Abstract

In the gut of patients with Crohn’s disease and patients with ulcerative colitis, the major forms of inflammatory bowel diseases (IBD) in humans, the tissue-damaging immune response is mediated by an active cross-talk between immune and non-immune cells. Accumulating evidence indicates also that cytokines produced by these cells play a major role in initiating and shaping this pathologic process. One such cytokine seems to be interleukin (IL)-21, a member of the common γ-chain-receptor family. IL-21 is produced in excess in the inflamed tissue of patients with IBD mostly by activated CD4+ T helper (Th) cells, follicular Th cells (TFH), and Natural killer (NK) T cells, which exerts regulatory effects on multiple cell types [10-13]. In this article, we review the available data supporting the pathogenic role of IL-21 in IBD.

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Key words: Interleukin-21; Gut; T cells; Epithelial cells; Fibroblasts

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De Nitto D, Sarra M, Pallone F, Monteleone G. Interleukin-21 triggers effector cell responses in the gut. World J Gastroenterol 2010; 16(29): 3638-3641 Available from: URL: http://www.wjgnet.com/1007-9327/full/v16/i29/3638.htm DOI: http://dx.doi.org/10.3748/wjg.v16.i29.3638

INTRODUCTION

The organized lymphoid tissue of the gastrointestinal tract contains large numbers of immune cells that are deputed both to protect from infectious diseases and to evoke immune tolerance [2,3]. Perturbations in this delicately balanced microenvironment can promote the collapse of tolerance, thus leading to chronic inflammation that alters the integrity and function of the gut [4]. This occurs for example in patients with Crohn’s disease (CD) and patients with ulcerative colitis (UC), the major forms of inflammatory bowel diseases (IBD) in humans [4]. In both these conditions, the tissue damage is mediated by an excessive and poorly controlled immune-inflammatory reaction directed against components of the normal bacterial flora [5,6]. Evidence also indicates that an active and dynamic interplay between immune and non-immune cells plays a major role in initiating and shaping this pathologic process, and that cytokines are essential mediators of this cross-talk [7-9]. One such cytokine seems to be interleukin (IL)-21, a product of activated CD4+ T helper cells co-expressing interferon-γ and follicular T helper cells. Moreover, both in vitro and in vivo studies indicate that excessive IL-21 production leads to the activation of multiple signaling pathways that expand and sustain the ongoing mucosal inflammation. In this article, we review the available data supporting the pathogenic role of IL-21 in IBD.

IL-21 IS MADE BY TH1 CELLS AND FOLLICULAR TH CELLS IN THE HUMAN GUT

Initial studies conducted in our laboratory showed that IL-21 protein is over-produced in the inflamed gut of
patients with CD and patients with UC as compared to normal controls. These data were confirmed by a recent study showing that IL-21 mRNA expression is increased in rectal mucosa from patients with active UC compared to UC patients in remission and healthy controls and that, in UC, IL-21 gene expression correlates with histological activity of the disease. A genome-wide association study for IBD has identified risk variants in the chromosomal 4q27 region harbouring the IL-2 and IL-21 genes, suggesting that polymorphism(s) in this region might contribute to regulation of IL-21 production/function \cite{14,16,18}. However, it is noteworthy that expression of IL-21 in the uninflamed mucosa of IBD patients does not differ from that seen in the normal colonic mucosa, and that peripheral blood T cells isolated from IBD patients and healthy controls express similar levels of IL-21. Therefore, it is plausible that up-regulation of IL-21 in IBD is strictly linked to the ongoing mucosal inflammation.

In both CD and UC, IL-21 is made by CD4+ but not CD8+ T cells. By flow-cytometry it was also shown that the majority of IL-21-producing CD4+ T-LPL co-express interferon (IFN)-γ, and to a lesser extent IL-17A, supporting the hypothesis that, in IBD, IL-21 is preferentially made by Th1 rather than Th17 cells \cite{19}. At the present time, it remains unclear how IL-21-positive cells co-expressing IFN-γ differentiate in the human gut. Since Th1 cells are abundant in the human gut, and particularly in CD mucosa \cite{20}-\cite{22}, it is conceivable that Th1 cells can acquire the ability to make IL-21 in response to specific stimuli. Indeed, we have recently shown that in vitro stimulation of intestinal lamina propria (LP) CD4+ T cells with IL-12, the major inducer of Th1 cell response, enhances the fraction of cells producing IL-21 or both IL-21 and IFN-γ \cite{19}.

IL-21 is also produced by TFH cells in the human gut, and the fraction of IL-21-producing TFH cells is significantly higher in CD than in UC and controls \cite{19}. Interestingly, activation of mucosal T cells with IL-12 leads to enhanced production of IL-21 by TFH cells \cite{19}, thus confirming that IL-12-driven signals positively regulate IL-21 production in the gut.

