Control of Intestinal Inflammation by Interleukin-10

Abhisake Kole and Kevin J. Maloy

Abstract Twenty years ago, the observation that mice genetically deficient in IL-10 spontaneously developed severe intestinal inflammation, revealed an essential role for IL-10 in the maintenance of intestinal homeostasis. In the intervening period much has been learned about the cellular and molecular factors that are involved in IL-10-mediated regulatory pathways. Elegant experiments with conditional cell-type specific knockout strains have illustrated that IL-10 acts on both myeloid cells and T cells within the intestine to suppress innate and adaptive inflammatory responses and enhance regulatory circuits. Although several distinct cellular sources of IL-10 have been identified in the gut, $CD4^+$ T cells are a crucial non-redundant source of IL-10 for the regulation of intestinal inflammation. Induction of IL-10 may represent an important means through which intestinal microbiota establishes mutually beneficial commensalism with mammalian hosts, but can be exploited by certain pathogens to facilitate infection. Recent genetic studies in humans have confirmed the essential role of IL-10 in preventing deleterious inflammation in the gut. A better understanding of the molecular pathways involved in IL-10 induction and function in the intestine may facilitate the development of novel therapies for inflammatory bowel disease (IBD).

Contents

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1 Introduction

Interleukin (IL)-10 was originally identified as a factor secreted from $CD4^+$ T helper type 2 (Th2) cells, with a potent ability to inhibit cytokine secretion from Th1 cells (Fiorentino et al. 1989). Since then, IL-10 has been found to be secreted from a wide variety of T cell populations, including Th1 cells (Del Prete et al. 1993; Jankovic et al. 2007), $Foxp3^-$ regulatory T (Tr1) cells (Groux et al. 1997), $Foxp3^+$ regulatory T (Treg) cells (Rubtsov et al. 2008) and $CD8^+$ intestinal intraepithelial T cells (Das et al. 2003). In addition, many other leukocytes have been reported to be able to secrete IL-10, including B cells (O'Garra et al. 1992), macrophages (Chirdo et al. 2005; Denning et al. 2007; Rivollier et al. 2012), dendritic cells (DCs) (Chirdo et al. 2005; de Saint-Vis et al. 1998), eosinophils (Kayaba et al. 2001) and neutrophils (Romani et al. 1997), as well as certain non-haematopoietic cells like epithelial cells (Cella et al. 2009; Jarry et al. 2008).

IL-10 acts not only to control Th1 cell function, but also to suppress the proinflammatory activities of a wide variety of other cell types. In the absence of IL-10, mice developed spontaneous inflammation at a variety of environmental surfaces including the skin, lungs and intestines (Kuhn et al. 1993; Rubtsov et al. 2008), suggesting that IL-10 is critical in suppressing aberrant immune responses to innocuous environmental antigens. In this chapter, we review the essential role of IL-10 in intestinal homeostasis, summarise the key cellular sources of IL-10 in the gut and discuss the multiple mechanisms through which IL-10 prevents intestinal inflammation.

2 IL-10 Inhibits Chronic Intestinal Inflammation

The mammalian intestinal tract contains the highest bacterial load in the body, particularly the large intestine, which harbours up to 10^{14} bacteria per gram of faecal content (Hooper et al. 2012). The intestinal immune system engages in a constant and dynamic dialogue with the intestinal microbiota to maintain a mutually beneficial state of intestinal homeostasis (Hooper et al. 2012). However, a breakdown of the regulatory immune networks that control responsiveness to the microbiota can result in aberrant inflammatory responses that in humans may manifest as inflammatory bowel diseases (IBD)—chronic inflammatory disorders that are extremely debilitating and have no current cure (Maloy and Powrie 2011). The first demonstration that IL-10 played an essential role in preventing immunopathology against

the commensal microflora was the observation that *Il10*$^{-/-}$ mice developed a spontaneous, progressive enterocolitis starting in the cecum, ascending colon and transverse colon, that subsequently extended to the descending colon and rectum and eventually also affected the small intestine (Berg et al. 1996; Kuhn et al. 1993). Genetic deletion of *Il10rb*, which encodes an essential subunit of the IL-10R (Moore et al. 2001), also led to spontaneous colitis similar to that seen in *Il10*$^{-/-}$ mice (Spencer et al. 1998). Conversely, treatment of *Il10*$^{-/-}$ mice with exogenous IL-10 prevented disease development when given to weanlings (Berg et al. 1996), although IL-10 treatment of adult *Il10*$^{-/-}$ mice with established disease attenuated, but could not fully reverse, clinical pathology. Intestinal inflammation in *Il10*$^{-/-}$ mice was mediated by Th1 cells, as adoptive transfer of Th1 cells isolated from the inflamed colons of *Il10*$^{-/-}$ mice into IL-10-sufficient mice recapitulated the disease seen in *Il10*$^{-/-}$ mice (Davidson et al. 1996). Furthermore, blockade of IFN-γ, the signature cytokine of Th1 cells, ameliorated disease in *Il10*$^{-/-}$ mice (Berg et al. 1996).

