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Immunomodulation of Crohn's disease using TNF-α neutralizing monoclonal antibodies*

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Introduction

Crohn's disease is an inflammatory disease that can affect the entire intestinal tract, and is characterized by transmural granulomatous inflammation. Patients with Crohn's disease frequently have intra- and extra-intestinal complications including fistulas, perforations, uveitis, arthritis, and liver disease. Inflammation in Crohn's disease often results in extensive fibrosis, and many patients undergo resections for strictures in the course of their disease. As yet, the cause of Crohn's disease is unknown, and medical treatment is symptomatic. Corticosteroids, mesalazine, and immunomodulating drugs such as cyclosporine, azathioprine, and methotrexate have proved to have beneficial effects in certain subgroups of patients with Crohn's disease, but none of these drugs is curative, and many are associated with serious toxicity. In recent years, studies on the abnormalities of mucosal immune responses in Crohn's disease have yielded new insights, and these efforts have been greatly boosted by the availability of various genetically manipulated mice that develop intestinal inflammation. This review discusses some of these developments, with particular emphasis on the implications for the development of novel intervention strategies.

Control of mucosal inflammatory responses

The normal intestinal mucosa contains many (potential) inflammatory cells, including neutrophils, monocytes, macrophages, natural killer (NK) cells and mast cells. When stimulated, small and large bowel epithelial cells, enterocytes, can also mount inflammatory responses, by expression of class II HLA antigens, and by secretion of cytokines such as IL-6, IL-7, IL-15, and a wide range of chemokines. Specific as well as non-specific mucosal immune responses are necessary for normal life. The inflammatory reaction to intestinal microbial pathogens is usually very efficient, and various immune deficiencies, concerning both the humoral and the cell-mediated systems, are complicated by viral, bacterial and protozoal infections.

However, in recent years the notion has emerged that anti-inflammatory responses may be equally important, and that a lack of control may be a major cause of inflammatory bowel disease. By clinical observation, immunohistochemical analysis of inflammatory infiltrates, and detection of soluble activation markers in patients with Crohn's disease, evidence has accumulated for a pivotal pathogenic role of T-lymphocytes. Various mouse models have further refined this concept and have identified CD4+ T-cells as the cell population that is critical for both initiation and control of immune-mediated mucosal inflammation.

The very early lesion in Crohn's disease is a tiny ulcer that is known to endoscopists as the aphtoid lesion (1). The aphtoid lesion is a result of inflammation of lymphoid aggregates that are scattered throughout the large bowel, or the larger Peyer's patches in the ileum. The lymphoid aggregates are covered by specialized epithelium, known as M-cells, that are able to transport and present luminal antigen. Aphtoid lesions can progress to frank ulcers, that may fuse to form the longitudinal ulcers that are characteristic for Crohn's disease. Mononuclear mucosal cells obtained from patients with Crohn's disease express activation markers, such as the IL-2 receptor alpha chain and secrete increased levels of cytokines such as IL-2 (2). In addition, using various techniques, increased mucosal production of various pro- and anti-inflammatory cytokines, including IL-1, IL-4, IL-6, IL-8, IL-10, IL-12, IFN-γ, and TNF-α has been reported (3). Clinical observations in patients with Crohn's disease also suggest an important role for T-cell activation. Patients with Crohn's disease who developed AIDS had a reduction of disease activity, and uncontrolled data suggest that T-cell apheresis may be of clinical benefit (4). Conversely, IL-2 treatment caused a severe increase of disease activity in a patient with Crohn's disease (5).

