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The interleukin-23 axis in intestinal inflammation

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Summary: Immune responses in the intestine are tightly regulated to ensure host protective immunity in the absence of immune pathology. Interleukin-23 (IL-23) has recently been shown to be a key player in influencing the balance between tolerance and immunity in the intestine. Production of IL-23 is enriched within the intestine and has been shown to orchestrate T-cell-dependent and T-cell-independent pathways of intestinal inflammation through effects on T-helper 1 (Th1) and Th17-associated cytokines. Furthermore, IL-23 restrains regulatory T-cell responses in the gut, favoring inflammation. Polymorphisms in the IL-23 receptor have been associated with susceptibility to inflammatory bowel diseases (IBDs) in humans, pinpointing the IL-23 axis as a key, conserved pathway in intestinal homeostasis. In addition to its role in dysregulated inflammatory responses, there is also evidence that IL-23 and the Th17 axis mediate beneficial roles in host protective immunity and barrier function in the intestine. Here we discuss the dual roles of IL-23 in intestinal immunity and how IL-23 and downstream effector pathways may make novel targets for the treatment of IBD.

Keywords: *IL-23, innate immune activation, intestinal inflammation, regulatory T cell, Th17*

Introduction

The inflammatory bowel diseases (IBDs), Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory disorders of the gastrointestinal (GI) tract affecting 0.2% of the population. The etiology of IBD is unknown, but results from clinical and experimental studies indicate a breakdown in intestinal homeostasis with the development of aberrant inflammatory responses to intestinal bacteria (1). IBD is a complex multifactorial disease that involves interactions between host genetic and environmental factors. Animal models of IBD have proved useful in the identification of immune pathological mechanisms and indicate that chronic inflammation may be the result of over-production of inflammatory responses or deficiencies in key negative regulatory pathways (1, 2). Recent therapeutic approaches involve blockade of inflammatory cytokines such as tumor necrosis factor-α (TNF-α). While this method has been shown to be highly effective in some cases, many patients are 'non-responders.' Furthermore, sustained neutralization of TNF-α may lead to enhanced susceptibility to

infection (3), highlighting the need for alternative, more specific therapeutic approaches.

Among the inflammatory cytokines implicated in IBD pathogenesis, recent attention has focused on the interleukin-12 (IL-12)-related cytokine IL-23 as a key driver of intestinal inflammation (4–8). IL-23 expression appears to be specifically increased in the intestine rather than systemically during intestinal inflammation (7), indicating a tissue-specific role in the inflammatory response. In addition, recent genetic studies in humans identified polymorphisms in the *IL23 receptor* (*IL23R*) gene that were associated with susceptibility to IBD (9, 10).

Here, we review recent findings from our laboratory and others showing that IL-23 is a pivotal player in intestinal homeostasis via its ability to orchestrate both T-cell-dependent and innate pathways of intestinal inflammation and to suppress regulatory T-cell responses in the intestine. Further understanding of these multiple activities of IL-23 may identify novel targets for therapeutic intervention in IBD.

Characteristics of the intestinal immune system

The GI tract has an enormous surface area (11) and represents the major site via which pathogens gain access to the body. The anatomical features of the GI tract are adapted to its roles in nutrient absorption and immune surveillance (11). In addition, the intestine is colonized by a large number and diverse array of microbial species (12), which thrive in specialized niches benefiting the host through breakdown of food products, provision of vitamins, and aiding in development of the immune system (12, 13).

The intestinal immune system is charged with the difficult task of mounting rapid protective immune responses to invading pathogens while avoiding inflammatory responses to the largely beneficial endogenous flora. To this end, there are a number of distinct structural and cellular features designed to accommodate these dual processes of host defense and tolerance.

Barrier function and immune surveillance

The intestine is covered by a layer of intestinal epithelial cells (IECs), joined together by tight junctions, which acts as a barrier to the luminal contents. Within the epithelial cell layer, goblet cells produce mucins that form a protective layer impeding bacterial attachment, while Paneth cells produce anti-microbial peptides such as defensins (14). Upon microbial stimulation, IECs themselves contribute to host protective immunity through the production of anti-microbial peptides, cytokines, and chemokines that influence the immune response (14). In addition, the epithelial cell layer contains large numbers of T cells, particularly $\gamma\delta^+$ and $\alpha\beta^+$ CD8$\alpha\alpha$-expressing T cells, which may also contribute to immune surveillance and prevent immune pathology (15).

The intestine contains a large number of leukocytes capable of mounting a range of different immune responses. Innate immune cells such as macrophages, dendritic cells (DCs), and polymorphonuclear cells are in the front line of defense and provide a rapid response to infection, preventing invasion by pathogens (11, 14). Adaptive effector functions mediated via B and T cells also play a key role in protective immunity through the production of immunoglobulin A (IgA) and IgG as well as the development of helper and cytotoxic T-cell responses (11, 16). The small and large intestines also contain forkhead box protein 3 $(Foxp3)^+$ regulatory T cells (Tregs) that contribute to the maintenance of intestinal homeostasis (17).

Adaptive responses are initiated within the associated organized lymphoid structures. Some of these are contained within the gut wall, such as the Peyer's patches (PPs) in the small intestine and cecal and colonic patches in the large intestine. Small isolated lymphoid follicles (ILF) are also present throughout the intestine (11). These are highly dynamic structures that can develop in response to environmental signals such as the endogenous intestinal flora (18). Furthermore, there is traffic of cells and antigens from the intestine through the lymph to the mesenteric lymph nodes (MLNs) (11). The latter play an important role in the compartmentalization of the intestinal immune response, ensuring local host protective immunity while preventing damaging systemic inflammatory responses (19). Antigen-presenting cells within the MLNs imprint expression of gut-homing receptors on antigen-activated T cells, allowing them to traffic to the intestine to mediate their effector function (11, 20).