Intestinal inflammation in *Il10*$^{-/-}$ mice is dependent on the presence of intestinal bacteria, although not all bacteria are equally able to induce colitis (Sellon et al. 1998). The opportunistic gram-negative bacterial pathogen, *Helicobacter hepaticus*, was shown to trigger the onset of inflammation in *Il10*$^{-/-}$ mice, although its presence was not strictly necessary (Devkota et al. 2012; Dieleman et al. 2000; Kullberg et al. 1998). *H. hepaticus*-induced typhlocolitis in *Il10*$^{-/-}$ mice, was dependent on Th1 cells and could be inhibited by blockade of IFN-γ (Kullberg et al. 1998). Similarly, *H. hepaticus*-infected wild type (WT) mice also developed typhlocolitis when treated with an anti-IL-10R antibody, and this was associated with robust Th1 and Th17 cell responses in the inflamed intestine (Kullberg et al. 2006). Surprisingly, germ-free (GF) *Il10*$^{-/-}$ mice that were monoassociated with *H. hepaticus* did not develop colitis. However, following colonisation with commensal *Lactobacillus reuteri*, *H. hepaticus* gained the ability to trigger colitis in GF *Il10*$^{-/-}$ mice, suggesting that interactions between microbial species enhanced the virulence of *H. hepaticus* (Whary et al. 2011). It is known that GF mice have underdeveloped mucosal immune systems (Macpherson and Harris 2004) and, since the immune response in *H. hepaticus*-infected *Il10*$^{-/-}$ mice was characterised by potent *H. hepaticus*-specific B and T cell responses (Kullberg et al. 2001, 1998; Whary et al. 2011), colonisation with bacteria capable of inducing lymphoid structures may be a prerequisite for *H. hepaticus*-induced inflammatory responses. In addition, colonization with *L. reuteri* greatly increased intestinal expression of the LPS receptor, TLR4, potentially facilitating increased detection of *H. hepaticus* upon subsequent infection (Whary et al. 2011). Alternatively, in specific pathogen-free (SPF) *Il10*$^{-/-}$ mice, colonization with *H. hepaticus* led to strain-specific blooms of distinct bacterial families, which correlated with the susceptibility of the strain to colitis development (Buchler et al. 2012). However, it remains to be tested whether *H. hepaticus* triggers inflammatory responses against other commensal bacteria.

Despite a strong link between inflammation in *Il10*$^{-/-}$ mice and *H. hepaticus* infection, *Il10*$^{-/-}$ mice maintained in certain *Helicobacter*-free facilities also

developed colitis (Dieleman et al. 2000), indicating that other constituents of the intestinal microbiota can also elicit inflammatory responses in the absence of IL-10. The gram-negative anaerobic bacterium, *Bilophila wadsworthia*, was recently identified as another colitogenic bacterium in *Il10*$^{-/-}$ mice (Devkota et al. 2012). *B. wadsworthia* bloomed in mice fed a diet rich in saturated milk-derived fats, but only caused colitis in the absence of IL-10. Furthermore, unlike *H. hepaticus*, monoassociation with *B. wadsworthia* in GF *Il10*$^{-/-}$ mice led to development of a Th1 cell-mediated colitis (Devkota et al. 2012). Taken together, these results suggest that IL-10 is an essential regulatory cytokine for suppressing inflammation due to dysregulation of microbial communities.

IL-10 was also shown to have protective roles in other non-spontaneous models of colitis. For example, in vivo activation of T cells with anti-CD3 mAb leads to massive induction of T cell-derived TNF-α and IFN-γ, and marked enteropathy (Zhou et al. 2004). However, anti-CD3 mAb treatment also led to induction of IL-10 (Durez et al. 1993; Ferran et al. 1994) that limited the enteropathy, as anti-CD3 treatment of *Il10*$^{-/-}$ mice led to increased levels of both TNF-α and IFN-γ, and to increased epithelial cell apoptosis and intestinal tissue damage, relative to that seen in IL-10-sufficient mice (Zhou et al. 2004). In another model, administration of piroxicam, a non-steroidal anti-inflammatory drug (NSAID) and inhibitor of prostaglandin synthesis, led to rapid, acute colitis in *Il10*$^{-/-}$ mice but not WT mice (Berg et al. 2002). Treatment with NSAIDs that did not inhibit prostaglandin synthesis did not induce colitis, suggesting that prostaglandins and IL-10 provide redundant anti-inflammatory effects. Finally, treatment with exogenous IL-10 has been shown to ameliorate the severity of disease in several diverse models of colitis, including the naive T cell transfer model of colitis (Powrie et al. 1994), acute colitis driven by dextran sulphate sodium (DSS)-induced disruption of the epithelial barrier (Qiu et al. 2013; Steidler et al. 2000), and granulomatous colitis induced by streptococcal peptidoglycan–polysaccharide polymers (Herfarth et al. 1996).

The role of IL-10 in human IBD, including Crohn's disease and ulcerative colitis, is currently under investigation and will be discussed elsewhere in this issue. Briefly, mutations and single nucleotide polymorphisms (SNP) in the genes for human IL-10 receptor led to development of paediatric IBD and were associated with ulcerative colitis (Franke et al. 2008; Glocker et al. 2009). However, not all loss-of-function SNPs in the IL-10R genes led to IBD. For example, a SNP in *IL-10R1* resulting in increased TNF-α production by monocytes, was found in equal frequency in both controls and IBD patients (Gasche et al. 2003). Additionally, IL-10 levels, as well as the frequency of IL-10^{+} myeloid cells, were equal in the colons of controls and IBD patients (Hart et al. 2005; Schreiber et al. 1995). However, IL-10 is induced upon treatment of IBD with steroids (Santaolalla et al. 2011) and steroid-refractory patients may benefit from treatment with exogenous IL-10 (Schreiber et al. 1995; van Deventer et al. 1997). Thus, these results suggest that IL-10 may be an effective molecule for dampening intestinal inflammation in humans. The mechanisms of action and sources of IL-10 in the intestine will be discussed in the following sections.