Activation of CD4+ T-lymphocytes by antigen-presenting cells can have various effects. Many T-cells will either die or become tolerant to the presented antigen. Apoptotic death of stimulated T-cells is necessary for a normal immune response and mutations that lead to functional inactivation of the receptor/ligand pathway that signals apoptosis result in auto-immune and lymphoproliferative phenotypes. The fraction of CD4+ lymphocytes that proliferates following antigen-dependent stimulation
can functionally differentiate into two types that are phenotypically characterized by production of different cytokines. T-helper 1 (Th1) lymphocytes secrete IL-2, interferon-γ (IFN-γ), lymphotoxin and TNF-α, whereas Th2 lymphocytes produce IL-4, IL-10 and IL-13 (6). These differences are important, because Th1 responses predominantly cause cellular immune responses and delayed type hypersensitivity reactions, whereas Th2 differentiation is linked to humoral immune responses and allergy. Recent studies have shed some light on the circumstances that direct differentiation of naive CD4+ cells, and these can be grouped into signals that are derived from the antigen presenting cells, and soluble signals, usually cytokines, that are secreted by T-lymphocytes or NK cells. Antigen presenting cells influence differentiation of stimulated CD4+ T-cells by expression of co-stimulatory molecules (B7.1 leading to Th1 – and B7.2 Th2 differentiation) and by secretion of cytokines (IL-12 causing Th1 – and IL-6 Th2 differentiation). Furthermore, IFN-γ secreted by T-lymphocytes and NK cells is a potent Th-1 stimulator, whereas IL-4 and IL-10 inhibit Th1 development and stimulated differentiation into the Th2 phenotype (Fig.).

Data from experiments using T-cell transfer or genetically manipulated mice support the concept that uncontrolled activation of CD4+ T-cells is a central event in the pathogenesis of Crohn’s disease. Transfer of CD4+ T-cells from BALB/C mice to SCID mice, that lack T- and B-cells, results in repopulation of various organs including the spleen, the small and the large bowel of the recipient animal. However, if only the CD45RBhigh subpopulation of CD4+ cells is transferred, a severe inflammatory disease of the entire colon, histopathologically characterized by transmural inflammation, fissures, occasional crypt abscesses and granulomas ensues (7). The mucosal lesion in CD45RBhigh transferred SCID mice is associated with increased production of IL-2 by isolated lamina propria T-cells, and by increased production of IFN-γ and TNF-α as detected by RT-PCR (8). Interestingly, treatment of CD45RBhigh transferred SCID mice by administration of IL-10 or anti-IFN-γ significantly reduced expression of both IFN-γ and TNF-α. Mice with a functionally inactive IL-10 gene (IL-10 knock-out mice) also develop an inflammatory disease of both the small and the large bowel, that is frequently complicated by adenocarcinomas (9).

Administration of trinitro-benzene-sulfonic acid (TBNS) and ethanol to rats and susceptible mouse strains also results in an inflammatory disease that depends on T-cell activation. Recent studies have suggested that the TBNS-dependent contact sensitization in these mice results in a loss of tolerance towards the indigenous intestinal flora. This is a very important finding because TBNS-induced colitis has been shown to be dependent on the expression of IL-12 and may be inhibited by antisense DNA therapy that targets nuclear factor κB (NFκB), a transcription factor for many pro-inflammatory cytokines (10, 11).

Finally, in many ‘immune-mediated’ animal models of IBD, including the IL-10 KO mouse and the CD45RBhigh transfer model, inflammation depends on the presence of intestinal bacterial antigens, because inflammatory bowel disease is less severe, or does not occur at all, in germ-free animals.

Taken together, these data from animal models indicate that T-lymphocytes are pivotal for the control of mucosal inflammation. Different subsets of CD4+ lymphocytes regulate cellular and humoral immune responses and differentiation of CD4+ lymphocytes is regulated by cytokines as well as yet ill-defined genetic factors.

**Inflammatory changes in Crohn’s disease**

A rapidly increasing body of evidence indicates that the early changes in Crohn’s disease consist of activation of T-cells present in lymphoid aggregates in the small or large bowel. For example, using immunohistochemistry, several investigators have demonstrated increased production of pro-inflammatory cytokines such as IFN-γ and TNF-α (12). Mucosal epithelial cells from patients with Crohn’s disease (and ulcerative colitis as well) express HLA class II molecules, which is additional, albeit indirect, evidence for increased production of IFN-γ (13). We have recently demonstrated that in Crohn’s disease the number of deep lamina propria infiltrating lymphocytes is increased when compared to either normal controls or patients with ulcerative colitis, and that these cells express IFN-γ (unpublished data). In contrast, no differences were detected in the number of IL-4 expressing mononuclear cells, that were prominently present in both Crohn’s disease and ulcerative colitis. These data confirm the notion that inflammation in Crohn’s disease is caused by an exaggerated Th1 response.