Through their ability to integrate signals from their environment and prime appropriate immune responses, DCs play a pivotal role in initiating and directing the nature of the immune response in the intestine (11, 21). Some DCs are strategically positioned within the subepithelial cell dome (SED) region of the PP, where they can sample the incoming antigen that is transported from the lumen by specialized epithelial cells termed microfold cells (M cells), which are interspersed in the epithelium overlying the PPs (21). In addition, DCs can also extend processes through epithelial cell tight junctions without disrupting the integrity of the epithelial cell barrier and sample the luminal contents (22). Antigen-bearing DCs can migrate to the MLN or PP where they can initiate a primary T-cell response (21). Intestinal DCs are a heterogeneous population that can mediate both tolerogenic and inflammatory responses to intestinal antigens (reviewed in 20). This flexibility in function may represent the activities of

distinct subsets. However, there is also accumulating evidence that intestinal DC function can be conditioned through exposure to local factors such as thymic stromal-derived lymphopoietin (TSLP), IL-10, transforming growth factor-β (TGF-β), or retinoic acid (RA) (20, 23).

Microbial interactions and repair mechanisms

The immune system is equipped with a variety of cell surface and cytoplasmic pattern recognition receptors (PRRs) that recognize conserved structures termed microbe-associated molecular patterns (MAMPs), which are expressed by a wide variety of microorganisms. A number of PRR families have been described including Toll-like receptors (TLRs), nucleotide oligomerization domain-containing protein (NOD)-like receptors (NLRs), RA inducible gene I (RIG-I)-like receptors (RLRs), and C-type receptors (24, 25). These PRRs are ancient host defense mechanisms that initiate complex signaling cascades leading to host protective responses through activation of transcription factors such as nuclear factor κB (NF-κB). The best characterized of these PRRs are the TLRs, which recognize a variety of products ranging from cell wall carbohydrates to nucleic acid and signal via two main adapter molecules, myeloid differentiation protein 88 (MyD88) and Toll/IL-1R (TIR)-domain-containing adapter-inducing interferon-β (TRIF). TLRs are expressed on a wide range of leukocytes in the intestine and can also be expressed by IECs (25).

TLRs play an important role in the initiation of intestinal inflammation. MyD88 signals are required for the development of spontaneous colitis in IL-10$^{-/-}$ mice, indicative of a role for TLR-mediated recognition of the endogenous gut flora in the onset of intestinal inflammation (26). NOD2, a member of the NLR family, is of particular interest in IBD, as mutations in the gene encoding NOD2 are associated with an increased risk of developing CD (27). NOD2 is expressed by DCs, macrophages, and Paneth cells and through its detection of the muramyl dipeptide component of peptidoglycan acts as a cytosolic bacterial sensor. NOD2 activation induces inflammatory cytokine production by myeloid cells and production of antimicrobial peptides by Paneth cells (27, 28). However, there is also evidence for an immune suppressive role through antagonism of TLR2 function (28). Although it is still not understood precisely how NOD2 mutations predispose to CD, this is a clear demonstration that defects in PRRs can precipitate the development of IBD.

The intestine is endowed with a remarkable ability to renew and repair damage to the epithelial surface via processes termed restitution and regeneration. Repair mechanisms are regulated by a variety of factors including cytokines and growth factors such as TGF-β, trefoil factors (29), and prostaglandins (30). MyD88 has also been shown to be important in the preservation of the epithelial barrier (30, 31), while NF-κB expression in gut epithelial cells is essential for maintenance of immune homeostasis (14). Thus, while PRR stimulation drives pro-inflammatory cascades associated with IBD development, they may also play critical roles in protection and repair in the intestine.

Recent studies have highlighted an important role for IL-23 in intestinal immunity. IL-23 expression and activity are enhanced in the intestine and it has been shown to contribute to host protective pathways in the gut. However, IL-23 is also a key driver of chronic intestinal inflammation in both mice and humans. Here, we discuss the double-edged sword of IL-23-mediated immunity in the intestine.

IL-23

Discovery and source

The discovery of the T-helper 1 (Th1)/Th2 paradigm and functional heterogeneity of helper T-cell responses (32) ushered in a new era in cytokine biology, prompting a search to identify upstream factors involved in the differentiation of distinct Th responses. The heterodimeric cytokine IL-12, composed of a p35 and a p40 subunit, was shown to play a key role in promotion of Th1 responses and host defense toward intracellular pathogens (33, 34). In addition, the IL-12-driven Th1 response was also implicated in the pathogenesis of a number of autoimmune and inflammatory diseases (34).

In 2000, a novel heterodimeric cytokine, termed IL-23, was discovered, which is comprised of IL-12p40 and an IL-23-specific p19 subunit (35). This forced a re-evaluation of the role of IL-12 in inflammatory disease. In the seminal study on the role of IL-23 in inflammation, Cua *et al.* (36) utilized IL-23-specific knockout mice to demonstrate that in experimental autoimmune encephalomyelitis (EAE) it was IL-23 rather than IL-12 which was the agent driving the inflammation. In the wake of the initial studies in EAE, IL-23 was identified as the causative agent in a number of inflammatory disorders in tissue including joint (37) and intestinal inflammation (5–8). In addition, IL-23 has been implicated in psoriasis (38, 39). IL-23 expression is increased in lesional psoriatic skin of humans and intra-dermal injection of IL-23 in mice induces psoriasis-like symptoms associated with increased expression of cytokines including IL-22, IL-17, and TNF-α (38, 40), indicating a key role for IL-23 in autoimmune inflammation in the skin.

