The opposing roles of IL-21 and TGFβ1 in chronic inflammatory bowel disease

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Abstract
There are large numbers of T-cells in the mucosa of the intestine in healthy individuals. The stimulus for their presence is the normal gut microbiota. For unknown reasons, in patients with IBD (inflammatory bowel disease), there is inappropriate and chronic activation of mucosal T-cells which leads to gut damage and severe morbidity. In one form of IBD, namely Crohn’s disease, the T-cells are probably responding to the microbiota. T-cell survival in the gut wall is dependent on pro-inflammatory cytokines and antibody-mediated inhibition of one of these cytokines, TNFα (tumour necrosis factor α), has shown efficacy in patients, thus encouraging investigations of other ways to control mucosal T-cell responses. In the present paper, we give a brief review of T-cell immunology in IBD and then discuss how two particular cytokines, namely IL-21 (interleukin 21), which is generally pro-inflammatory and important in gut T-cell survival and in maintaining Th17 cells, and TGFβ1 (transforming growth factor β1), which is generally immunosuppressive, play opposing roles in gut inflammation.

Introduction
The gut is one of the most vulnerable sites in the body to infectious disease. The necessity of eating and drinking means that pathogens have ready access to the interior of the body. Stomach acid provides some protection, as does the mucus layer; however, pathogens can live on the surface of the gut, inside the epithelial cells lining the gut, and, by crossing the single layer of absorptive epithelium, gain access to the tissues. Unsurprisingly, the small and large intestine are richly endowed with lymphoid tissue in the form of aggregates of lymphoid tissue (Peyer’s patches and appendix), and solitary lymphoid follicles where immune responses can be generated. It is estimated that the normal human gut contains 300 Peyer’s patches and 30000–50000 solitary follicles [1]. The intervening mucosa is also densely infiltrated with T-cells, macrophages and IgA plasma cells which provide the effecter limb of mucosal immunity.

Since the Second World War, there has been a dramatic increase in chronic inflammatory diseases of various tissues. Examples include psoriasis in the skin, asthma in the airways, rheumatoid arthritis in the joints, Type 1 diabetes in the pancreas and multiple sclerosis in the brain. The gut is no exception, and approximately 1 in 500 individuals in developed countries have idiopathic IBD (inflammatory bowel disease). There are two main forms of IBD: UC (ulcerative colitis) and Crohn’s disease. The former is primarily a neutrophilic superficial lesion affecting the colon, whereas the latter is a mononuclear-dominated lesion, characterized by granuloma, deep fissuring ulcers and fibrosis. Crohn’s disease can affect any part of the gut [2].

There has been huge advances in understanding the immune processes which lead to the development of these diseases, although it is fair to say that most of the progress has been in Crohn’s disease, rather than UC, which is still poorly understood. In Crohn’s disease, it would appear that chronic inflammation is driven by T-cells responding not to pathogens, but to the normal microbiota of the gut [3,4]. The events which lead to initial sensitization are still not clear, but part of the reason lies in genetic variants of molecules which control microbial sensing and microbial degradation, such as the peptidoglycan-sensing intracellular receptor Nod2 (nucleotide-binding oligomerization domain-containing 2) and the autophagy protein ATG16L1 [5]. However, it is important to emphasize that these variants only account for a very small fraction of the susceptibility and are only moderate risk factors, so much more work needs to be done before there is any understanding of the sensitizing phases of IBD.

The intestinal microbiota is also a rich source of molecules which can regulate and initiate mucosal immune responses. Many bacteria ferment fibre to produce short-chain fatty acids such as butyrate, which, as well as being an energy source for colonocytes, is a potent inhibitor of histone deacetylation, affecting transcription of inflammatory and anti-inflammatory genes [6]. It is presumed, but not known, that the T-cells in Crohn’s disease are responding to antigens from the microbiota. Finally, gut bacteria are a source of many pathogen-associated molecular patterns which can stimulate innate immune responses, especially when the intestinal barrier is broken. Gut bacteria are also a highly resilient...
and stable population. They comprise approximately 1000 different species, uniquely adapted to survive in the gut, and essential for gut health, so they are extremely difficult to remove.

The alternative approach to study IBD is therefore to investigate the local immune response to identify pathways important for T-cell survival, inhibition of which might dampen inflammation. The most spectacular success of this strategy has been the advent of anti-TNFα (tumour necrosis factor α) antibodies to control Crohn’s disease and more recently UC.