**Immunomodulation of Crohn’s disease**

Standard immunomodulatory therapy of steroid-refractory patients with Crohn’s disease consists of administration of azathioprine, methotrexate, or cyclosporin (14). It should be noted that, although all these drugs are assumed to have some immunomodulatory effect, these have only been characterized in detail for cyclosporine. This drug acts by interfering with the calcineurin-dependent activation

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**Fig. 1** Factors that determine T-lymphocytes differentiation.
of NF-AT, a transcription factor that is necessary for transcription of the IL-2 gene. Through this mechanism, cyclosporine prevents (antigen-dependent) T-cell activation. At a higher concentration, cyclosporine also inhibits the activity of another transcription factor, NFkB, that is important for the transcription of many pro-inflammatory cytokines, including TNF-α. Modulation of inflammatory responses by cyclosporine has been investigated in various animal models, and some studies suggest that the effects of this drug on humoral and cellular immune responses are dose-dependent, the latter situation requiring high-dose treatment. In fact, in some models low-dose cyclosporine boosted Th-1 type immune responses. These experimental data should be kept in mind when reviewing the experience with cyclosporine treatment in Crohn’s disease, and it is interesting that the only controlled study that reported a positive outcome used high-dose cyclosporine (15, 16).

We have used anti-CD4 monoclonal antibodies in patients with Crohn’s disease in order to investigate the safety and potential efficacy of this rather non-specific immunosuppressive approach. Twelve patients with steroid-refractory Crohn’s disease received daily infusions of a mouse/human chimeric anti-CD4 antibody (cMT-412). This antibody depletes the number of circulating CD4-cells, and interferes with CD4-dependent activation of T-cells. Indeed, a one-week course of daily antibody infusions caused a long-lasting depletion of circulating CD4-cells, and dose-dependently decreased the Crohn’s disease activity index (CDAI) (17). We also demonstrated a reduction of HLA-DR expression by epithelial cells following anti-CD4 treatment, which is highly suggestive of a reduction of mucosal IFN-γ production (13). Although no opportunistic infections occurred, a major problem precluding wide-spread clinical use of the antibody used is the resulting CD4-depletion which in some patients was sustained for more than 12 months following treatment.

TNF was first identified as a factor that caused hemorrhagic necrosis of certain tumors in BCG-treated mice and independently as a mediator of the metabolic changes in chronic parasitic disease (18–20). Soon it became clear that TNF also caused many of the deleterious systemic inflammatory changes in sepsis, and TNF-neutralizing antibodies prevented the mortality caused by infusion of Gram-negative bacteria in primates. These findings provided a rationale for development of anti-TNF antibodies for treatment of severe sepsis. However, subsequent clinical trials failed to demonstrate a beneficial effect of anti-TNF antibodies in sepsis. In fact, it became clear that in some animal models of sepsis, i.e. septic peritonitis, TNF had a protective role (21). TNF was also demonstrated to be critical for clearance of intracellular bacteria, and for the formation of granulomas in mycobacterial disease (22). These data prompted further research of the potential immunomodulatory effects of TNF-neutralizing strategies, and several studies reported a beneficial effect of such interventions in delayed-type hypersensitivity reactions, and in animal models of arthritis. These data inspired us to investigate the potential beneficial effects of TNF-neutralizing antibodies in Crohn’s disease. The first patient to be treated with anti-TNFα antibodies was a girl with severe steroid-refractory Crohn’s colitis (23). She was treated with two bolus injections of the mouse/human chimeric antibody cA2, and responded in a remarkable manner. The Crohn’s disease activity index (CDAI) normalized, and virtually all mucosal ulcers healed. A small open label trial in patients with active Crohn’s disease confirmed this observation, and in addition many other inflammatory parameters, including circulating IL-6, CRP, and secreted phospholipase A₂ normalized rapidly following administration of anti-TNFα antibodies (24). Recently, a controlled clinical trial of cA2 therapy has been completed, in which 108 patients were enrolled, and the results indicate that in patients with severe Crohn’s disease anti-TNF induces clinical responses in 70% (25). This trial also had a prolonged treatment phase, in which patients were re-randomized to receive either repeated administrations or placebo. These data have not yet been published in full detail, but the preliminary analysis indicates that remissions induced by anti-TNF therapy can be maintained in most patients. To date very little is known about potential (long-term) toxicity of anti-TNF therapy. Some patients have developed acute hypersensitivity reactions during, and shortly following infusion, very similar to those observed when infusion with other immunoglobulin preparations has been given, but in most cases these have not necessitated premature cessation of treatment. Following repeated administration of anti-TNFα antibody many patients develop human-anti-chimeric-antibodies (HACA), that are expected to reduce the half-life of the circulating antibody, and may be responsible for adverse responses. However, I have administered cA2 to several patients that in retrospect were found to have HACAs at the time of infusion, without apparent adverse responses. The most serious possible long-term complication of anti-TNF therapy is the development of lymphomas as it has been reported in several patients with rheumatoid arthritis. It should be noted that patients with (auto)immune disease have an increased incidence of lymphoproliferative disease, and at present it is not known whether the anti-TNF treatment contributed to the induction of lymphomas. In conclusion, the clinical studies thus far reported indicate that neutralization of TNF using human/mouse chimeric antibodies had an important therapeutic effect in patients with therapy-refractory Crohn’s disease.