IL-23 is expressed by cells of the innate immune system such as DCs and macrophages in response to PRR stimulation,

endogenous signals like prostaglandin E2, and stimulation via CD40L, demonstrating a potential role for T cells in reinforcement of the IL-23 response (7, 34). There appears to be differential regulation of IL-12 and IL-23 by myeloid cells in response to TLR signaling as the former is strongly induced following TLR4, TLR3, or TLR8 stimulation (34). By contrast, stimulation of TLR2 alone or in combination with NOD2 stimulation induces expression of IL-23 (34, 41). Additionally, dectin-1 agonists, such as curdlan, induce a striking production of IL-23 (42). Indeed, zymosan, a component of the *Saccharomyces cerevisiae* cell wall and a TLR2 and dectin-1 agonist, induces IL-23 production as well as promoting Th17 responses *in vivo* (43), suggesting that certain microbes possess a 'MAMP signature' that specifically activates the IL-23 axis. The preferential production of IL-23 in the gut may therefore be a function of the pattern of PRR expression on intestinal immune cells as well as the nature of the PRR stimuli present in the intestinal lumen.

A functional receptor for IL-23 was identified a couple of years after the discovery of the cytokine (44). The receptor is also a heterodimer and unsurprisingly shares one subunit, IL-12Rβ1 (binds IL-12p40), with the IL-12 receptor, with its own specific subunit IL-23R, and is expressed on T cells, natural killer cells, DCs, and macrophages. IL-23 signaling is mediated predominantly through the signaling adapter molecule signal transducer and activator of transcription 3 (STAT3) (34, 44).

IL-23 and Th17 responses

In early studies, IL-23 was shown to induce interferon-γ (IFN-γ) production from activated T cells, suggesting overlapping function with IL-12 (35). However, more recent work has focused on its ability to promote a novel subset of IL-17-producing $CD4^+$ helper T cells termed Th17 cells (37, 45). Th17 cells have been implicated in the pathogenesis of several autoimmune diseases including EAE (43, 45, 46) and collagen-induced arthritis (37). The strong link between IL-23 and the Th17 response *in vivo* suggested that IL-23 was involved in the differentiation of Th17 cells. However, a series of papers identified TGF-β and IL-6, rather than IL-23, as the key cytokines directing Th17 cell development (47–49). Subsequently, it was shown that other pro-inflammatory mediators such as IL-1 (50) and IL-21 could substitute for IL-6 during Th17 differentiation (51–53). A similar developmental pathway has also been described for human Th17 cells (54, 55).

At the molecular level, both the transcription factor RORγT (46) and STAT3 (51, 56–58) were identified as key factors in Th17 differentiation. More recently, other transcription factors including an additional member of the ROR family, RORα, IRF4 (59), and the aryl hydrocarbon receptor (60, 61) have also been implicated in this pathway. Although not required for Th17 differentiation, IL-23 does appear to be an important control point in the Th17 response. In the absence of IL-23, there is a reduction in the accumulation of Th17 cells *in vivo* in response to inflammatory stimuli, suggesting a role for IL-23 in the expansion and/or maintenance of the Th17 response (37, 45).

It is now evident that Th17 cells are a heterogeneous population producing a number of cytokines in addition to IL-17 including IL-6, IL-17F, IL-21, IL-22, and TNF-α (45). Recently, it was shown that in the absence of IL-23, Th17 cells demonstrated reduced production of inflammatory cytokines and increased secretion of IL-10, which correlated with an impaired ability to transfer EAE (62). These results indicate that IL-23 may be an important factor in selecting inflammatory Th17 effector functions providing a mechanism by which the Th17 response can adapt to environmental conditions.

IL-23 in models of intestinal inflammation

In the last decade, a large number of animal models of intestinal inflammation have been used to study the etiology and pathogenesis of IBD (reviewed in 2). The majority of models involve chronic inflammation and develop spontaneously in mice with genetic alterations that disrupt normal functioning of components of the intestinal immune system such as epithelial cells, innate immune cells, and helper T-cell subsets. There are also models of acute inflammation that develop following administration of chemicals such as DSS that disrupt the integrity of the epithelial cell surface. These models tend to be self-limiting, characterized by epithelial cell hyperplasia and recruitment of innate immune cells, and may represent a dysregulated repair response. Although none of the mouse models are identical to CD and UC, they reflect the heterogeneity of the human disease, which also involves a spectrum of acute and chronic inflammation. These models also illustrate the complex interplay between the diverse cell types of the intestinal immune system and the gut flora that actively control intestinal homeostasis.

In our laboratory, we have developed both T-cell-dependent and T-cell-independent models of colitis to probe adaptive and innate immune interactions that mediate intestinal inflammation. Our results indicate a pivotal role for IL-23 in the inflammatory response.

IL-23 and innate intestinal inflammation

The first evidence that IL-23 could mediate intestinal inflammation independent of effects on T cells came from an acute

model of colitis (7). In this model, RAG $^{-/-}$ mice administered an agonistic anti-CD40 monoclonal antibody developed intestinal inflammation accompanied by a systemic inflammatory response including splenomegaly, increases in serum cytokines, wasting disease, and inflammatory infiltrates in the liver. Increases in TNF-α and IFN-γ were present in the spleen and intestine and blockade of these cytokines led to amelioration of systemic and mucosal inflammation. By contrast, analysis of the functional role of IL-12 family cytokines using RAG $^{-/-}$ mice deficient in IL-12 or IL-23 revealed differential roles for these cytokines. Systemic disease was driven by IL-12 and was independent of IL-23, whereas IL-23 but not IL-12 played a non-redundant role in colitis. These results revealed a striking compartmentalization of the response and indicated a pivotal role for IL-23 in the intestine.

