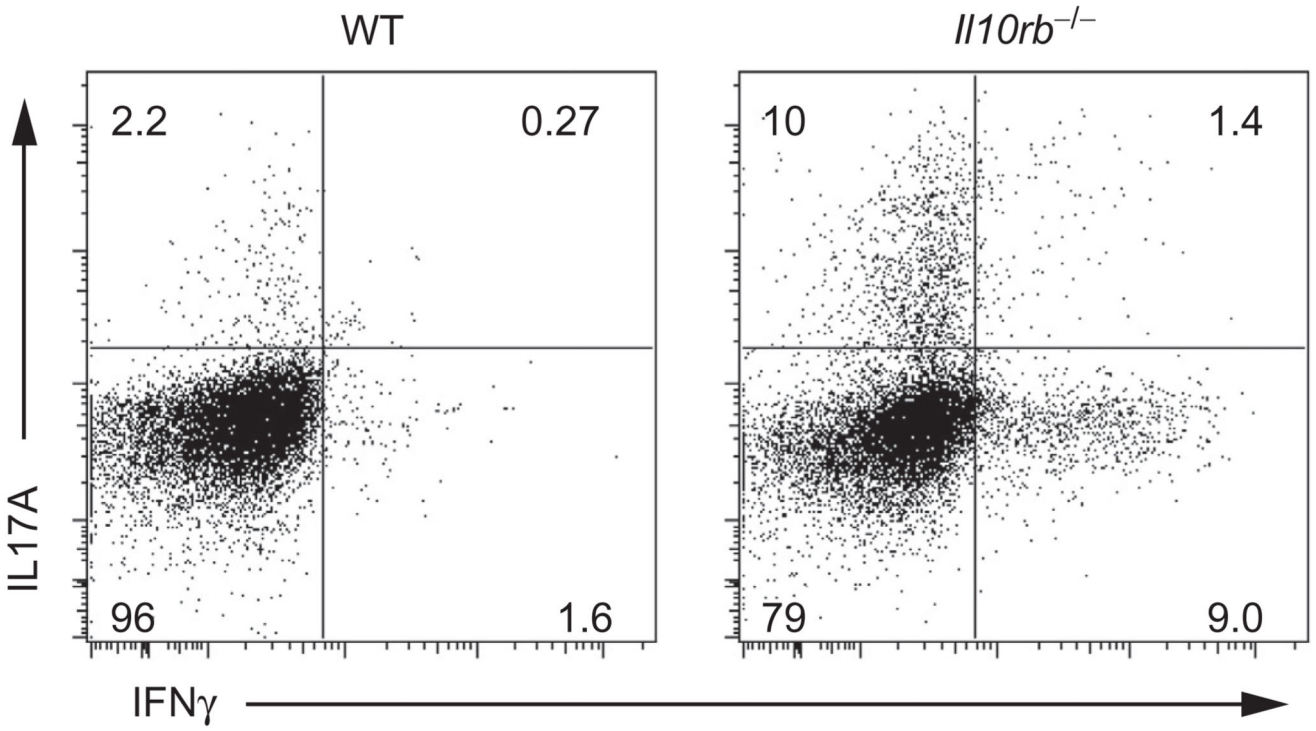

Figure 5.3.

Increase in IL17A$^+$ and INFγ^+ CD4$^+$ T cells in the lamina propria of *Il10rb*$^{-/-}$ mice. Lamina propria cells of 4-month-old WT and *Il10rb*$^{-/-}$ mice were obtained, and stimulated with phorbol myristate acetate (PMA) and ionomycin for 4 h, in the presence of GolgiStop. Plots gated on CD4$^+$ T cells.

