T-cell-directed therapies in inflammatory bowel diseases

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ABSTRACT

Gut inflammation occurring in patients with IBDs (inflammatory bowel diseases) is associated with exaggerated and poorly controlled T-cell-mediated immune responses, which are directed against normal components of the gut flora. T-cells accumulate in the inflamed gut of IBD patients as a result of multiple mechanisms, including enhanced recruitment of cells from the bloodstream, sustained cell cycling and diminished susceptibility of cells to undergo apoptosis. Activated T-cells produce huge amounts of cytokines, which contribute to amplify and sustain the ongoing mucosal inflammation. Strategies aimed at interfering with T-cell accumulation and/or function in the gut have been employed with clinical success in patients with IBDs. In the present article, we review the available results showing that T-cell-directed therapies are useful to dampen the tissue-damaging immune response in IBDs.

INTRODUCTION

CD (Crohn’s disease) and UC (ulcerative colitis) are the major chronic IBDs (inflammatory bowel diseases) in humans. Although the aetiology of both CD and UC is unknown, evidence has accumulated to demonstrate that IBD-related mucosal inflammation develops in genetically predisposed individuals as a result of an exaggerated mucosal immune response directed against luminal antigens [1]. Detailed analysis of the immuno-inflammatory molecules produced in the inflamed gut of IBD patients has, however, revealed that CD and UC have considerably different pathophysologies, even though non-specific mediators of tissue damage are produced in excess in both diseases. It is also becoming evident that, during IBDs, tissue damage is mediated by an active cross-talk between immune and non-immune cells, and that T-cells are key players in this pathogenic process [2]. This concept is supported by several lines of evidence. First, in both CD and UC, the tissue injury occurs in areas that are heavily infiltrated with activated CD4+ and CD8+ T-lymphocytes. These cells are recruited from the bloodstream as a result of enhanced production of chemoattractants within the inflammatory microenvironment and regulated expression of adhesion molecules on vascular endothelium and integrins on T-cells [3] (Figure 1). The increased mucosal accumulation of CD4+ T-cells also relies on a sustained cell cycling and diminished susceptibility of cells to undergo apoptosis, the programmed cell death that follows activation and helps maintain immune homoeostasis by preventing expansion of effector cells [4,5] (Figure 1). Secondly,
Figure 1  Putative mechanisms involved in the accumulation of T-cells in the gut of patients with IBDs
T-cells (T) are recruited from the bloodstream as a result of enhanced synthesis of chemokines by epithelial cells, APCs and stromal cells, and interaction between adhesion molecules (e.g. MadCAM-1 and ICAM-1) on vascular endothelium and integrins (e.g. α4β7 and LFA-1) on T-cells. In the gut lamina propria, T-cells undergo proliferation and become resistant against apoptotic stimuli.

studies conducted in various mouse models of IBDs have shown that most immunoregulatory events in mucosal inflammation are controlled by T-cells, that mucosal T-cell activation is antigen-dependent and the responsible antigens originate from intestinal bacteria [6–8]. Thirdly, reductions in CD4+ T-cell numbers may favour clinical remission in IBD patients. This can occur, for example, in patients undergoing bone-marrow transplantation or infected with HIV [9,10]. Fourthly, IBDs may associate with other T-cell-mediated diseases [e.g. psoriasis, MS (multiple sclerosis), rheumatoid arthritis and celiac disease], raising the possibility that these diseases may share the same T-cell-mediated pathogenic mechanism(s) [11,12]. Finally, therapeutic interventions targeting T-cells or T-cell-derived cytokines have already shown great promise in the treatment of IBD patients [13].

In the present article, we will review results showing that compounds interfering with mucosal T-cell accumulation and/or T-cell functions are useful in attenuating gut inflammation and in halting the progression of the inexorable tissue damage and loss of function associated with IBD.