This may in part reflect differential production of IL-23 relative to IL-12 in the gut. Indeed, in the anti-CD40 model there was a marked accumulation of activated DCs in both the spleen and intestine; however, there was greater induction of IL-23p19 mRNA compared with IL-12p35 mRNA in activated colonic DCs. Furthermore, colonic DCs expressed higher levels of IL-23 upon activation than their counterparts in the spleen. In support of this observation, Becker *et al*. (63) reported constitutive IL-12p40 activity associated with IL-23 expression among small intestinal *lamina propria* DCs in a process dependent on the intestinal flora. Together, these results suggest that some intestinal DCs are primed to preferentially produce IL-23. Whether this represents the activities of distinct DC subsets in the intestine versus the spleen or is the consequence of local tissue conditioning of the DCs is not known.

To further investigate the role of IL-23 in innate intestinal inflammation, we turned to a model of intestinal bacterial infection in immune deficient RAG $^{-/-}$ mice (5, 64). *Helicobacter hepaticus* is a Gram-negative microaerophilic bacterium that colonizes the crypts of the cecum and the colon (65). In lymphocyte-replete mice, it establishes a lifelong infection, but mice are resistant to disease in part due to activation of immune suppressive IL-10-producing T cells (66). However, infection of susceptible strains such as 129 SvEv RAG2 $^{-/-}$ mice with *H. hepaticus* leads to the development of typhlitis (inflammation of the cecum), colitis (64, 67, 68), and in some cases colon cancer (69).

Intestinal symptoms are accompanied by systemic inflammation, which manifests itself as splenomegaly and liver inflammation with a marked accumulation of neutrophils and myeloid cells in the spleen and intestine (64). Blockade of IL-23p19 inhibited typhlitis and colitis, suggesting a key functional role for IL-23 in bacteria-driven intestinal inflammation (5). By contrast with the anti-CD40 model, neutralization of IL-23 also inhibited the *H. hepaticus*-driven systemic inflammation. This may be explained by the fact that CD40 ligation will directly initiate an inflammatory cascade in the spleen independent of the intestine, whereas in *H. hepaticus* infection, the inciting stimulus is contained primarily within the intestine, such that the splenic immune activation may be secondary to the intestinal response. These studies highlight the role of IL-23 in orchestrating an innate inflammatory cascade in the gut and show that both T-cell-derived and bacterial signals promote IL-23 production *in vivo*.

IL-23 drives expression of Th17 signature cytokines by innate cells in the gut

The ability of IL-23 to differentially induce intestinal inflammation suggests the presence of tissue-specific IL-23-dependent effector pathways. In innate colitis, IL-23 was found to control the production of a number of inflammatory cytokines such as IL-1β, IL-6, TNF-α and IFN-γ and both TNF-α and IFN-γ were found to play a functional role (7). This finding is consistent with the ability of IL-23 to induce production of these inflammatory cytokines by activated DCs and macrophages (34). In addition, in both anti-CD40 and *H. hepaticus*-induced colitis, there was a highly localized induction of the Th17 cytokines IL-17, IL-17F, and IL-22 (5, 7, authors' unpublished observations) in the intestine, which was not found in the spleen. The functional relevance and cellular source of these cytokines is currently unclear. However, the increase in IL-17 is consistent with the prominent neutrophil infiltrate characteristic of *H. hepaticus* infection. The inflammatory response to the intestinal pathogen *Citrobacter rodentium* has been shown to be IL-23 dependent and associated with induction of Th17 cytokines (49, 70). Recent findings indicate that IL-22, rather than IL-17, played a key protective role and that DCs are a source of IL-23-dependent IL-22 (70). These results identify the induction of Th17 signature cytokines from innate immune cells as an important component of IL-23 function in the gut.

IL-23 and T-cell-mediated colitis

An early study demonstrated that blockade of IL-12p40 but not IFN-γ could attenuate established colitis in IL-10 $^{-/-}$ mice. This finding suggested that in this spontaneous model, IL-12p40 possessed activity outside the IL-12(p70)/IFN-γ axis (71). IL-23 has since been shown to play a key role in T-cell-dependent colitis in a number of models including spontaneous and *H. hepaticus*-induced colitis in mice deficient in IL-10 or IL-10 signaling, respectively, suggesting that the IL-12p40 activity is due to IL-23 (6, 8). Similarly, adoptive transfer of

$CD4^+CD45RB^{hi}$ T cells into $RAG^{-/-}$ mice did not result in colitis in IL-23-deficient $RAG^{-/-}$ hosts, whereas disease developed normally following T-cell transfer into IL-12-deficient $RAG^{-/-}$ recipients (5). Systemic signs of disease, including wasting disease, splenomegaly, and liver inflammation, could be mediated by either IL-12 or IL-23 but were dependent on IL-12p40. Together, these results indicate that IL-23 plays a highly specific role in the intestinal inflammatory response, whereas either IL-12 or IL-23 is sufficient to drive the systemic response. A number of pro-inflammatory cytokines were increased in the inflamed colon including IFN-γ, TNF-α, IL-1β, and IL-17, reflecting activation of both Th1 and Th17 cells. In the absence of IL-23, both Th1 and Th17-associated cytokines were reduced, suggesting that IL-23 promotes both types of response in the intestine (5). It has been shown recently that blockade of IL-23 was sufficient to resolve established colitis, indicating that IL-23 contributes to both the induction and maintenance of intestinal inflammation (4).