**IL-21 ENHANCES INFLAMMATORY PATHWAYS IN THE GUT**

A large body of evidence supports the concept that excessive production of IL-21 in the gut has deleterious consequences for the host. IL-21 is highly produced in the gut of wild-type mice with dextran sulfate sodium (DSS)- and trinitrobenzene sulfonic acid-relapsing (TNBS)-induced colitis. Notably, IL-21-deficient mice are largely protected against disease in both models. Amelioration of both DSS- and TNBS-induced colitis in IL-21-knockout mice is associated with a marked decrease in Th17-related molecules, such as IL-17 and IL-17F. Administration of IL-21R/Fc, a fusion protein that binds to IL-21 and prevents it activating cell-surface receptors, in wild-type mice attenuates DSS-colitis, confirming the pro-inflammatory role of IL-21 in this model. A similar scenario emerges from studies in human IBD \cite{19}. Stimulation of intestinal mucosal T cells with IL-21 results in enhanced activation of transcription factors (i.e. Stat3, Stat4 and T-bet) and marked synthesis of IFN-γ and IL-21 itself \cite{16}. Moreover, treatment of CD mucosal cells with IL-21R/Fc reduces Stat4 and T-bet and inhibits IFN-γ production. Neutralization of IL-21 in CD mucosal cell cultures leads also to a decreased expression of IL-17A. Taken together these data indicate that IL-21 is able to expand Th1 and Th17 cell responses in the gut, even though further experimentation is needed to elucidate the basic mechanism by which IL-21 exerts these regulatory effects.

Initially described as an important regulator of the function of immune cells \cite{23,24}, IL-21 has been recently shown to also regulate the activity of non-immune cells. Gut myofibroblasts and epithelial cells express constitutively IL-21R and are able to respond to IL-21 \cite{25,26}. In particular, stimulation of colonic myofibroblasts with IL-21 enhances the synthesis of matrix metalloproteinases (MMPs), a family of proteases that are supposed to participate in the tissue damage and remodelling occurring in IBD \cite{24,28}. The IL-21-driven induction of MMPs can be potentiated by tumor necrosis factor α, and associates with no change in the production of tissue inhibitors of MMPs. Regulation of MMPs by IL-21 does not however seem to occur at the transcriptional level, because stimulation of fibroblasts with IL-21 does not alter the MMP RNA expression. Additionally, the intracellular level of MMP proteins is not increased by IL-21, and the IL-21-induced MMP synthesis is not affected by inhibitors of gene transcription and de novo protein synthesis. Therefore, it is plausible that IL-21 preferentially increases the secretion of either pre-constituted or newly synthesized MMPs. The in vivo relevance of these findings relates to the demonstration that supernatants of CD mucosal cells induce myofibroblasts to secrete MMP and this is partially inhibitable by IL-21R/Fc. IL-21 induces activation of mitogen activated protein kinases in colonic epithelial cells thereby promoting the secretion of macrophage inflammatory protein (MIP)-3α, a chemokine up-regulated on the inflamed gut epithelium of IBD patients and involved in the recruitment of T cells in the gut mucosa. In line with these observations, blockade of endogenous IL-21 in cultures of IBD mucosal explants reduces MIP-3α synthesis by epithelial cells.

**IL-21 INHIBITS REGULATORY T CELL DIFFERENTIATION AND MAKES CD4+ T CELLS RESISTANT TO TREGS-MEDIATED IMMUNE-SUPPRESSION**

Regulatory T cells (Tregs) play an important role in maintaining homeostasis and preventing autoimmunity in various organs, including the gut. Tregs specifically express the transcription factor forkhead winged helix transcription factor gene (Foxp3), which is also functionally required for their regulatory activity \cite{29}. In addition to naturally occur-
ring Tregs that are produced by the thymus as a functionally distant and mature population of T cells. Tregs can arise in the periphery upon conversion of CD4+CD25- T cells into Foxp3-positive-CD4+CD25+ cells in response to activating stimuli and transforming growth factor (TGF)-β[30,31]. This phenomenon seems to occur in the normal gut, where TGF-β1 synergizes with other regulatory molecules (e.g., IL-10, retinoic acid) in mounting an effective counter-regulatory response[16,37]. However, if activation of naïve CD4+CD25- T cells occurs in the presence of TGF-β1 and inflammatory stimuli, they tend to differentiate into effector Th17 cells rather than into Tregs[38]. IL-21 seems to accomplish this function, given that it can cross-regulate Tregs induction and direct the development of Th17 cells[39]. Interestingly, colitis induced by the transfer of naïve T cells into severe combined immunodeficient mice is suppressed by TGF-β1-induced Tregs generated in vitro in the absence of IL-21 but not by T cells generated in the presence of TGF-β1 and IL-21[40]. By contrast, these latter T cells exacerbate colitis with increased expression of IL-17 and a reduced number of Foxp3-expressing cells in the gut mucosa[40]. Consistent with this is the demonstration that blockade of IL-21 associates with high numbers of Foxp3-positive Tregs and reduced tissue damage in the colon and small intestine of mice with acute graft vs host disease[41].

IL-21 is also able to counteract the regulatory effects of Tregs by providing human CD4+ T cells signals that raise their threshold for suppression by Tregs[42]. Collectively these observations delineate another mechanism by which IL-21 contributes to amplify the ongoing inflammation in IBD.

CONCLUSION

There is no doubt that IL-21 modulates the activity of several cell types that orchestrate the tissue damage in IBD, and that blockade of IL-21 signalling attenuates the ongoing mucosal inflammation in experimental models of IBD. Therefore, it is conceivable that the near future will witness the use of novel therapeutic strategies aimed at inhibiting IL-21 activity in IBD. However, some important issues related to the blockade of IL-21 function must be taken into consideration before moving into the clinic. For instance, we should not forget that IL-21 plays a decisive role in the control of B cell and plasma cell function, and that IL-21 signalling may attenuate the course of IgE-mediated disease, and that IL-21 plays a decisive role in the coordination of lymphocyte function.

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