3 Regulatory Activities of IL-10 in the Intestine

3.1 Effects on Innate Immune Cells

The IL-10 receptor, composed of *Il10ra* and *Il10rb*, is expressed on both innate and adaptive immune cells as well as non-hematopoietic cells (Moore et al. 2001). Upon engagement of IL-10R, most cells use the Janus kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway to control the transcription of IL-10-responsive genes. STAT3 is an essential protein downstream of IL-10 in this pathway, although interactions with other STAT proteins may mediate cell- or tissue-specific roles (Moore et al. 2001).

STAT3 is indispensable for both the anti-proliferative and anti-inflammatory effects of IL-10 on macrophages (Takeda et al. 1999). Mice with a myeloid cell-specific deletion of STAT3 (LysM-cre/*STAT3*$^{fl/fl}$) developed a spontaneous enterocolitis characterised by excessive Th1 cell activity similar to that seen in *Il10*$^{-/-}$ mice (Takeda et al. 1999). Peritoneal macrophages isolated from these conditional STAT3 mutants were unresponsive to IL-10. IL-10 failed to reduce LPS-induced TNF-α and IL-6 secretion, induce expression of the anti-inflammatory suppressor of cytokine signalling 3 (SOCS3) or inhibit macrophage cell growth in conditional STAT3 mutants (Kobayashi et al. 2003; Takeda et al. 1999). In addition, CD11c^{+} cells from the colon lamina propria (LP) of LysM-cre/*STAT3*$^{fl/fl}$ mice secreted significantly greater amounts of IL-12p40, both spontaneously and upon ex vivo LPS stimulation, which was required for development of spontaneous colitis (Kobayashi et al. 2003). Furthermore, TLR4$^{-/-}$ conditional STAT3 mutants did not produce IL-12p40 and were also protected from spontaneous colitis and LysM-cre/*STAT3*$^{fl/fl}$ mice also failed to develop colitis when crossed onto the RAG$^{-/-}$ background (Kobayashi et al. 2003). Taken together, these results suggest that in the absence of IL-10 signalling, colon myeloid cells secrete excess IL-12p40 in response to LPS and stimulate IFN-γ production from CD4^{+} T cells. IFN-γ signals via STAT1, and STAT1/STAT3 double mutants were partially protected from spontaneous colitis (Kobayashi et al. 2003), suggesting that IFN-γ may feed back onto colon myeloid cells to further exacerbate colitis in the absence of IL-10-mediated counter-regulation.

Other cytokines, such as IL-6, also signal through STAT3 and thus, phenotypes observed in conditional STAT3 mutants may not be fully attributable to a lack of IL-10 signalling. However, myeloid cell-specific deletion of IL-10R also led to increased secretion of IL-12p40, TNF-α and IL-1β upon stimulation with LPS (Pils et al. 2010). Intestinal macrophages are normally anergic to TLR stimulation in both the human and mouse and produced IL-10, but no detectable IL-12 or IL-23, upon LPS stimulation (Monteleone et al. 2008; Rivollier et al. 2012; Smythies et al. 2005). Hyporesponsiveness to TLR stimulation was dependent on constitutive IL-10 secretion by the colonic macrophages, as when stimulated with either LPS or CpG in the presence of an anti-IL-10R neutralising antibody, they regained the ability to produce IL-12p40 (Monteleone et al. 2008). Similarly, LP

macrophages isolated from *Il10*$^{-/-}$ mice produced IL-12 and IL-23, even without exogenous stimulation (Rivollier et al. 2012). In addition to suppressing production of IL-12 family cytokines by colonic macrophages and DC, in the Peyer's patches, IL-10 was one of the host factors responsible for suppressing pro-inflammatory type I interferon secretion from plasmacytoid DCs (Contractor et al. 2007).

Intestinal inflammation in *Il10*$^{-/-}$ mice was dependent on MyD88 signalling in colon myeloid cells. MyD88 signalling is downstream of most TLRs, which further supports the notion that IL-10 suppresses intestinal inflammation by promoting the anergic phenotype of LP macrophages and DCs. By crossing *Il10*$^{-/-}$*MyD88*$^{fl/fl}$ mice to mice expressing Cre recombinase in various cellular compartments, Hoshi et al. were able to assess whether TLR signalling on specific cells was necessary for the development of colitis in *Il10*$^{-/-}$ mice (Hoshi et al. 2012). When MyD88 was conditionally ablated in either LysM-expressing cells (monocytes, macrophages, neutrophils and some DCs) or CD11c^{+} cells (DCs and some macrophages), *Il10*$^{-/-}$ mice were protected from the development of spontaneous colitis (Hoshi et al. 2012). Furthermore, the spontaneous production of IL-1β, IL-6, IL-12p40 and TNF-α in the colon LP of *Il10*$^{-/-}$ mice was completely abrogated when myeloid cells were deficient in MyD88 signalling (Hoshi et al. 2012). Secretion of IFN-γ and IL-17 were also abrogated, suggesting that MyD88-dependent myeloid cell activation in the absence of IL-10 promotes the differentiation of pathogenic CD4^{+} T cells in the LP. Similarly, in human colon explant cultures, blockade of IL-10 also led to increased secretion of IFN-γ and IL-17 (Jarry et al. 2008).