There is abundant evidence that Th1 cells contribute to T-cell-mediated colitis. *H. hepaticus*-driven disease in IL-10-deficient mice (66) as well as T-cell transfer colitis (72) are both dependent on IFN-γ. In addition, T-bet (73) and IRF-1 (74), both of which play key roles in Th1 development, are required for development of colitis. However, there is also evidence that Th17 cells can play a pathogenic role. Firstly, Th17 cells accumulate in the inflamed colon in T-cell-dependent IBD and other models (75). Secondly, depletion of factors involved in Th17 differentiation, such as IL-6 (76) or IRF-4 (77), inhibits colitis. Finally, transfer of intestinal bacteria-reactive Th17 cells to severe combined immunodeficiency (SCID) mice led to the development of IL-23-dependent colitis (4). Additionally, intestinal DCs are potent inducers of Th17 differentiation (78, 79), suggestive of a link between Th17 and intestinal immune responses.

Although clearly capable of inducing intestinal inflammation, it is not clear which Th17 cell effector cytokines mediate the inflammatory response. Initial studies focused on IL-17 production, and it was reported that blockade of IL-17 in combination with IL-6 could ameliorate colitis in IL-10-deficient mice (8). However, studies from our own laboratory (75) and others (80) have shown that IL-17 production by T cells was dispensable for T-cell transfer colitis. Although IL-17 and IL-17F have overlapping functions, there is evidence for differential activities in the gut (81). In an acute model of colitis, IL-17 mediated a protective role (81, 82), while IL-17F was pathogenic (81). In light of these results, it would be of interest to establish the role of IL-17F in chronic colitis. Another potential candidate is IL-21, which in addition to its effects on Th17 differentiation has been shown to induce MIP3α expression that acts as a chemoattractant for $\alpha4\beta7^+$ T cells as well as promoting the release of tissue-destructive matrix metalloproteinases from gut fibroblasts (83). In addition, IL-22 has been reported to play a pathogenic role in psoriasis (40, 84), but, as discussed below, the limited information available suggests a more protective role in the intestine (70, 85). Collectively, these results indicate that both Th1 and Th17 cell responses can contribute to chronic intestinal inflammation and that in the intestine, IL-23 is permissive for both types of response.

IL-23 and the Th17/Treg pathway

Treg cells control intestinal homeostasis

Early studies showed that T-cell transfer colitis could be inhibited by Tregs present within the $CD4^+CD45RB^{lo}CD25^+$ population of normal mice (17). $CD4^+CD25^+$ Tregs express high levels of the transcription factor Foxp3 and play a non-redundant role in immune homeostasis (86). Foxp3 is required for the development and function of Treg cells, as loss of its function in mice results in a lack of Treg cells and the development of a fatal autoimmune and inflammatory disease. Mutations in the human *FOXP3* gene leads to the onset of a similar condition termed immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX). IPEX patients develop type 1 diabetes, allergy, and enteropathy, indicating the importance of Foxp3-mediated pathways in the control of immune pathology in humans (87, 88). $Foxp3^+$ Tregs can be imprinted with their suppressive function in the thymus (86) and have been termed naturally arising regulatory T cells (nTregs). It is now evident that naive T cells can acquire Foxp3 expression, so called induced Tregs (iTregs) following T-cell activation in the presence of TGF-β (89, 90). Recent studies indicate that the gut is a preferential site for development of $Foxp3^+$ Tregs due to the presence of a specialized subset of $CD103^+$ DCs that promote Foxp3 expression via a TGF-β and RA-dependent mechanism (91, 92).

IL-23-mediated inhibition of regulatory pathways

The mutual requirement for TGF-β in Th17 and Treg development suggested a shared developmental pathway for these T-cell subsets. Other factors like IL-2 (86) and the vitamin A metabolite RA (91–94) promote Treg development and appear to inhibit Th17 development (58, 94, 95). Conversely, IL-6 (48, 49) and IL-21 (51–53) induce the differentiation of Th17 cells and inhibit Foxp3 expression. These results suggest a model in which the peripheral development of Th17 and iTreg cells is governed by local environmental conditions. This reciprocal relationship also suggests that cytokines may be