Table 5.1

Identified mutations in *IL10RA*, *IL10RB*, and *IL10* leading to infantile and very early onset IBD

Mutation	Clinical manifestation	Age of onset	References
IL10R1: p.G141R (homozygous)[a]	Severe enterocolitis, enterocutaneous fistulas, Perianal abscesses, chronic folliculitis	<1 year	Glocker et al. (2009)
IL10R1: p.T84I (homozygous)[a]	Severe pancolitis	3 months	Glocker et al. (2009)
IL10R1: p.R101W (homozygous)[a]	Severe and progressive colitis	< 3 months	Kotlarz et al. (2012)
IL10R1: p.I169T	Severe and progressive colitis, folliculitis, atopic dermatitis	< 3 months	Kotlarz et al. (2012)
IL10R1: p.I169T (homozygous)	Severe and progressive colitis, folliculitis, atopic dermatitis	< 3 months	Kotlarz et al. (2012)
IL10R1: p.R262C (homozygous)	Pancolitis, perianal disease, no small intestinal inflammation, but granulomas present	1 month	Begue et al. (2011)
IL10R1: p.P206X (homozygous mutation in 5′ splice donor site, leading to premature stop codon)	Fevers, skin folliculitis, bloody diarrhea and colonic erosions, perianal disease with fistula, joint effusions	< 5 months	Moran et al. (2013)
IL10R1: p.G125R (homozygous)[a,b]	Severe colitis. Both patients had family members with fatal infantile colitis	1 month	Engelhardt et al. (2013)
IL10R1: p.L125R (homozygous)[a]	Severe colitis, recurrent infections	1 month	Engelhardt et al. (2013)
IL10RA: Ex1_3del (homozygous deletions)	Severe colitis, enteritis, otitis media, urinary tract infections. Cousin with neonatal onset diarrhea, died in infancy	2 months	Engelhardt et al. (2013)
IL10R1: p.Y57Y; p.V23fsX31 (compound heterozygote)	Colitis, sinusitis	3 months	Engelhardt et al. (2013)
IL10R1: p.T84I; p.R101W (compound heterozygote)	Neonatal onset CD with severe growth failure and nonspecific inflammation from rectum to descending colon	<2nd week of life	Mao et al. (2012)
IL10R1: pY57C; R117C (compound heterozygote)	Severe and progressive colitis, arthralgia, arthritis, Kawasaki disease	<3 months	Kotlarz et al. (2012)
IL10R2: p.W159X (homozygous)[c,d]	Proctitis, folliculitis, perianal abscesses, recurrent infections, impaired wound healing, multiple enterocutaneous fistulas.	3 months	Glocker et al. (2009) and Kotlarz et al. (2012)
IL10R2: p.C66Y	Severe and progressive colitis, folliculitis	<3 months	Kotlarz et al. (2012)

Mutation	Clinical manifestation	Age of onset	References
IL10R2: p.R117H (homozygous)	Severe colitis, recurrent ear infections, oral ulcers, toxic megacolon, perforation, peritonitis. Brother died of severe enteropathy	3½ years old	Engelhardt et al. (2013)
IL10R2: p.G193R (homozygous)	Intestinal adhesions, colitis, fatal septicemia. Had two siblings with fatal colitis	10 days	Engelhardt et al. (2013)
IL10RB: c.C52T (in 3′ UTR) (homozygous)	Severe progressive colitis, folliculitis	<3 months	Kotlarz et al. (2012)
IL10R2: p.W204X; p.S230X (compound heterozygote)	Severe and progressive colitis, dermatitis	<3 months	Kotlarz et al. (2012)
IL10R2: p.W204X (homozygous)	Severe and progressive colitis, folliculitis	< 3 months	Kotlarz et al. (2012)
IL10R2: p.E141X (homozygous)	Pancolitis, perianal lesions, no small intestinal inflammation, but granulomas	3 months	Begue et al. (2011)
IL10RB c.331+907_574del (homozygous deletion)	Severe and progressive colitis, folliculitis	< 3 months	Kotlarz et al. (2012)
IL10R2 deletion p. W18fsX29 (homozygous)	Colitis, recurrent otitis media	1 month	Engelhardt et al. (2013)
IL10R2: p.L59fsX72 (homozygous)	Severe colitis, bronchitis, oral ulcers, gingivitis. Two siblings and an uncle with fatal colitis	1 month	Engelhardt et al. (2013)
IL10: p.G153N (homozygous)[c]	Severe and progressive colitis	< 3 months	Kotlarz et al. (2012)
IL10: p.G113R (homozygous)[b,c,d]	Bloody diarrhea. Female afflicted with perianal, rectovaginal fistulas	3 and 11 months	Glocker et al. (2010)

[a] Underwent HSCT with clinical remission.

[b] Identified in two patients.

[c] Identified in three patients.

[d] Two patients underwent HSCT, both with clinical remission.