IL-10 also reduces the expression of co-stimulatory molecules on macrophages (Ding et al. 1993; Ding and Shevach 1992). After initial priming in the lymph nodes, CD4^{+} T cells receive secondary signals in the tissue that fully activate them and promote cytokine secretion (McLachlan et al. 2009). In the intestines, however, IL-10 suppressed expression of CD80 and CD86 on CX$_3$CR1^{hi} macrophages, rendering them unable to activate effector CD4^{+} T cells (Kayama et al. 2012). Thus, CX$_3$CR1^{hi} cells, which comprise approximately 70 % of the MHC II^{+} myeloid cells in the LP (Rivollier et al. 2012), sequestered CD4^{+} T cells from CX$_3$CR1^{-} DCs, which expressed higher levels of CD80 and CD86. Accordingly, transfer of CX$_3$CR1^{hi} cells from WT, but not STAT3$^{-/-}$ mice, ameliorated T cell-dependent colitis (Kayama et al. 2012). In vitro, CX$_3$CR1^{hi} cells inhibited DC-driven CD4^{+} T cell proliferation by a mechanism that was dependent on IL-10 signalling in CX$_3$CR1^{hi} cells, but not on IL-10 secretion by CX$_3$CR1^{hi} cells.

The studies above highlight the important role of IL-10 in suppressing pro-inflammatory cytokine production from normally anergic myeloid cells. In conjunction with reducing the co-stimulatory and antigen-presenting capacity of these cells (de Waal Malefyt et al. 1991; Ding et al. 1993; Ding and Shevach 1992), IL-10 has profound effects on controlling T cell expansion and T cell-mediated immunopathology. However, IL-10 has also been shown to be involved in regulation of innate immune-mediated colitis. Infection of RAG$^{-/-}$ mice on a 129 background with *H. hepaticus* leads to chronic IL-23-dependent typhlocolitis that is driven by the accumulation and activation of innate lymphoid cells (ILC)

(Buonocore et al. 2010; Maloy et al. 2003). Transfer of $CD4^+CD25^+$ T cells inhibited the development of *H. hepaticus*-induced innate typhlocolitis and was fully dependent on IL-10 production by this regulatory T cell subset (Maloy et al. 2003). However, it is still unclear whether IL-10 acts directly on ILCs to suppress their inflammatory potential or indirectly via myeloid cells to suppress secretion of IL-23, a key cytokine in ILC activation (Buonocore et al. 2010).

3.2 Effects on Adaptive Immune Cells

Though IL-10 can clearly control adaptive immune responses indirectly via signalling on antigen-presenting cells, IL-10 also signals directly onto $CD4^+$ T cells. Although naïve $CD4^+$ T cells and many effector $CD4^+$ T cells do not express IL-10R, $Foxp3^+$ regulatory T (Treg) cells and $IL\text{-}17A^+$ $CD4^+$ T cells both expressed IL-10R on the cell surface (Chaudhry et al. 2011; Huber et al. 2011).

IL-10 promotes the maintenance, expansion and function of Treg cells. In the T cell adoptive transfer model of colitis, $CD4^+$ $CD45RB^{hi}$ naïve T cells transferred into lymphopenic hosts expand in response to IL-23 and microbiota-derived signals, seed the LP, and induce severe colitis (Ahern et al. 2010; Feng et al. 2010; Powrie et al. 1993). Co-transfer of Treg cells prevents immunopathology caused by $CD45RB^{hi}$ T cells (Powrie et al. 1993). The ability of Treg cells to inhibit development of colitis was dependent on IL-10 signalling on the Treg cells (Murai et al. 2009). Thus, *Il10rb*$^{-/-}$ Treg cells failed to prevent development of colitis as did WT Treg cells transferred into *Il10*$^{-/-}$*/RAG*$^{-/-}$ mice (Murai et al. 2009). This study further demonstrated that *Il10rb*$^{-/-}$ Treg cells and WT Treg cells transferred into *Il10*$^{-/-}$*/RAG*$^{-/-}$ both lost their suppressive function due to a failure to maintain expression of Foxp3 (Murai et al. 2009). Continued expression of Foxp3 is required for Treg cells to maintain their suppressor function (Williams and Rudensky 2007).

Another example illustrating the importance of intrinsic IL-10 signals for Treg cell activity in the gut was the observation that Treg cell-specific ablation of *Il10ra* in lymphoreplete mice resulted in the development of severe, spontaneous colitis (Chaudhry et al. 2011). In this case, however, IL-10R-dependent Treg cell function was not due to maintenance of Foxp3 expression, as *Il10ra*$^{-/-}$ Treg cells maintained Foxp3 expression for up to 3 months after *Il10ra* was deleted (Chaudhry et al. 2011). In this model, IL-10R was functional in naïve $CD4^+$ T cells and only deleted upon commitment to the $Foxp3^+$ Treg cell lineage. Thus, it is possible that IL-10R signalling at the time of T cell priming is necessary for Treg cell commitment, and that in its absence, Treg cells become vulnerable to plasticity due to transient or impermanent expression of Foxp3. Consistent with this hypothesis, a recent study found that a subset of non-Treg cells transiently express Foxp3 (Miyao et al. 2012). Thus, it is also possible that in lymphopenic settings, IL-10 promotes the expansion of committed Treg cells over non-Treg cells transiently expressing Foxp3.

