



Review

Immunotherapy of Inflammatory Bowel Diseases: Current Concepts and Future Perspectives

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Abstract. The etiology and the pathogenesis of inflammatory bowel diseases (IBD), e.g. Crohn's disease and ulcerative colitis are still not completely understood. However, there is growing evidence that an alteration of the mucosal immune system towards luminal antigens in a genetically susceptible host plays a key role in the pathogenesis of IBD. In particular, cytokines produced by intestinal epithelial cells, lamina propria macrophages and CD4⁺ T cells appear to contribute to the initiation and perpetuation of intestinal inflammation in IBD. This review focuses on the role of the mucosal immune system in the pathogenesis of IBD and potential novel immunotherapeutic strategies for chronic intestinal inflammation. Such strategies include recombinant antiinflammatory cytokines, neutralizing antibodies or fusion proteins, antisense oligonucleotides and adenoviral gene transfer.

Key words: Crohn's disease; ulcerative colitis; cytokines; immunotherapy; gene transfer.

The primary function of the small bowel is to absorb nutrients into the circulation¹⁴. Since the bowel is exposed to numerous bacterial and dietary antigens during the course of its activity, there is a strong need for control elements such as the epithelial barrier and the mucosal immune system to prevent harmful reactions^{9, 10}. The latter system is well developed and comprises lymphocyte populations in the peyer's patches, intestinal epithelial cells, intraepithelial lymphocytes and macrophages and lymphocytes in the lamina propria (LP)²⁸. Based on the above observations, this system has to maintain immunoregulatory mechanisms that down-regulate immune responses while retaining the ability to respond to pathogenic organisms. One mechanism that helps to maintain this crucial balance is the down-regulation of mucosal T cell responses upon activation of the T cell receptor.

There is growing evidence that alterations of the mucosal immune system may contribute to the pathogenesis of inflammatory bowel diseases (IBD) that comprise two major forms: ulcerative colitis (UC) and Crohn's disease (CD)^{23, 28}. One aspect of the altered immunoregulation in IBD patients is a hyperresponsiveness of lamina propria mononuclear cells to mucosal antigens, in particular to otherwise less immunogenic or harmless products of the intestinal flora. It was shown that normal mucosal T cells mount lower *in vitro* proliferative responses to common microbial antigens than do peripheral T cells, whereas IBD mucosal T cell responses are equal to that of peripheral T cells. In addition, whereas lamina propria mononuclear cells from normal individuals do not respond to components of the resident intestinal flora by cell proliferation, such response is found in IBD patients¹⁰. These data indicate

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an important role of the bacterial microflora in the pathogenesis of IBD. This hypothesis is supported by studies in mice models of colitis, showing that chronic intestinal inflammation is prevented when mice are kept under germfree conditions^{12, 15, 27}. In addition, these findings are underlined by the fact that manifestations of both CD and UC are most frequently seen in gut areas with the highest bacterial concentrations (ileum, colon), that CD relapses are prevented by diversion of the fecal stream and that increases in gut permeability precede acute flares in CD^{10, 14}. Finally, it was recently shown that exposure of uninvolved gut to luminal contents from diseased areas in CD may induce histologic signs of CD⁸. Based on these data, an uncontrolled reaction of the mucosal immune system to luminal, most likely bacterial, antigens appears to be an important factor in the pathogenesis of IBD.