Why does anti-TNF antibody work in Crohn’s disease?

The remarkable effects of anti-TNF antibody therapy in Crohn’s disease have inspired many investigators to speculate about the mechanisms of action. Because not much TNF (if any) can be detected in the blood of patients with Crohn’s disease (26), the target of this treatment is probably located within the intestinal mucosa. It remains uncertain whether cA2 in vivo primarily binds to secreted TNF or to membrane-bound TNF. It is conceivable that anti-TNF affects the function of T-cells or monocytes, either by
crosslinking membrane-bound TNF or by killing TNF expressing cells, i.e. through complement activation. Whatever the primary mechanism is, the secondary (beneficial) effects are evident. For example, the increased expression of chemokines within the intestinal mucosa disappears following treatment, thereby reducing recruitment of monocytes and T-cells, circulating cytokines and sPLA_2 levels return to normal, and the abnormally increased intravascular thrombin formation in Crohn’s disease is diminished.

Future strategies

Although anti-TNF therapy is a breakthrough in the treatment of severe Crohn’s disease, for several reasons other strategies are still required. Although patients have been successfully treated for up to 44 weeks it is uncertain whether long-term treatment with monoclonal antibodies will be feasible, and the development of HACAs may disallow maintenance therapy. Several non-antibody TNF-neutralizing proteins have been designed, that derive the potential to bind TNF from the use of TNF receptor binding domains. Although these molecules exclusively contain human protein sequences, some have been found to be immunogenic, probably as a consequence of neo-epitopes exposed at fusion points. The production of TNF can also be inhibited using pharmacological approaches, by i.e. use of phosphodiesterase inhibitors, NF/KB inhibitors, or TNF-convertase (metalloproteinase) inhibitors (27-32). A potential disadvantage of TNF-convertase inhibitors is that, although they diminish TNF secretion, the expression of membrane-bound TNF may, in some cases, increase. In addition the metalloproteinase inhibitors that are now available are not entirely specific for TNF, but also inhibit cleavage of other proteins, including the TNF receptors. Hence, the clinical efficacy of these approaches can not be predicted from their effects on TNF production, and clinical studies are needed.

Conclusion

The role of various cytokines in regulating mucosal inflammation in inflammatory bowel disease has become apparent in recent years. Neutralization of TNF using monoclonal antibodies has resulted in dramatic clinical responses in patients that were refractory to standard immunosuppressive therapy. The formation of HACAs or yet unforeseeable toxicity may prevent long-term treatment with monoclonal antibodies, and additional approaches are therefore needed. Various drugs have been demonstrated to reduce TNF production, but assessment of their efficacy in Crohn’s disease awaits clinical testing.

References


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