Though not required for the maintenance of Foxp3 expression in lymphoreplete mice, IL-10 signalling on Treg cells was necessary for their control of $CD4^+$ T cell

accumulation (Chaudhry et al. 2011). As in myeloid cells, the effects of IL-10 were dependent on STAT3 phosphorylation as Treg cell-specific ablation of STAT3 also resulted in spontaneous intestinal inflammation (Chaudhry et al. 2009). Intestinal inflammation in Treg cell-specific IL-10R or STAT3 conditional mutants was accompanied by a selective increase in the frequency of IL-17A-producing $CD4^+$ T cells, but not IFN-γ-producing $CD4^+$ T cells (Chaudhry et al. 2009, 2011).

IL-10 signalling on Treg cells induced their secretion of IL-10, which was critical for the control of Th17 cells during intestinal inflammation (Chaudhry et al. 2011; Huber et al. 2011). Th17 cells, but not naïve or Th1 cells, expressed the IL-10R and responded to IL-10 with a reduction in their rate of proliferation. Accordingly, $CD4^+$ T cell-specific dysfunction in IL-10 signalling led to increased frequencies of IL-$17A^+$ cells (Chaudhry et al. 2011; Huber et al. 2011). Thus, although IL-10 controls Th1 cells indirectly via suppression of pro-inflammatory cytokine secretion from myeloid cells, IL-10 is able to control Th17 cell-mediated intestinal inflammation via direct signalling on the T cell.

IL-10 has been shown to potentiate not only the function of Treg cells, but also their expansion. In a model of oral tolerance, it was shown that after a priming phase in the mesenteric lymph nodes (MLNs), Treg cells homed to the intestinal LP, where they expanded in response to IL-10 from CX_3CR1^{hi} macrophages (Hadis et al. 2011). Similarly, in human gut sections, $Foxp3^+$ Treg cells were found to be in close contact with IL-10-producing non-T cells (Uhlig et al. 2006). In T cell transfer colitis, treatment with anti-IL-10R completely abrogated the ability of Treg cells to cure colitis induced by $CD45RB^{hi}$ $CD4^+$ T cells (Liu et al. 2003; Uhlig et al. 2006). This was partly due to the inability of Treg cell-derived IL-10 to signal onto non-Treg cells, but *Il10*$^{-/-}$ Treg cells also partially suppressed colitis, suggesting that anti-IL-10R treatment inhibited some Treg cell-mediated mechanisms of immune suppression that were independent of their ability to secrete IL-10.

4 Sources of IL-10 in the Intestine

As noted above, many different cells are capable of producing IL-10. Their anatomical location and interactions with other cells determine which cell type is the critical source of IL-10 under various circumstances. In the second section, we summarise recent work elucidating the factors governing IL-10 production from intestinal immune cells and the relative contributions of each cell type to IL-10-mediated regulation of intestinal inflammation.

4.1 Innate Sources of IL-10

Intestinal macrophages, characterised by high expression of CD11b, F4/80 and CX_3CR1, are robust producers of IL-10 (Chirdo et al. 2005; Denning et al. 2007;

Rivollier et al. 2012). In contrast, DCs, characterised by CD11c expression and intermediate to low expression of F4/80 and CX_3CR1, are not believed to be major producers of IL-10 in the LP (Denning et al. 2007; Rivollier et al. 2012). Recent data from a human IL-10 transgenic mouse also confirmed that macrophages, and not DCs, were the principal source of innate immune cell-derived IL-10 in the intestine (Ranatunga et al. 2012). Steady-state macrophage production of IL-10 was partially dependent on the presence of commensal bacteria since germ-free mice showed an approximately 50 % reduction in IL-10 (Rivollier et al. 2012). Consistent with this finding, it was recently shown that the commensal bacterium, *Clostridium butyricum*, specifically induced IL-10 from intestinal macrophages during DSS-induced colitis (Hayashi et al. 2013). Production of steady-state IL-10 was MyD88-independent, while induction of IL-10 by *C. butyricum* during inflammation was dependent on TLR2 and MyD88 (Hayashi et al. 2013; Rivollier et al. 2012). This discrepancy can be explained by the finding that most steady-state macrophages do not express TLRs, while monocyte-derived cells recruited during inflammation express TLR2 (Platt et al. 2010). Infiltrating monocyte-derived cells normally exhibit an inflammatory phenotype (Rivollier et al. 2012), but signals from *C. butyricum* may attenuate their colitogenic potential. Indeed, colonization with *C. butyricum* lessened the severity of DSS-induced colitis by a mechanism dependent on myeloid cell-derived IL-10 (Hayashi et al. 2013).

Commensal-dependent but MyD88-independent production of constitutive IL-10 may instead be downstream of the TRIF signalling pathway. Downstream of TLR3, TLR4 and cytosolic DNA sensors, TRIF connects microbiota-derived signals to the production of interferon (IFN)-β. We recently found that myeloid cells from mice lacking the IFN-α/β receptor (IFNAR) produced significantly lower amounts of IL-10 both with and without stimulation with TLR ligands (Kole et al. 2013). Similarly, ex vivo treatment of LP myeloid cells with an anti-IFNAR neutralising antibody also inhibited IL-10 production (Kole et al. 2013). Furthermore, type I interferons enhanced CD40L-induced IL-10 secretion (Luft et al. 2002). These result position type I interferons as an essential regulator of IL-10 production by intestinal macrophages under both steady-state and inflammatory conditions.