INHIBITORS OF T-CELL TRAFFICKING IN THE GUT MUCOSA

T-cell trafficking in mucosal surfaces is mostly mediated by interactions between integrins (e.g. α4β7), a family of transmembrane glycoproteins expressed on the leucocyte surface, and cognate endothelial ligands, which include members of the immunoglobulin superfamily of adhesion molecules, VCAM-1 (vascular cell adhesion molecule-1), MAdCAM-1 (mucosal addressin cell adhesion molecule-1) and ICAM-1 (intercellular adhesion molecule-1) [14]. Compounds that inhibit the interaction between lymphocyte integrins and endothelial adhesion molecules have been used to reduce homing of T-cells into the gut lamina propria of IBD patients [3]. One such inhibitor is natalizumab, a humanized IgG4 monoclonal antibody directed against the α4 integrin subunit which was initially used commercially for treatment of MS [15]. In two large Phase III studies, natalizumab was effective in inducing and maintaining remission in patients with active luminal CD, regardless of whether patients were or were not refractory to anti-TNF-α (tumour necrosis factor-α) antibodies [16,17]. Since some patients receiving natalizumab treatment developed progressive multifocal leucoencephalopathy, a rare opportunistic infection of the central nervous system caused by the JC virus [18], the drug was temporarily withdrawn from the market. In 2008, the FDA (Food and Drug Administration) reapproved natalizumab for the treatment of moderate-to-severe active CD in patients who have had an inadequate response to, or are unable to tolerate, conventional therapies. More recently, vedolizumab (formerly MLN0002), a humanized α4β7 antibody, and rhuMAb Beta7, a humanized IgG1 monoclonal antibody
targeting the β7 integrin subunit, have been introduced for the treatment of active IBDs. Two Phase II studies showed that vedolizumab is significantly more effective than placebo in inducing short-term clinical responses in patients with UC and patients with CD [19,20]. Two Phase III trials evaluating vedolizumab therapy in inducing and maintaining remission in both UC and CD are currently in progress. A Phase I trial evaluating safety of rhuMAb Beta7 in patients with active ulcerative colitis is currently ongoing.

Alicaforsen (ISIS-2302) is an antisense oligonucleotide designed to inhibit the expression of ICAM-1, an endothelial molecule that selectively binds the integrin LFA-1 (leucocyte function-associated antigen-1) expressed on the leucocyte surface. Despite initial and promising results [21], a large randomized placebo-controlled study demonstrated that alicaforsen is ineffective in inducing clinical remission in patients with CD [22].

CCX282-B is a small molecule that has been developed to selectively antagonize the activity of CCR9 (chemokine receptor 9), a T-cell-surface molecule that promotes lymphocyte homing to small intestinal mucosa upon its binding with epithelial cell-derived CCL25 (chemokine ligand 25). Oral administration of this drug has been evaluated in a Phase II trial in patients with moderate-to-severe CD, with encouraging preliminary results [23]. Phase III trials of this compound in patients with CD are currently ongoing.

**INHIBITORS OF T-CELL PROLIFERATION**

TCR (T-cell-receptor) engagement by antigen/MHC ligand initiates a complex signalling cascade, which triggers the activation of various transcription factors [e.g. NF-xB (nuclear factor xB)] and promotes the release of calcium from the endoplasmic reticulum into the cytoplasm. Increases in calcium levels lead to the activation of calmodulin, a calcium-sensor protein, which, in turn, activates the serine/threonine phosphatase calcineurin [24]. Activated calcineurin then rapidly dephosphorylates and promotes the nuclear translocation of the transcription factor NFATc (nuclear factor of activated T-cells, cytoplasmic) [25]. Once in the nucleus, NFATc initiates the transcription of several genes involved in T-cell activation (e.g. cell proliferation and cytokine production) [26]. It is noteworthy that pharmacological inhibitors of calcineurin (i.e. cyclosporin and tacrolimus) have been shown to have some efficacy in IBDs. In particular, cyclosporin, a cyclic non-ribosomal peptide originally extracted from the fungus Beauveria nivea, is effective in the control of CD-related perianal fistulas; however, a very high rate of recurrence occurs after drug withdrawal [27]. More convincing results were seen in UC, where cyclosporin is effective as a rescue therapy in steroid-intolerant or steroid-resistant patients with severe disease [28]. However, cyclosporin treatment associates with a very high rate of adverse events, such as opportunistic infection, hypertension, electrolyte imbalances, paraesthesiae, tremor and renal dysfunction, which limit its long-term use [29]. Tacrolimus, a macrolide lactone, has been successfully employed in the treatment of patients with fistulizing CD and patients with severe steroid-refractory UC [30,31]. As with cyclosporin, tacrolimus administration can have serious adverse effects (e.g. nephrotoxicity).

More recently two monoclonal antibodies directed against the α chain of the IL (interleukin)-2 receptor (namely CD25) have been developed to antagonize IL-2 biological effects and mimic the activity of calcineurin inhibitors. Findings obtained with daclizumab, a humanized anti-CD25 antibody, showed no clinical and endoscopic effect of this antibody over placebo in patients with moderately active UC [32]. By contrast, two small uncontrolled trials showed that basiliximab, a chimaeric anti-CD25 monoclonal antibody, can be effective in active steroid-resistant UC [33,34]; a Phase II trial is currently in progress to assess the efficacy of basiliximab in UC. Whether basiliximab is effective in CD remains unknown.