Role of Cytokines in the Pathogenesis of IBD

There is now strong evidence that LP CD4⁺ T cells play a key role in the pathogenesis of IBD^{5, 28}. The pathogenic role of LP T cells in IBD is most likely due to an altered activation status with consecutive cytokine release and macrophage activation. Several groups have thus studied Th1 (IFN- γ , IL-2) and Th2 (IL-4, IL-5) cytokine production by mucosal T cells in IBD. Fuss et al.¹³ analyzed cytokine production by purified LP CD4⁺ T cells from gut specimens from IBD patients upon cell stimulation with anti-CD2/CD28. There was a decreased IL-2 production of LP CD4⁺ T cells in patients with CD, but not UC, after accessory pathway stimulation. However, BRESE et al.⁴ found that lesional CD tissues contained increased numbers of IL-2 secreting cells, whereas UC tissues did not. These unequivocal data could be due to technical differences or may reflect the fact that IL-2 production in CD is dependent on the stage of disease. An alternative explanation is that basal levels of IL-2 production are increased in CD, whereas the capacity to further induce IL-2 production is reduced. The expression and production of IFN- γ , another and perhaps more definitive Th1 cytokine than IL-2, in CD, but not UC, was found to have increased^{1, 4}. Conversely, the study by Fuss et al.¹³ showed an increased production of IFN- γ by LP CD4⁺ T cells in CD, but not UC, when cells were stimulated via accessory signalling pathways.

With regard to Th2 cytokine production in IBD, Fuss et al.¹³ described decreased production of IL-4 by LP CD4⁺ T cells in both CD and UC. Such decrease of IL-4 production was associated with reduced numbers

of IL-4 producing cells, as shown by elispot analysis. Furthermore, IL-5 production by LP CD4⁺ T cells in CD was found to be decreased, whereas an increased IL-5 production was observed in UC. In summary, these data suggest that mucosal T cell responses in CD are associated with a Th1-like cytokine response (IFN- γ), whereas UC is associated with an IL-5 response.

To determine possible reasons for this divergent cytokine production by LP T cells between CD and UC, various groups have looked at IL-12 and IL-18 production by LP mononuclear cells and macrophages^{17, 19, 21}. Both cytokines have been shown to augment IFN- γ responses in T cells at the transcriptional level by activating the transcription factors STAT-4 and AP-1, respectively². The functionally active IL-12 (p35/p40) heterodimer and IL-18 were both found to be increased in the LP of patients with CD, but not UC. Since IL-12 is a potent inducer of Th1 T cell differentiation, these data provide reason to believe that IL-12 could be a key cytokine for Th1 T cell differentiation in CD, whereas lower IL-12 levels in UC in the presence of the IL-12-antagonising cytokine EBI3 may favor a different T cell differentiation in this disease⁶. Furthermore, high levels of IL-18 may contribute to an activation of Th1 effector cells in CD. Interestingly, high expression of IL-18 was also seen in intestinal epithelial cells, suggesting that also these cells, may have an important immunoregulatory role in IBD.

In addition to IL-12 and IL-18, the expression and production of various other cytokines produced by mononuclear cells and macrophages have been analyzed²². A decrease of the IL-1R antagonist/ IL-1 ratio was found in patients with IBD whose levels correlated inversely with severity of the disease. In addition, various groups described increased levels of various proinflammatory cytokines (IL-1, IL-6, IL-8, TNF) in inflamed CD and UC tissue samples compared to control patients. Thus, mononuclear cells in the gut show profound alteration of cytokine profiles. The differences between CD and UC may provide a rational basis for a specific immunotherapy in IBD patients.

Novel Animal Models of Chronic Intestinal Inflammation: a Milestone for the Development of New Immunotherapeutic Strategies

Several novel animal models of chronic intestinal inflammation have been established in the last 5 years which have provide new insights into the immunopathogenesis of IBD¹². These models comprise inducible models in otherwise healthy animals with normal im-

mune systems, adoptive transfer models, spontaneously occurring models and models created by gene manipulation. The latter group is the most rapidly growing group and comprises rats carrying transgenes for HLA-B27 and β_2 -microglobulin, transgenic mice for STAT-4, T cell reconstituted Tg ϵ 26 mice transgenic for the human CD3 ϵ gene, mice carrying a dominant negative N-cadherin mutant, knockout mice for the All-rich region of the TNF gene and mice in which the genes for IL-2, IL-10, Gi α_2 and the α or β chain of the T cell receptor have been inactivated by homologous recombination^{15, 30}. These models clearly showed that modulation of cytokine levels in the inflamed gut may cause persistent mucosal inflammation. One very interesting finding in the last years was that most models appear to be mediated by Th1 T cells that produce large amounts of IFN- γ . This excessive Th1 response can be counterregulated by Th2/Tr1 (IL-10) and Th3 (TGF- β) cytokine responses^{18, 24, 26}. However, recent evidence suggests that also Th2 cytokines such as IL-4 may cause intestinal inflammation in some of the above models^{3, 16}.