As mentioned above, CX_3CR1^{hi} LP macrophages were an important source of IL-10 for Treg cell expansion in the gut (Hadis et al. 2011). CX_3CR1, the receptor for fractalkine, was not only a marker of intestinal macrophages, but was also necessary for their secretion of IL-10. Expansion of Treg cells by IL-10-producing CX_3CR1^{hi} macrophages was a prerequisite for dissemination of Treg cells to peripheral sites and the establishment of oral tolerance (Hadis et al. 2011). Thus, $CX_3CR1^{-/-}$ mice were unable to mount tolerance against ingested antigens, but could be rescued by the adoptive transfer of CX_3CR1^{+} antigen-presenting cells (Hadis et al. 2011). Similarly, adoptive transfer of LP $CD11b^{+}CD11c^{+}F4/80^{+}$ cells, which also express CX_3CR1 (Rivollier et al. 2012), rescued Treg cells transferred into $Il10^{-/-}/RAG^{-/-}$ mice from loss of Foxp3 expression (Murai et al. 2009).

The importance of innate immune cell-derived IL-10 is questionable, however. For example, myeloid cell-specific ablation of IL-10 (LysM-cre/$Il10^{fl/fl}$) did not

result in the development of spontaneous colitis (Siewe et al. 2006) and *Il10*$^{-/-}$/*RAG*$^{-/-}$ mice did not develop worse colitis upon transfer of naive CD45RB^{hi} CD4^{+} T cells (Murai et al. 2009). In contrast, transfer of bulk splenic CD4^{+} T cells into *Il10*$^{-/-}$/*RAG*$^{-/-}$ recipients did lead to worse colitis, that was characterised by increased levels of IL-12p40 and concomitant increases in IFN-γ and IL-17 secretion (Liu et al. 2011). Innate sources of IL-10 were also necessary for TGF-β signalling and SMAD3 phosphorylation on CD4^{+} T cells (Liu et al. 2011). Thus, although dispensable for controlling the colitogenic potential of naïve CD4^{+} T cells, innate leukocyte-derived IL-10 may restrain the inflammatory phenotype of effector and/or memory CD4^{+} T cells.

4.2 Adaptive Sources of IL-10

Unlike myeloid cell-specific deletion of IL-10, CD4^{+} T cell-specific deletion of IL-10 (CD4-cre/*Il10*$^{fl/fl}$) resulted in development of spontaneous colitis (Roers et al. 2004). It is important to note, however, that CD4-cre/*Il10*$^{fl/fl}$ mice were positive for *Helicobacter ganmani* while the *Helicobacter* status of LysM-cre/*Il10*$^{fl/fl}$ mice was not divulged (Roers et al. 2004; Siewe et al. 2006). As mentioned earlier, *Il10*$^{-/-}$ mice administered piroxicam develop an acute colitis due to inhibition of prostaglandin synthesis (Berg et al. 2002). *RAG*$^{-/-}$ mice reconstituted with bulk CD4^{+} T cells from *Il10*$^{-/-}$ mice, but not WT mice, were also susceptible to piroxicam-induced colitis (Blum et al. 2004), providing further evidence that CD4^{+} T cells are a crucial source of IL-10 for the control of intestinal inflammation.

IL-10 can be produced by many different subsets of CD4^{+} T cells in the gut, including Foxp3^{+} and Foxp3^{-} cells. In the small intestine, IL-10 was produced by Foxp3^{-} intraepithelial cells, whereas in the colon and small intestine LP, IL-10 was produced by both Foxp3^{+} and Foxp3^{-} LP cells (Kamanaka et al. 2006; Maynard et al. 2007). Human IL-10 in a transgenic mouse model was also expressed highly in CD4^{+} Foxp3^{+} T cells from the colon LP (Ranatunga et al. 2012). Cell tracking studies demonstrated that Foxp3^{+} IL-10-producing cells were derived from both Foxp3^{+} and Foxp3^{-} thymic precursors, while Foxp3^{-} IL-10-producing cells were derived only from Foxp3^{-} precursors (Maynard et al. 2007).

When IL-10^{-} splenic CD4^{+} T cells were transferred into *RAG*$^{-/-}$ mice, they gained the ability to produce IL-10 after homing to either the small intestinal epithelium or the LP of the large intestine, suggesting that T cells receive local signals that stimulate their production of IL-10 (Kamanaka et al. 2006). Although capable of inducing IL-10 from Treg cells, IL-10 signalling was not strictly required for the development of IL-10^{+} CD4^{+} T cells in the intestine (Chaudhry et al. 2011; Maynard et al. 2007). Instead, TGF-β, a cytokine constitutively expressed in the LP (Babyatsky et al. 1996), was required for IL-10 expression in CD4^{+} T cells (Maynard et al. 2007). In contrast, both retinoic acid and IL-23 produced by mucosal DCs inhibited IL-10 production by intestinal Treg cells (Ahern et al. 2010; Maynard et al. 2009).