**INDUCERS OF T-CELL APOPTOSIS**

There is evidence that in IBDs, and particularly in CD, mucosal T-cell accumulation is partly dependent on a reduced rate of programmed cell death (apoptosis) [35]. The molecular mechanisms underlying this phenomenon are complex and not fully understood; however, there is evidence that some cytokines over-produced in IBD tissue can prolong T-cell survival by enhancing the expression of various anti-apoptotic factors [e.g. Bcl-2, Bcl-XL and Flip (Fas-associated death domain-like IL-1β-converting enzyme-inhibitory protein)] [35–39] (Figure 2). Therefore restoring the susceptibility of T-cells to apoptosis is a major goal in the therapeutic armamentarium of IBD patients. In this context, several studies have shown that the clinical benefit of various pharmacological agents commonly used in the treatment of IBDs is linked to their ability to enhance mucosal CD4+ T-cell apoptosis. One such compound is AZA (azathioprine). Both AZA and its derivative 6-MP (6-mercaptopurine) are currently used to induce and maintain remission in patients with CD as well as in patients with UC [40,41]. AZA treatment has steroid-sparing effects, and associates with the attenuation or resolution of the mucosal inflammation. It has been shown that treatment of CD patients with AZA led to a marked increase in apoptotic T-cells in the intestinal lamina propria [42]. Interestingly, AZA-induced lymphocyte apoptosis requires T-cell co-stimulation with CD28, and is mediated by the specific blockade of the activation of the GTPase
Rac1. This inhibits, in turn, the transcription of Rac1 target genes, such as MEK (mitogen-activated protein kinase/extracellular-signal-regulated kinase), NF-κB and Bcl-Xₐ, and activates the mitochondrial pathway of apoptosis [42]. More recently, additional mechanisms of action of thiopurine derivatives in controlling IBD-related inflammation have been proposed. In particular, it was shown that AZA/6-MP significantly inhibited both T-cell proliferation and memory response respectively in vitro and in vivo [43].

Antibodies to TNF-α are second-line agents for the treatment of patients with IBDs. Clinical trials have demonstrated that intravenous therapy with infliximab, an anti-TNF-α human/mouse chimaeric monoclonal antibody, is able to induce and maintain the clinical response in adult and paediatric patients with moderate-to-severe active luminal CD [44,45], and patients with moderate-to-severe active UC [46]. There is also evidence that infliximab induces and maintains remission in patients with CD-related perianal fistulas [47,48]. More recently, it has been reported that adalimumab, a fully humanized anti-TNF-α monoclonal antibody, displays clinical efficacy in adult patients with luminal and fistulizing CD [49,50]. Clinical trials for adalimumab in UC are currently ongoing. Several reports have demonstrated that treatment with anti-TNF-α increases the rate of apoptosis in the intestinal lamina propria compartment and that this effect associates with mucosal healing [51]. However, the mechanisms by which anti-TNF-α antibodies induce T-cell death is still a matter of debate. In a preliminary report, Scallon et al. [52] showed that infliximab binds transmembrane TNF-α and kills TNF-α-expressing cells via both complement activation and antibody-dependent cell-mediated cytoxicity. More recently, two independent groups reported that infliximab-induced T-cell death was completely prevented by the broad caspase inhibitor Z-VAD-FMK (benzyloxy carbonyl-valyl-alanyl-aspartyl-fluoromethane), thus suggesting that this drug activates the caspase-dependent pathway of apoptosis after binding to surface TNF-α [53,54]. It has also been shown that infliximab promotes accumulation of toxic ROS (reactive oxygen species) in lymphocytes and up-regulates the pro-apoptotic factors Bax, Bak and p21 proteins [55].