The role of activated T-cells in IBD

T-lymphocytes in the gut mucosa in IBD are thought to be activated via co-stimulatory molecules expressing antigen-presenting cells, peptide antigens from the flora, cytokines produced by antigen-presenting cells, inflamed epithelium and T-cells themselves [3]. Signature cytokines are produced by these cells in active IBD, allowing classification into helper T-cell subsets. Lamina propria T-cells in Crohn’s disease produce higher amounts of Th1 cytokines such as IFNγ (interferon γ) and express the prototypic Th1 transcription factor T-bet [7]. Consistent with this, there is high expression of IL-12, the major Th1-inducing cytokine in humans, in Crohn’s disease mucosa [8]. In UC, however, T-cells may produce Th2 cytokines such as IL-5 and IL-13 [9]. In addition to prototypical Th1 or Th2 cytokines, large amounts of disease perpetuating cytokines such as TNFα and IL-6 are produced by lamina propria T-cells in IBD and by cells of the innate immune system [10].

A T-cell subset which makes IL-17 and IL-22 (Th17 cells) has recently been described in mice and humans [11]. It has been proposed that IL-17 is important in the protection of mucosal surfaces from bacterial infections and is pathogenic in some autoimmune diseases, such as experimental autoimmune encephalitis. In mice, the development of Th17 cells in the small intestine requires the presence of a specific commensal microbe, namely a segmented filamentous bacterium [12]. It is not clear whether specific types of bacteria are required for the appearance of Th17 cells in humans. A number of recent studies have analysed the presence of this Th17 cell subset in the gut. In IBD patients, Th17-inducing cytokines such as IL-6 and IL-21 and Th17-related cytokines such as IL-17A/F and IL-22 are present at increased levels in the inflamed mucosa [13–15]. Th17-related transcription factors including STAT3 (signal transducer and activator of transcription 3), IRF4 (interferon-regulatory factor 4) and ROR (retinoic acid receptor-related orphan receptor) A/C are up-regulated in CD4+ T-cells isolated from IBD mucosa [16,17]. The situation is far from clear, however, because other studies suggest that IL-17A may possess anti-inflammatory properties [18]. To make matters more complex, T-cells which make IL-17A also often express a related cytokine, IL-17E. Studies in the DSS (dextran sulfate sodium)-induced colitis model show that IL-17F deficiency results in reduced colitis, whereas IL-17A-knockout mice develop more severe disease, thus suggesting that IL-17F rather than IL-17A is crucial in sustaining DSS colitis [19].

Table 1 summarizes the controversy over whether IL-17 is protective or pathogenic in the gut.

The ultimate proof of principle for a critical role of a cytokine in IBD is whether neutralization in vivo, in patients, usually with a monoclonal antibody, is of therapeutic benefit. Disappointingly, neutralization of IFNγ or IL-12/23 in Crohn’s disease has not been an overwhelming success [20].

The potential role of IL-21 in IBD

IL-21 is a four-helix-bundle cytokine made by activated CD4+ T-cells, activated NKT (natural killer T) cells and follicular helper T-cells [21,22]. IL-21 functions are mediated by a heterodimeric receptor, formed by the common γ-chain subunit, shared with IL-2, IL-4, IL-7, IL-9, IL-13 and IL-15 receptors, and its own receptor (designated IL-21R). IL-21R is expressed on a variety of immune cells, including T-, B-, NK (natural killer) and dendritic cells, and non-immune cells (e.g. epithelial cells, fibroblasts and endothelial cells). IL-21 regulates activation, proliferation and survival of both CD4+ T- and B-cells, the functional activity of CD8+ T-cells and NK cells, and is able to limit the peripheral differentiation of Treg cells (regulatory T-cells) and to counteract their suppressive properties. In vitro studies also suggest that IL-21 can negatively regulate the maturation and function of dendritic cells.

IL-21 is markedly overexpressed in the gut in Crohn’s disease [22]. IL-21 is produced mostly by CD4+ T-cells co-expressing IFNγ [24]. In contrast, only a small fraction of IL-21-producing CD4+ T-cells co-express IL-17A, thus indicating that, in the human gut, IL-21 is produced preferentially by Th1 rather than Th17 cells. Activation of CD4+ T-lymphocytes from normal gut with an anti-CD3 antibody and exogenous IL-12, the major Th1-inducing factor in Crohn’s disease, increases the proportion of IL-21-secreting Th1 cells, whereas blockade of endogenous IL-12 in cultures of Crohn’s disease mucosal cells significantly reduces IL-21 production [23]. On the other hand, blocking IL-21 in cells from Crohn’s disease patients with antibodies or soluble receptor fusion proteins inhibits IL-17A and IFNγ production [23,25].