Intestinal $CD4^+$ T cells also receive antigenic stimulation from the resident microbiota. IL-10^+ Treg cells in the LP were characterised by high surface expression of CD44 and low expression of CD62L, consistent with antigen-experienced effector or memory cells (Ranatunga et al. 2012). Several distinct types of intestinal bacteria, including both commensals and pathogens, have been shown to promote IL-10^+ Treg cell development in the intestine. Indigenous *Clostridium* species induced IL-10 production from colonic $FoxP3^+$ Treg cells, which may be partially dependent on their induction of TGF-β from intestinal epithelial cells (Atarashi et al. 2011). Persistent colonization of WT mice with opportunistic pathogen, *H. hepaticus*, led to the induction of both $CD25^+$ and $CD25^-$ IL-10-producing $CD45RB^{lo}$ Treg cells that suppressed *H. hepaticus*-induced intestinal inflammation (Kullberg et al. 2002). The pathogen, *Yersinia enterocolitica*, also induced IL-10^+ Treg cells by a mechanism dependent on TLR2/6 signalling (DePaolo et al. 2012). TLR2/6 was previously shown to recognise LcrV from *Yersinia* to induce IL-10 from myeloid cells and $Foxp3^-$ $CD4^+$ T cells (Depaolo et al. 2008). Finally, polysaccharide A from the commensal bacterium, *Bacteroides fragilis*, directly engaged TLR2 on $Foxp3^+$ $CD4^+$ T cells to induce IL-10 production (Round et al. 2011). Taken together, these studies suggest that induction of IL-10^+ Treg cells in the intestine may represent an important pathway through which commensal microbiota establishes beneficial mutualism with their mammalian host, but that certain pathogens may exploit this mechanism to facilitate infection.

In normal healthy mice, the antigen-experienced $CD45RB^{lo}$ $CD4^+$ T cell fraction contains several regulatory T cell populations that can suppress $CD4^+$ T cell-induced colitis via an IL-10-dependent mechanism (Asseman et al. 1999; Powrie et al. 1993). However, $CD4^+CD25^+$ Treg cells within the $CD45RB^{lo}$ population did not require IL-10 to suppress naive $CD45RB^{hi}$ $CD4^+$ T cell transfer colitis in $RAG^{-/-}$ hosts (Murai et al. 2009). However, adoptively transferred $Il10^{-/-}$ $CD45RB^{lo}$ T cells themselves elicited colitis in $RAG^{-/-}$ recipients, suggesting that IL-10 from $CD45RB^{lo}$ Treg cells is required for the suppression of colitogenic cells also contained within the antigen-experienced $CD45RB^{lo}$ population (Asseman et al. 1999). Accordingly, inflammatory $Foxp3^-$ $CD45RB^{lo}$ $CD4^+$ T cells were controlled by Treg cell-derived IL-10, while control of naive $CD45RB^{hi}$ $CD4^+$ T cells was IL-10 independent (Kamanaka et al. 2011). In addition, $CD45RB^{lo}$ $CD4^+$ T cell-induced colitis was driven by IL-22, a cytokine expressed by Th17 cells (Liang et al. 2006), providing further evidence that IL-10 directly inhibits Th17 cell pathogenicity.

Although dispensable for the prevention of $CD45RB^{hi}$ $CD4^+$ T cell-mediated colitis, IL-10 was required for Treg cell cure of established colitis in this model (Liu et al. 2003; Uhlig et al. 2006). IL-10 was also required for Treg cell control of immunopathology induced by *H. hepaticus* in a T cell-independent colitis model, or after infection with *Toxoplasma gondii* (Hall et al. 2012; Maloy et al. 2003). Thus, Treg cells preventing colitis by inhibiting T cell priming in the lymph nodes (Schneider et al. 2007) do so independently of IL-10, while Treg cell-mediated immunosuppression at sites of inflammation require IL-10. Consistent with this

hypothesis, IL-10$^+$ Treg cells accumulate in the colon LP during the cure of colitis (Uhlig et al. 2006). Furthermore, Treg cell-specific ablation of IL-10 resulted in spontaneous inflammation not only in the colon, but also in the lungs and skin (Rubtsov et al. 2008). Importantly, however, systemic, multi-organ, fatal autoimmunity, as observed in Foxp3$^{-/-}$ mice (Brunkow et al. 2001), did not develop in mice containing IL-10-deficient Treg cells, indicating that Treg cell-derived IL-10 was specifically important for the suppression of inflammation at sites where immune cells directly interact with environmental antigens.

Foxp3$^-$ IL-10$^+$ CD4$^+$ T (Tr1) cells are another regulatory subset with the potential to suppress colitis (Groux et al. 1997). Tr1 cells could be found amongst the intestinal epithelial lymphocytes as well as in the colon LP (Kamanaka et al. 2006; Maynard et al. 2007). Induction of Tr1 cells is dependent on chronic T cell activation (Groux et al. 1997) and is influenced by several cytokines including IL-27, type I interferons and IL-10. DC-derived IL-10 and IL-27 were both shown to induce differentiation of naïve CD4$^+$ T cells into a Foxp3$^-$ IL-10-producing phenotype (Awasthi et al. 2007; Groux et al. 1997; Wakkach et al. 2003). Type I interferons potentiated the TCR stimulation-dependent and IL-10-dependent differentiation of Tr1 cells (Corre et al. 2013; Levings et al. 2001), either via direct signalling on CD4$^+$ T cells or indirectly via signalling on antigen-presenting cells (Dikopoulos et al. 2005).