Visilizumab is a humanized IgG₂ monoclonal antibody that specifically binds to the invariant CD3ε chain of the TCR. This antibody has been selectively engineered in the Fc region to decrease binding on surface Fc receptors located on immune cells [56]. As a result of these changes, visulizumab reduces T-cell activation, complement fixation, cytokine release and recruitment of APCs (antigen-presenting cells). Visilizumab can induce dose- and time-dependent apoptosis of lamina propria T-cells isolated from patients with UC or patients with CD through a caspase 3/8-dependent mechanism [57]. The therapeutic efficacy of visilizumab has been evaluated in patients with severe UC. In a preliminary study, 32 patients with severe steroid-refractory UC were assigned to receive two intravenous infusions of visiluzumab. More than 80% of patients demonstrated a clinical response, and 41% achieved clinical remission [58]. In another study, patients were randomly assigned to receive intravenous visilizumab at 5, 7.5, 10 or 12.5 μg · kg⁻¹ of body weight · day⁻¹ for 2 consecutive days. For all treatment groups, response and remission rates ranged between 50% and 71%, and 5% and 35% respectively [59]. Results from ongoing Phase III trials in patients with UC and in patients with both luminal and fistulizing CD are currently pending. However, visilizumab administration has been associated with a very high rate of infusion-related side effects, the most common of which is the cytokine-release syndrome, characterized by fever, headache, chills, arthralgias, nausea and vomiting. Initial experiences have demonstrated that this syndrome, which is linked to polyclonal T-cell activation, may occur in up to 90% of subjects and frequently leads to dose reduction and even to a premature halting of the therapy [58,59].

Another cytokine involved in the control of lamina propria T-cell apoptosis is IL-6 [60]. To induce its biological effects, IL-6 needs to bind a circulating receptor (IL-6R); the IL-6–IL6R complex is then recognized by the membrane-bound receptor subunit gp130. This
T-cells in inflammatory bowel diseases

Distinct patterns of cytokines are made by Th cells in IBD tissue

In CD, naïve T-cells differentiate along the Th1 pathway under the stimulus of IL-12; however, additional signals, such as those provided by IL-23 and IL-21, are necessary to expand Th1 cell responses. Th1 cells make IFN-γ, TNF-α and IL-21. By contrast, in the inflamed colon of patients with UC, there is a predominant accumulation of Th2 cells that make IL-4, IL-5 and IL-13. Factors involved in the differentiation of Th2 cells are not fully understood, but IL-4 synthesized by non-T-cells could contribute. In both CD and UC, the intestinal mucosa is massively infiltrated with Th17 cells, which make IL-17 (both IL-17A and IL-17F), IL-21 and IL-22. Th17 cells differentiate from naïve T-lymphocytes under the stimulus of TGF-β1 (transforming growth factor-β1) and IL-6; IL-23 and IL-21 contribute to maintain/expand Th17 cell populations.

Interaction triggers the activation of the transcription factor STAT3 (signal transducer and activator of transcription 3), and the production of several intracellular anti-apoptotic factors, such as Bcl-2 and Bcl-XL (Figure 2). Consistently, blockade of IL-6 activity enhances gut lamina propria lymphocyte apoptosis and, in vivo in mice, injection of an anti-IL-6R antibody reduces colonic inflammation [38]. A small placebo-controlled Phase I/II randomized clinical trial showed that a humanized anti-IL-6R monoclonal antibody (MRA, also known as tocilizumab) was effective in inducing remission in patients with moderate-to-severe active luminal CD. Interestingly, the therapeutic benefit of MRA was associated with a significant increase in the percentage of apoptotic lamina propria mononuclear cells [61].

Inhibitors of T-cell activation and differentiation

T-cell activation in response to TCR engagement relies on the concomitant delivery of co-stimulatory signals provided by binding of lymphocyte surface molecules [e.g. CD28 and CD40L (CD40 ligand)] to their cognate receptors on the APC surface (e.g. CD80, CD86 and CD40). TCR engagement without co-stimulation leads to a state of profound T-cell unresponsiveness, known as T-cell anergy [62]. Thus selective inhibition of T-cell co-stimulation could be a novel therapeutic approach to limit T-cell activation and dampen mucosal inflammation in patients with IBDs. Abatacept is a recombinant fusion protein composed of a fragment of IgG1 and the extracellular domain of CTLA-4 (cytotoxic T-lymphocyte antigen-4), a molecule which binds the APC receptors CD80 and CD86 with high affinity, thus preventing CD28-mediated co-stimulation of T-cells [63]. Abatacept is approved in the U.S.A. for patients with rheumatoid arthritis who failed any other type of disease-modifying drug, and in Europe for those who failed other disease-modifying drugs including TNF antagonists. Phase III studies of abatacept are in progress in CD and UC. No Phase I or II studies have been performed in patients with IBDs.