In the light of present knowledge on the redundancy of cytokines in IBD tissue, it is highly unlikely that neutralization of IL-21 would be therapeutic in patients. However, IL-21 may regulate additional inflammatory pathways in the inflamed gut which raises enthusiasm for its therapeutic implementation. Intestinal lamina propria fibroblasts and epithelial cells constitutively express IL-21R and respond to IL-21 by making inflammatory molecules. Following IL-21 stimulation, colonic fibroblasts secrete large amounts of MMPs (matrix metalloproteinases), including interstitial collagenase (MMP1) and stromelysin (MMP3), a family of enzymes involved in ulceration by degrading the extracellular matrix and which are abundantly expressed around gut ulcers in IBD [26,27]. Traditionally, it has
Table 1 | Conflicting evidence over whether IL-17A is protective or pathogenic in the gut

<table>
<thead>
<tr>
<th>Species</th>
<th>Finding</th>
<th>Conclusion</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Human</td>
<td>IL-17A is highly overexpressed by T-cells in Crohn’s disease and UC</td>
<td>Inconclusive</td>
<td>[14]</td>
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<tr>
<td></td>
<td>FoxP3 (^{+}) (\text{Treg}) cells in human IBD make IL-17A</td>
<td>Protective</td>
<td>[40]</td>
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<tr>
<td>Mouse</td>
<td>IL-17A-null mice develop more severe DSS-induced colitis</td>
<td>Protective</td>
<td>[19]</td>
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<td></td>
<td>IL-17A-null mice are protected from DSS-induced colitis</td>
<td>Pathogenic</td>
<td>[41]</td>
</tr>
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<td></td>
<td>IL-17A-deficient T-cells produce more severe colitis in immune-deficient recipients</td>
<td>Protective</td>
<td>[18]</td>
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<tr>
<td></td>
<td>IL-17A-deficient T-cells produce identical colitis to wild-type T-cells in immune-deficient recipients</td>
<td>No role</td>
<td>[42]</td>
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<td>IL-17A-deficient T-cells produce identical colitis to wild-type T-cells in immune-deficient recipients</td>
<td>No role</td>
<td>[42]</td>
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<td>IL-17A-null mice are protected from TNBS colitis</td>
<td>Protective</td>
<td>[43]</td>
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<td></td>
<td>Anti-IL-17A therapy does not ameliorate gut pathology in immune-deficient hosts receiving T-cells from IL-10-null mice with colitis</td>
<td>No role</td>
<td>[44]</td>
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<td></td>
<td>Anti-IL-17A therapy ameliorates pathology in Helicobacter gastritis</td>
<td>Inconclusive</td>
<td>[45]</td>
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<td>Mice colonized with SFB generate strong gut Th17 responses and are protected from pathology in Citrobacter rodentium infection, but have fewer bacteria</td>
<td>Inconclusive</td>
<td>[46]</td>
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<td></td>
<td>IL-17A-null mice have higher numbers of colon bacteria during Citrobacter rodentium infection, and more mucosal pathology</td>
<td>Inconclusive</td>
<td>[47]</td>
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</tbody>
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*In Citrobacter rodentium and Helicobacter infections, the severity of mucosal pathology during infection is a function both of innate and adaptive immunity, and bacterial colonization levels. Thus a manipulation which generates more effective immunity and reduces pathogen numbers will also reduce pathology, and vice versa, because they are dependent variables.

been considered that MMP production in the gut in IBD is driven by TNFα and IL-1β, but it is now clear that many other factors are involved. IL-21 also induces colonic epithelial cells to produce CCL20, a chemokine involved in the recruitment of α4β7-positive T-cells in the gut mucosa (Figure 1) [28,29]. The functional relevance of these findings relates to the demonstration that CCL20 is up-regulated on the inflamed gut epithelium of IBD patients, and the inflamed intestine of mice with chemically induced colitis [30,31]. Specifically, CCL20 is produced in excess in mice with DSS-induced colitis and mice with TNBS (trinitrobenzene sulfonic acid)-induced colitis. Treatment of mice with an anti-CCL20 neutralizing antibody significantly reduces tissue damage [31]. Notably, IL-21-deficient mice are largely protected against colitis, and are unable to up-regulate Th17-associated molecules during gut inflammation [25]. Clinical studies using monoclonal anti-IL-21 antibodies in IBD will determine whether IL-21 is indeed critical in human gut inflammation.

**Strategies to allow endogenous immunosuppressive pathways to dampen IBD**

One of the most interesting aspects of gut immunology is the maintenance of the ability to be able to respond to pathogens, but to be able to control the immune response against the microbiota. There is absolutely no doubt that the normal microbiota are hugely immunogenic. In germ-free animals, there is poor development of gut-associated lymphoid tissue, including Peyer’s patches which only contain primary follicles. Unlike conventionally colonized mice, few T-cells can be found infiltrating the intestinal lamina propria in germ-free animals and there is an almost complete absence of secretory IgA. Intraepithelial lymphocyte numbers are very low. Colonization of germ-free mice with microbes rapidly restores immune populations to the levels seen in conventionally reared mice.