IL-27 also acted on effector Th1, Th2 or Th17 cells to induce their transition into an IL-10-producing self-regulating CD4$^+$ T cell (Fitzgerald et al. 2007; Stumhofer et al. 2007). Infection with *Toxoplasma gondii* resulted in fatal Th1 cell-mediated necrosis of the small intestine in the absence of IL-10 (Suzuki et al. 2000) and a subsequent study showed that Th1 cells themselves were a critical source of protective IL-10 during *T. gondii* infection (Jankovic et al. 2007). Although it was not determined in this study whether IL-27 was required for induction of IL-10 during toxoplasmosis, *Il27*$^{-/-}$ mice also succumbed to fatal immunopathology (Hall et al. 2012).

Mucosal myeloid cells are specialised for the induction of Foxp3$^-$ IL-10$^+$ CD4$^+$ T cells. They were shown to constitutively produce type I interferons and type I interferon signalling was essential for optimal production of both IL-10 and IL-27 (Kole et al. 2013). However, the main producers of these three cytokines were resident non-migratory LP macrophages, suggesting that they may be more important in adapting the phenotype of CD4$^+$ T cells locally in the LP rather than priming a distinct lineage. In contrast, migratory DCs presenting antigens from apoptotic intestinal epithelial cells were shown to be able to prime IL-10$^+$ CD4$^+$ T cell responses in the MLNs (Jang et al. 2006).

CD8$^+$ T cells can also be induced to produce IL-10. It was recently reported that naïve CD8$^+$ T cells cultured in the presence of IL-4 acquired an IL-10-producing phenotype and adoptive transfer of IL-10$^+$ CD8$^+$ T cells ameliorated colitis induced by 2,4,6-trinitrobenzene sulphonic acid, a hapten that elicits severe, acute colitis and diarrhoea (Zhao et al. 2013). Furthermore, previous studies had identified a population of CD8$^+$ T cells expressing the CD8αα homodimer that were specifically located in the small intestinal intraepithelial layer, which

recognised self-antigen and responded via the production of IL-10 (Saurer et al. 2004). Other studies indicated that both $CD4^+$ and $CD4^-$ subsets of $CD8\alpha\alpha^+$ intra-epithelial T cells were able to suppress Th1-mediated colitis in an IL-10-dependent manner (Das et al. 2003; Poussier et al. 2002). As was shown in vitro, expression of IL-10 in $CD4^+$ $CD8\alpha\alpha^+$ T cells was dependent on IL-4 and expression of the transcription factor, GATA3 (Das et al. 2003). Remarkably, $CD8\alpha\alpha^+$ T cells retained their tolerogenic phenotype even upon recognition of viral antigens (Saurer et al. 2004). Taken together, these data suggest that intraepithelial $CD8\alpha\alpha^+$ T cells constitute a discrete population of intestinal lymphocytes that may also mediate immunoregulatory activity through the secretion of IL-10.

Finally, B cells are also a functional source of IL-10. Although B cell-specific deletion of IL-10 did not result in spontaneous colitis (Madan et al. 2009), $IL\text{-}10^+$ B cells accumulated in the MLNs and LP of mice with chronic intestinal inflammation (Mizoguchi et al. 2002). Furthermore, several studies have provided in vivo evidence that IL-10-secreting B cells can regulate intestinal inflammation. Thus, adoptive transfer of WT B cells, but not $Il10^{-/-}$ B cells, suppressed spontaneous development of colitis in genetically susceptible strains (Mizoguchi et al. 2002), inhibited T cell transfer colitis (Schmidt et al. 2012) and attenuated DSS-induced colitis (Yanaba et al. 2011). As with macrophages and $CD4^+$ T cells, B cell secretion of IL-10 was partially dependent on signals derived from the microbiota (Schmidt et al. 2012). Thus, B cells may be a non-essential source of IL-10 that can contribute to intestinal immunoregulation.

5 Concluding Remarks

The diversity of cells that can produce and respond to IL-10 shows its central role in immune regulation. Development of spontaneous colitis in mice with Treg cell-specific deletion of IL-10 (Rubtsov et al. 2008) positions Treg cells as the critical source of IL-10 for maintenance of intestinal homeostasis. Similarly, spontaneous colitis in mice with defects in myeloid cell or Treg cell IL-10R signalling identifies them as the critical responders to IL-10 (Chaudhry et al. 2011; Takeda et al. 1999). Other sources of IL-10, though not essential for homeostasis, can also suppress intestinal inflammation.

Mice with defects in IL-10 and IL-10 signalling develop colitis primarily at sites of interaction with environmental antigens such as the resident microbiota, suggesting that IL-10 functions to maintain commensalism. Furthermore, spontaneous colitis is dependent on the presence of commensal bacteria, which elicit immunopathology in the absence of IL-10. Accordingly, several commensal bacterial species induce IL-10 production from innate and adaptive immune cells, as well as expression of the IL-10R in the intestines (Mirpuri et al. 2012).

The use of IL-10 for the treatment of IBD has not been as successful as hoped. However, this may be due to a hyporesponsiveness to IL-10 conferred by genetic mutations in the IL-10R gene (Glocker et al. 2009), or to ineffective delivery of

IL-10 to sites of inflammation such as the intestinal LP. Further work must be done to properly harness the immunosuppressive potential of IL-10 observed in multiple animal models of intestinal inflammation.

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