Several studies indicate that lamina propria T-cells derived from patients with CD and patients with UC have distinct functional phenotypes and exhibit different patterns of cytokine production. For example, CD tissue contains high levels of TNF-α and IFN-γ (interferon-γ), two cytokines predominantly synthesized by activated Th1 (T-helper type 1) cells and involved in macrophage activation [64] (Figure 3). By contrast, in UC tissue, there is a predominant synthesis of IL-5 and IL-13,
two cytokines made by Th2 (Th type 2) cells [65,66] (Figure 3). However, mounting evidence suggests that this classic Th1/Th2 paradigm in IBDs may be overly simplistic, and the hypothesis that these two pathways are always mutually exclusive has recently been challenged. Additionally, in the last few years, work from many laboratories has contributed to show that in both CD and UC there is exaggerated synthesis of additional cytokines, which are produced by a distinct subset of Th lymphocytes, known as Th17 cells [67] (Figure 3). Polarization of Th cells along the Th1, Th2 or Th17 pathway relies on the activity of additional molecules that are released within the inflammatory microenvironment (Figure 3). For example, differentiation of Th1 cells is strictly dependent on the presence of IL-12, a heterodimeric cytokine that is produced by APCs mostly in response to bacterial stimulation [68]. IL-12 shares the p40 subunit with IL-23, a cytokine involved in the expansion of both pathogenic Th1 and Th17 cells responses [69] (Figure 3).

The importance of the IL-12-driven Th1 signalling pathway in immuno-mediated injury in the gut was initially supported by studies in murine models of IBDs showing that administration of a monoclonal antibody directed against the IL-12/p40 subunit reduced the ongoing Th1 cell response and ameliorated CD-like colitis induced in mice by intrarectal administration of 2,4,6-trinitrobenzene sulfonic acid [70]. These observations led to the hypothesis that antibodies to IL-12 could also be beneficial in patients with active CD. To address this issue, two large placebo-controlled phase II clinical trials have been carried out, with the purpose of evaluating the safety and efficacy of monoclonal antibodies directed against the p40 subunit of IL-12 (ABT-874 and ustekinumab) in patients with active CD [71,72]. The clinical response and remission rates after 7 weeks of treatment with ABT-874 were 75% and 38%, respectively, compared with 25% and 0% for the group receiving placebo. Treatment with anti-IL-12 was associated with decreases in the secretion of IL-12, IFN-γ and TNF-α by mucosal cells and with significant improvement in mucosal histological scores [73]. The other study showed that ustekinumab was more effective than placebo in inducing a clinical response in patients with moderate-to-severe CD [73]. No relevant adverse effects were observed after treatment with anti-IL-12 antibodies. As pointed out above, IL-12 shares the p40 subunit with IL-23. Thus, at the present time, it is difficult to establish whether the therapeutic effect of anti-IL-12/p40 antibody is due to either the selective blockade of IL-12/IL-23 or concomitant neutralization of both IL-12 and IL-23. Indeed, IL-23, rather than IL-12, appears to be required for the development of some forms of intestinal inflammation in mice [74].

Apilimod mesylate, formerly known as STA-5326, is an oral inhibitor of IL-12 and IL-23 production. This compound has been shown to reduce the Th1 cell response and experimental intestinal inflammation induced by transfer of pathogenic CD4+CD45RBhigh T-cells in SCID (severe combined immunodeficiency) mice [75]. On the basis of these results, a randomized double-blind placebo-controlled clinical trial has recently been carried out to evaluate the efficacy of apilimod mesylate in patients with moderate to severe active CD [76]. Apilimod was well-tolerated but did not demonstrate efficacy over placebo in patients with CD [76].

In the last few years, various antibodies directed against the signature Th17 cytokine IL-17A have been developed and tested in models of inflammation [77]. AN417, a monoclonal antibody against IL-17, is currently being studied in Phase II clinical trials in patients with moderate-to-severe CD and the results are currently pending.

CONCLUSIONS

In recent years, progress in basic and translational research has led to a better understanding of the role of T-cells in the pathogenesis of IBDs. This has also fostered the advent of novel therapeutic strategies to attenuate T-cell-driven inflammation and to halt the progression of the inexorable gut damage and loss of function associated with IBDs. Blockade of T-cell recruitment in the gut and/or T-cell function is at the forefront of this new era with the success of several biological compounds, including immunosuppressors and anti-cytokine antibodies. These therapies are, however, not effective in all patients and efficacy may wane. Moreover, the use of some T-cell-directed therapies has been partly hampered by the occurrence of serious adverse events, and the therapeutic effect seen in pre-clinical studies has not been always confirmed by studies in humans. Thus a new challenge is to identify additional T-cell targets which could be selectively regulated from a therapeutic point of view, as well as to establish which patients will benefit from which therapy.

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T-cells in inflammatory bowel diseases


Received 11 January 2010/16 February 2010; accepted 25 February 2010
Published on the Internet 30 March 2010, doi:10.1042/CS20100027