The regulation of immune responses to microbes is highly complex and it is likely that there is no single pathway which takes precedence over others. This is beautifully exemplified by the fact that mice with targeted disruption or overexpression of immune genes often develop a spontaneous colitis [32,33]. Examples include IL-10- or IL-10R (IL-10 receptor)-deficient mice, IL-2-deficient mice, mice overexpressing IL-12 and mice overexpressing TNFα. TGFβ1 (transforming growth factor β1) seems, however, to be a particularly important cytokine. TGFβ1-null mice die early in life with severe systemic inflammation including a colitis; in the transfer model of colitis, T~reg~ cells producing TGFβ1 seem particularly important; and gut epithelial cells produce large amounts of active TGFβ1 [3]. Consistent with this, activated TGFβ1 is a potent inhibitor of inflammatory responses.

In the canonical signalling pathway, activated TGFβ1 receptors phosphorylate and activate the Smad2 and Smad3 transcription factors, which form heterodimeric complexes with Smad4, enter the nucleus and regulate the activity of target genes, either directly by binding to cognate DNA consensus sites or indirectly by interaction with other...
IL-21 plays a number of different roles in inflamed gut

IL-21 is produced mostly by CD4\(^+\) T-cells producing IFN\(\gamma\), but helps to keep both Th1 and Th17 cells alive. It activates epithelial cells to make CCL20, helping to recruit more T-cells into the inflamed gut. Finally, it induces fibroblasts to produce MMPs which destroy the extracellular matrix and basement membrane, so the epithelial cells detach and die.

Smad7 expression in effector T-cells determines whether a T-cell is susceptible to TGF\(\beta\)\(^1\)-mediated suppression

In the absence of Smad7, TGF\(\beta\)\(^1\) is an effective inhibitor of pro-inflammatory T-cells. However, in the presence of Smad7, TGF\(\beta\)\(^1\) signalling is blocked, and T-cells continue to make pro-inflammatory cytokines. TGF-\(\beta\)R, TGF\(\beta\)1 receptor.

Transcription factors. In Th1 cells, for example, TGF-\(\beta\)\(^1\) is markedly immunosuppressive [34]. Like many signalling pathways, Smads are also subject to regulation. In particular, the inhibitory Smad, Smad7, when expressed at high levels in cells, prevents Smad2/3 binding to the TGF\(\beta\)1 receptor, and also results in its ubiquitination and degradation. The relevance of this observation to IBD is that Smad7 is very highly overexpressed in T-cells from patients with IBD so there is disruption of TGF\(\beta\)1 signalling marked by a block in the phosphorylation of Smad3 due to high levels of Smad7. Knockdown of Smad7 in vitro with a Smad7 antisense oligonucleotide leads to restoration of TGF\(\beta\)1 signalling and, importantly, inhibition of ongoing pro-inflammatory cytokine production by endogenous TGF\(\beta\)1 [35–38].

Teleologically, disrupting TGF-\(\beta\)1-mediated immunosuppression is advantageous. During anti-pathogen inflammatory responses, there are markedly elevated levels of TGF\(\beta\)1. So some mechanism must exist to prevent the dampening of inflammation at a time when inflammation is beneficial. Smad7 serves this role. However, when the pathogen is
eliminated, endogenous TGFβ1 can help to heal the lesions. In IBD, however, where there is no pathogen and the host is mistaken into attacking the normal microbiota akin to a pathogen, Smad7 is deleterious, because it prevents endogenous TGFβ1 from dampening inflammation.

We have produced a transgenic mouse which overexpresses Smad7 in T-cells [39]. In vitro, T-cells from these animals fail to phosphorylate Smad2/3 when cultured with TGFβ1. Importantly, when transferred into immunodeficient mice, Smad7 transgenic T-cells produced a severe colitis which is not inhibited by Treg cells. To confirm this in humans, we were able to show that blood Treg cells, although able to suppress the proliferation of T-cells from the gut of normal patients, failed to inhibit the proliferation of T-cells from the gut of patients with Crohn’s disease [39]. However, when Smad7 was knocked down in Crohn’s disease gut T-cells, blood Treg cells were effective at dampening proliferation (Figure 2).

Collectively, these results show that endogenous TGFβ1 is capable of dampening ongoing gut inflammation as long as the inflammatory cells are responsive because they lack Smad7. This result also has major implications for immunotherapy with Treg cells because it shows that not only does one require a Treg cell, but also that the target cells need to be responsive to regulation.

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**References**


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