In addition, the new animal models offered interesting ways to test immunotherapeutic strategies (Table 1). Administration of recombinant antiinflammatory cytokines such as IL-10 (but not IL-4) was shown to be effective in the treatment of colitis in SCID mice after CD4⁺ T cell transfer²⁵. Furthermore, antibodies to pathogenic cytokines have been commonly used in colitis models. For instance, it was shown that IL-12 is essential for disease perpetuation in many Th1 colitis models, as treatment with antibodies to IL-12 suppressed or even abrogated disease activity by induction of T cell apoptosis¹⁹. In addition, antibodies to IFN- γ and TNF were shown to be effective in Th1-mediated mucosal inflammation, as disease was prevented or reduced by administration of specific neutralizing antibodies²⁵.

Table 1. Immunotherapeutic strategies in experimental bowel inflammation

Recombinant cytokines: IL-4, IL-10, TGF- β
Antibodies: anti-IFN- γ , anti-TNF, anti-IL-12, anti-IL-6R
Receptors: soluble TNF receptor
Antisense DNA: anti-NF- κ B, anti-ICAM-1
Adenoviral gene transfer: IL-4

As discussed above, regulatory T cell populations (Th2, Tr1, Th3) may prevent development of colitis by production of cytokines whose functions antagonize those of Th1 T cells. Thus, another approach for the treatment of Th1-mediated intestinal inflammation consisted in the induction of regulatory Th2 and Th3 cells

by the induction of oral tolerance mechanisms^{12, 18}. In summary, these data suggest that the mucosal balance between pro- and antiinflammatory cytokines is a key regulator in chronic intestinal inflammation. Thus, modulation of IL-12, TNF and TGF- β levels may have relevance for treatment of IBD. Such modulation may be performed by the administration of recombinant cytokines, cytokine receptors or neutralizing antibodies. However, some recent studies indicate that antisense DNA approaches and adenoviral gene transfer should also be considered to modulate gene expression in the gut^{20, 31}. For instance, it was shown that antisense DNA to the transcription factor NF- κ B suppresses proinflammatory cytokine production by LP cells and consequently intestinal inflammation.

Immunotherapy in IBD Patients: Current Status and Future Perspectives

Based on the above data, it appeared to be possible to design rational immunotherapies in IBD patients (Table 2). Initial trials focused on the application of recombinant human IL-10 to CD patients⁷. While such treatment appeared to be safe and well tolerated, only a minority of the patients clinically responded to the therapy. Similar data have been obtained with recombinant human IL-11. However, several very promising studies in the last years have shown that systemic administration of anti-TNF antibodies may be effectively used in CD^{11, 29}. In particular, there was a significant reduction in clinical activity scores in steroid-refractory CD patients upon antibody treatment. Furthermore, there was a marked reduction in the number of draining fistulas in anti-TNF treated patients. Although some questions with regard to safety and toxicity remain to be answered, these data indicate that antibodies to TNF appear to be very promising for treatment of chronic active CD. In addition to anti-TNF antibodies, anti-IL-12 antibodies will soon be available for phase II studies in CD, thus providing another potentially interesting option for immunotherapy in IBD patients.

Table 2. Current Immunotherapy in IBD patients

Recombinant cytokines: IL-10, IL-11, IFN- α
Antibodies: anti-TNF, anti-IL-12

Taken together, various novel immunotherapies are currently being tested in IBD patients. Although no final evaluation can be made so far, the data suggest that such therapies may find their way into routine application in the near future.

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