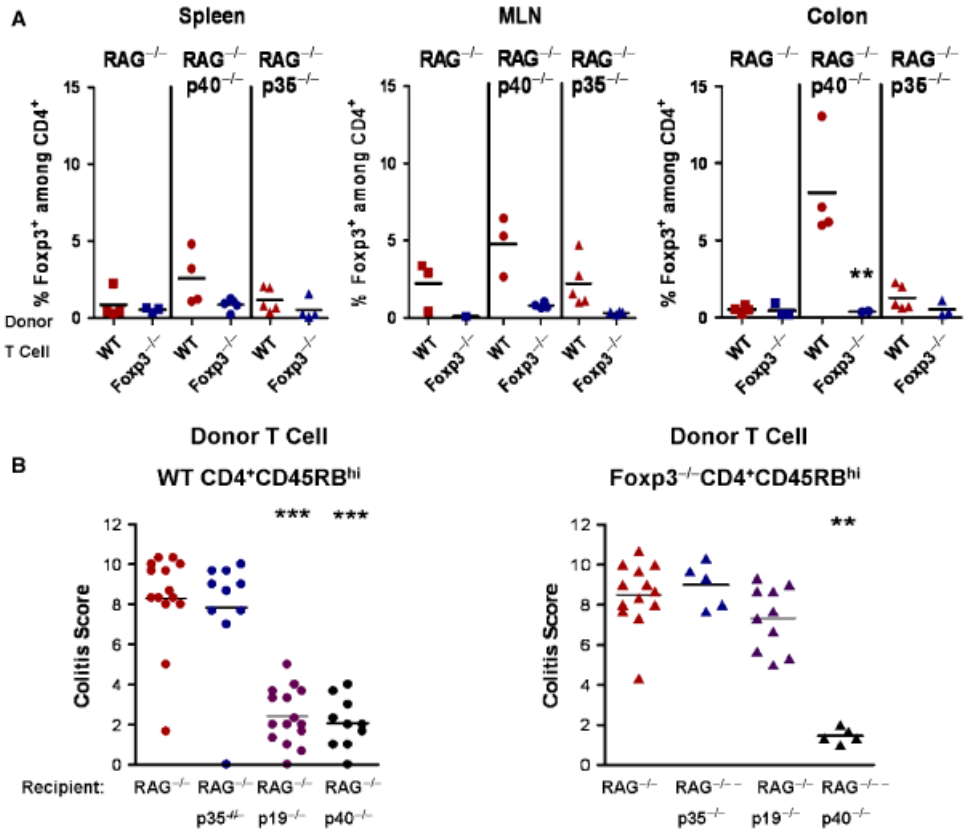

***Fig. 1.* (A) Foxp3 expression in the spleen, MLNs, and colon of mice transferred with either wildtype or Foxp3 $^{-/-}$ T cells.** IL-12p40 but not IL-12p35 is required for restraining Foxp3 responses, in accordance with a role for IL-23p19 in iTreg inhibition. $^{**}P < 0.01$ and $^{***}P < 0.001$. (B) Colitis scores of IL-12 family deficient RAG $^{-/-}$ upon transfer with either wildtype or FoxP3 $^{-/-}$ naïve T cells. IL-23p19 and IL-12p40 are required for colitis in the presence of iTreg (wildtype T cells). In the absence of iTreg generation (Foxp3 $^{-/-}$ T cells), IL-12p40, but neither IL-12p35 nor IL-23p19 alone, is required for disease. Part of the data presented is reproduced from *Immunity* 2008;28:559–570 with permission.

permissive for inflammation indirectly through the suppression of the Treg response as has been established for IL-6 in the development of EAE (53).

Recently, we have analyzed the relationship between IL-23 and intestinal Treg responses. We found that neutralization of TGF-β or IL-10 in IL-23-deficient RAG $^{-/-}$ hosts transferred with colitogenic T cells led to intestinal inflammation in what was normally a resistant strain (75). Although the resulting inflammation was not equivalent to that observed in IL-23-sufficient mice, it suggested that intestinal inflammation was not completely dependent on IL-23 when particular immune suppressive pathways were removed. Resistance to T-cell transfer colitis in IL-23-deficient RAG $^{-/-}$ mice was associated with a marked increase in the frequency of Foxp3^{+} Tregs in the MLN and colon compared with IL-23-sufficient RAG $^{-/-}$ mice. This increased Treg frequency is also found in IL-12p40 RAG $^{-/-}$ but not in IL-12p35 RAG $^{-/-}$ mice (*Fig. 1A*), confirming a role for IL-23 but not IL-12 in constraining intestinal Treg responses. This increase was not observed in the spleen and may explain the development of wasting disease and splenomegaly in T-cell-restored IL-23-deficient RAG $^{-/-}$ mice (5).

To test the functional role of Foxp3^{+} cells that emerged in the intestine in the absence of IL-23, we transferred Foxp3-deficient CD4^{+}CD45RB^{hi} cells into IL-23-deficient RAG $^{-/-}$ mice. Strikingly, this approach resulted in disease with a very similar incidence and severity to T-cell transfer colitis in IL-23-sufficient RAG $^{-/-}$ mice (75), while IL-12p40-deficient RAG $^{-/-}$ mice, lacking both IL-12 and IL-23, were still resistant to wasting disease and colitis (*Fig. 1B*). These results indicate that IL-23 promotes intestinal inflammation by repressing Treg cells in the intestine and that IL-12-dependent mechanisms are sufficient to mediate colitis in the absence of Tregs. IL-23 had little effect on pre-formed Tregs, suggesting that it suppresses Treg differentiation in the intestine. This idea may explain the decreased Th1 response in IL-23-deficient mice, as an increase in Treg responses will suppress a number of immune responses including the differentiation and expansion of Th1 cells.

IL-6 has been shown to promote inflammation by desensitizing T cells to Treg-mediated suppression (96) and via inhibition of TGF-β-mediated differentiation of Tregs from naive precursors (48, 49). As has been described previously (76), we found that inhibition of the IL-6R pathway prevented development of colitis (unpublished observations) and that this too was linked to an increased frequency of $CD4^+Foxp3^+$ cells (75). However, unlike the tissue-specific induction of Treg cells observed in the absence of IL-23, removal of IL-6 signaling led to an increased frequency of Treg cells in both the spleen- and gut-associated lymphoid tissue, suggesting a more widespread mechanism of action. Further studies are required to determine whether the increase in frequency of $Foxp3^+$ cells contributes to the resistance to colitis observed in the absence of IL-6. Together, the data indicate that by contrast with IL-6, IL-23 plays a highly specific role in restraining Treg responses in the intestine, thereby promoting local inflammatory responses and host protective immunity.

Molecular mechanism of IL-23-mediated Treg inhibition

Currently, the mechanism via which IL-23 acts to inhibit Tregs is not known, but the tissue specificity may be explained by the increased production of IL-23 in the intestine. However, addition of IL-23 to cultures of antigen-activated T cells failed to inhibit TGF-β-mediated Foxp3 induction, suggesting that it is not a straightforward direct effect (75). Alternatively, IL-23 may act indirectly via induction of other cytokines that directly suppress Treg differentiation or through inhibition of the ability of $CD103^+$ DCs to induce Tregs. However, IL-23 was not essential for the production of a number of inflammatory cytokines linked to Foxp3 suppression, including IL-6 (48, 49), IL-21 (51–53), or IL-27 (97), as these cytokines were upregulated in the inflamed intestine of IL-23-deficient mice (75).

Another possibility is that T-cell responsiveness to IL-23 is enhanced in the gut environment and that these conditions are not reproduced *in vitro*. IL-23R expression has been described to be very sensitive to the concentration of TGF-β. Low levels of TGF-β promote IL-23R expression in the presence of IL-6 or IL-21. Conversely, higher concentrations of TGF-β inhibit IL-23R expression. Interestingly, IL-23 was shown to suppress Foxp3 expression in T cells transduced with the IL-23R, indicating that in the presence of sustained IL-23R expression, IL-23 can directly suppress Foxp3 (98). In this study, it was proposed that Th17 and iTregs can arise from a common intermediate that expresses both RORγT and Foxp3 (98). Molecular characterization revealed a direct physical interaction between Foxp3 and RORγT that played a functional role in the ability of Foxp3 to inhibit RORγT-driven IL-17 production (98, 99). In these elegant studies, a model is proposed in which Th17 and iTreg differentiation is regulated in a cell-intrinsic fashion via direct interactions between Foxp3 and RORγT and that cytokines modulate differentiation by influencing the balance of Foxp3 and RORγT within a cell. $Foxp3^+RORγT^+$ cells could be found in the intestine (98, 100), raising the possibility that IL-23 acts on this intermediate cell to directly suppress Treg cell differentiation.

Nevertheless, transfer of Foxp3-deficient T cells to lymphocyte-deficient hosts did not result in a major skew toward the Th17 lineage, as would be predicted in a cell-intrinsic model (75). Instead, our results favor a model in which IL-23 either directly or indirectly inhibits Treg cell differentiation in a cell-extrinsic manner, leading to the emergence of effector responses that would otherwise have been suppressed. Several recent studies have identified the gut-associated lymphoid tissue as a preferential site of Treg induction (20). Our results are consistent with this idea and show in addition that iTreg cells play a functional role in the control of the inflammatory response. Furthermore, they identify IL-23, like TGF-β and RA, as an additional factor that influences the balance between tolerance and immunity in the intestine.

IL-23 in intestinal host defense and repair

Although most studies have focused on the pathogenic role of IL-23 in chronic inflammation, it is evident that IL-23 and associated Th17 effector cytokines also mediate host protective immunity (101–103). IL-17 is a potent stimulator of granulopoiesis and drives neutrophil recruitment through induction of chemokines such as CXCL8 (IL-8) (104). This property of IL-17 renders it important in host defense against a number of infections including *Klebsiella pneumoniae* (103, 105), *Candida albicans* (106), and *Toxoplasma gondii* (107). IL-22, whose expression appears to be stringently linked to IL-23, has more recently been described as a crucial factor in the resistance to *K. pneumoniae* (108) and *C. rodentium* infection (70) through the induction of anti-microbial peptides and maintenance of epithelial barrier function. Similarly, in models of both acute and chronic colitis, IL-22 mediates protection via induction of mucin production from colonic epithelial cells (85).

Consistent with these results, a tissue protective role for IL-23 and IL-17 was also observed in models of acute intestinal inflammation (81, 82, 109). In these models, there is disruption of the epithelial cell barrier and exposure of the immune system to an increased bacterial load. In this setting, IL-23 and

associated Th17 cytokines may be protective through their ability to promote production of anti-microbial peptides and initiate epithelial repair mechanisms to limit bacterial penetrance.

The data suggest a complex role for IL-23 in intestinal immunity mediating both protective and pathologic functions. Under homeostatic conditions, low levels of IL-23 may contribute to intestinal barrier function and microbicidal activity though IL-17 and IL-22. Treg cells may complement this activity through production of TGF-β that directly promotes epithelial repair mechanisms (29) and induces non-inflammatory Th17 responses (62). In the presence of a strong inflammatory stimulus, there is increased production of IL-23 and other inflammatory mediators that inhibit Treg responses leading to the development of chronic inflammation involving the activation of Th1 and inflammatory Th17 responses (*Fig.* 2).

IL-23 and human IBD

In accordance with the animal models, there is also a large body of data supporting a role for IL-23 in human IBD. CD is associated with elevations in both IL-12 and IL-23 (110, 111) as well as increases in IFN-γ and IL-17 production by T cells and non-T cells (111–114). Increased expression of IL-22 (115) and IL-21 (83) was also found in the inflamed intestine of IBD patients. Recent attention has focused on a population of $CD14^+$ macrophages that are abundant in CD patients (114). These cells expressed high levels of IL-23 and TNF-α in response to stimulation with commensal bacteria and preferentially produced IFN-γ rather than IL-17 in response to IL-23 stimulation. IFN-γ functioned in a positive feedback loop to promote the differentiation of these high-IL-23-producing inflammatory macrophages. These results indicate that intestinal inflammation in humans is associated with accumulation of innate immune cells that are prone to IL-23 production in response to stimulation with commensal flora. In addition, they provide a clear positive association between IL-23 and IFN-γ in the intestinal inflammatory cascade. Altogether, data in human IBD provide evidence for activation of Th1 and Th17-associated cytokines produced by both T cells and innate cells. Importantly, clinical trials of an anti-IL-12p40 antibody proved promising in the treatment of CD, providing direct evidence of a functional role for IL-12 and/or IL-23 in CD (116).

The spotlight was focused on the IL-23 axis following the publication in 2006 (9) of a genome-wide association study that identified *IL-23R* as a susceptibility gene in IBD. This study identified a number of single-nucleotide polymorphisms (SNPs) across the IL-23R gene associated with development (but not severity) of IBD. Interestingly, the SNPs identified were associated with both CD and UC, indicating that they were more associated with general GI inflammation than specific forms of IBD (117). This work has been followed by reports of

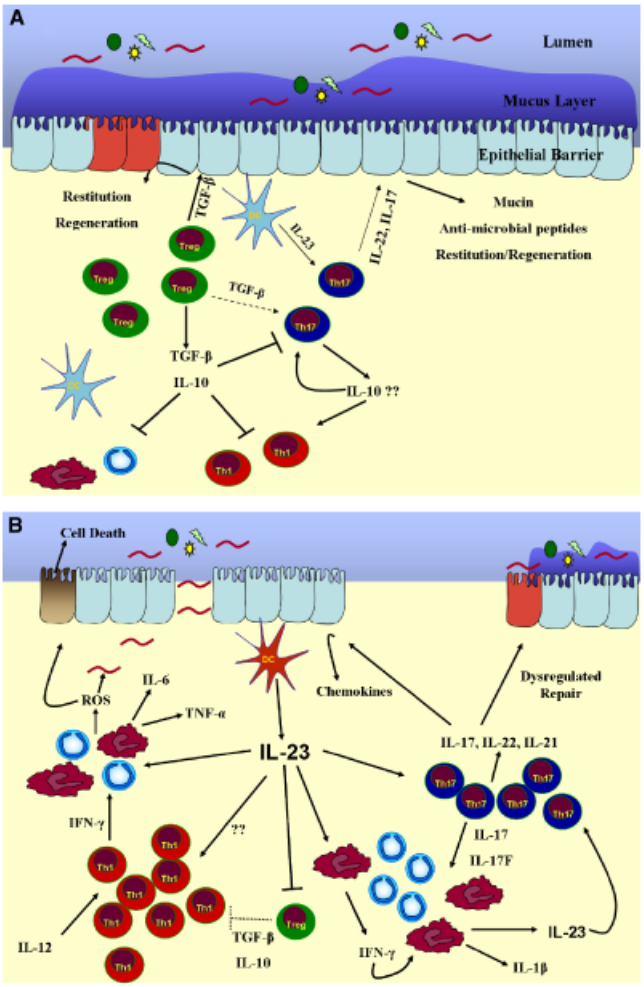

***Fig.* 2. The IL-23 axis and control of the balance between immune homeostasis and pathology in the intestine.** (A) Under steady state conditions, the intestine is populated by an array of immune cells, including a significant population of Tregs that prevent aberrant immune responses through production of molecules such as TGF-β and IL-10, despite the presence of *Th1* and *Th17* cells. TGF-β also plays a key role in the restitution and regeneration of the epithelium. In addition, it supports the differentiation of small numbers of Th17 cells, which produce cytokines like IL-17 and IL-22 in response to the low levels of IL-23. These direct mucin and anti-microbial peptide production, promoting host defense and preserving the epithelial barrier. Maintenance of an intact epithelial barrier prevents translocation of the microbial flora, thus avoiding excessive immune activation. (B) Breakdown of intestinal immune homoestasis leads to upregulation of IL-23 expression. This inhibits the accumulation of Tregs, allowing the expansion of Th1 and Th17 cells as well as leading to innate immune activation. IL-23 also promotes a pathogenic phenotype from Th17 cells, inhibiting IL-10 production and promoting expression of pro-inflammatory mediators. Significant damage to the epithelium due to this immune response allows the translocation of flora from the lumen into the underlying *lamina propria*, further exacerbating the inflammation and potentially activating the systemic immune response. Over-production of protective factors from Th17 may lead to a dysregulated repair response that may compound host damage (ROS, reactive oxygen species).

mutations in the area of the *STAT3* gene (10) and in *IL-17* and *IL-17F* (118) as enhancing the risk of developing IBD.

Although a number of susceptibility variants in the *IL23R* gene were identified, the molecular mechanism underlying their role in disease is unknown. One study has linked the disease-causing mutations with increased serum levels of IL-22 (119), suggesting that dysregulated IL-23 responses could elicit a Th17 signature response leading to disease, supporting the idea that the mutations are a gain of function. Somewhat at odds with these results, NOD2 mutations in DCs have been linked to a decreased ability to prime Th17 responses *in vitro* (41), linked to reduced IL-23 production upon stimulation with TLR2 and NOD2 agonists, suggesting that a failure to upregulate IL-23 may be harmful rather than protective. Clearly, further work is required to identify how mutations in *IL23R* affect IL-23-mediated effector functions in the intestine and the impact of this on host protective and immune pathological pathways.

Conclusions

The last number of years has seen a surge in our knowledge of the mechanisms underlying inflammatory diseases such as IBD. The combination of human genetics with animal models provided complementary approaches to identify key pathogenic pathways, such as the IL-23 axis, which, with its tissue-specific expression pattern, represents an attractive therapeutic target. Indeed, blockade of IL-23 is expected to not only inhibit chronic inflammatory pathways in the intestine but will also promote Treg responses invoking dominant tolerance and immune homeostasis in the gut. However, we cannot ignore the emerging roles of IL-23 and Th17-associated cytokines in host protective immunity and barrier function in the gut. Further insight into the role of particular Th17 cytokines in intestinal homoestasis could illuminate how distinct IL-23-driven responses may mediate protection against infection versus chronic immune pathology in the intestine.

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