Biological therapies of inflammatory bowel disease

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Introduction

The cause of chronic inflammation in inflammatory bowel disease is unknown, and damage to the intestinal mucosa leads to a rather restricted repertoire of lesions. As a result, unambiguous phenotyping of inflammatory bowel diseases has been difficult. Because until recently no useful animal models for inflammatory bowel disease were available, following the introduction of corticosteroids in the 50-ies, research concerning the pathogenesis and treatment of inflammatory bowel disease did not yield important new breakthroughs for many years. In the last decade, new techniques became available that enabled measurement of the expression of inflammatory mediators in the mucosa of patients with inflammatory bowel disease, and (many) transgenic and knock-out mice were generated that, sometimes surprisingly, had inflammatory bowel disease as a major phenotype (1). As a result, current knowledge of the pathogenesis of mucosal inflammation has dramatically increased, and because such knowledge can be rapidly translated into clinical interventions, there is no shortage of phase I and II intervention studies in inflammatory bowel disease. In at least one case, this development has led to the introduction of a biological therapy for Crohn's disease. Because the rapidly expanding knowledge of mucosal inflammation, as well as the avelange of new intervention studies have made it difficult for the practising gastroenterologist to keep up-to-date, in this short overview, I attempt to bring these developments within a logical and clinically useful framework.

Mechanisms of mucosal inflammation

The gastrointestinal mucosal compartment is one of the most important human immunological organs and contains about half of all immunocytes. An important difference with these other immunological compartments is the proximity of a vast number of bacteria within the intestinal lumen, many times the number needed to kill the host. No doubt, the intestinal mucosal system is an example of a well-balanced symbiosis, in which intestinal bacteria are necessary for the normal development of the (mucosal and the systemic) immune system, and for the nutrition of the intestinal epithelium. It should be noted that the mucosal immune system is able to differentiate between pathogenic and non-pathogenic bacteria. In fact, bacterial pathogenicity could be redefined as the ability to activate the mucosal immune system. On the other hand, the mucosal immune system is in a constant state of low-grade inflammation, and apparently, well-regulated mechanisms control the inflammatory pressure.

The initiation route of the mucosal inflammatory reaction is not known, but two mechanisms seem to be of importance : 1) antigen-dependent T-lymphocyte proliferation and 2) chemokine-mediated epithelium-dependent inflammation.

Antigen-dependent stimulation

It is known that lamina propria (CD4+) lymphocytes can be activated by antigen-presenting cells, leading to mucosal inflammation (Fig. 1) and in several animal models, the responsible antigens seem to be derived from intestinal bacteria (2-4). It is assumed that such activation constantly occurs, but because of the existence of several counter-regulatory mechanisms, this normally does not initiate inflammation. Currently identified counter-regulatory mechanisms include (Fig. 2) (5) :

- Tolerance : Tolerance can be induced when T-lymphocytes are activated through the T-cell receptor in the absence of simultaneous co-stimulation (for example through the interaction of B7 and CD28) or altered co-stimulation (for example through interaction of CTLA-4 with CD28) (6-8). Mucosal tolerance is a major problem in the development of orally active vaccines, but can be used therapeutically in T-lymphocyte dependent diseases when the origin of the causal antigen is known (for example in animal models of arthritis, uveitis, encephalitis) (9-14). At present it is not known whether defect in mucosal tolerance plays a role in the pathogenesis of inflammatory bowel diseases.
- Apoptosis of activated T-lymphocytes : Antigen-dependent activation of T-lymphocytes can either result in (IL-2 dependent) proliferation or in suicidal apoptosis. In normal circumstances, most activated T-lymphocytes will express both Fas (the death receptor) and Fas-ligand, and this causes programmed cell

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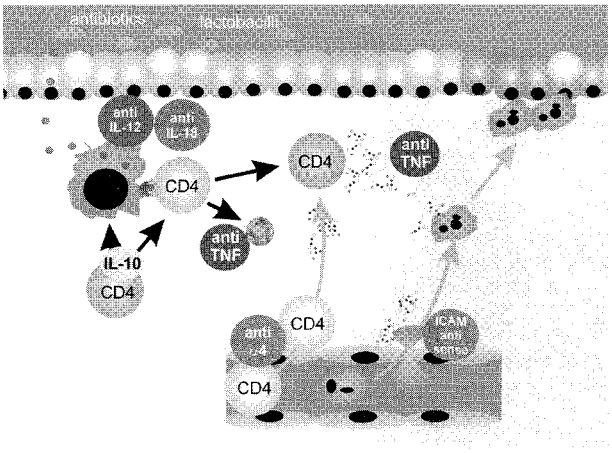

Fig. 1.

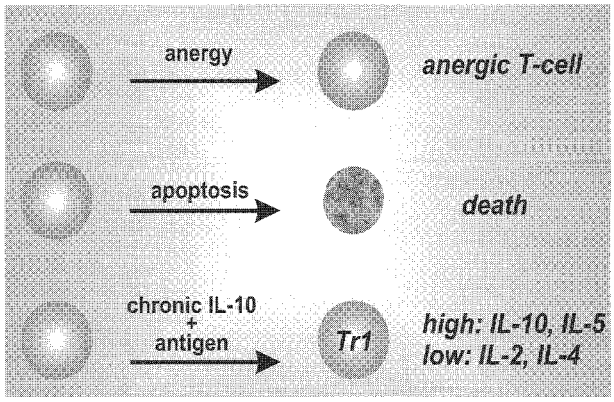

Fig. 2. — Mechanisms of peripheral T-cell tolerance.

death through activation of caspases and induction of mitochondrial leakage (5,15-17). Genetic defects in this pathway have been described and are phenotypically characterized by inflammatory diseases and the occurrence of lymphomas (18-22). It has been recently reported that lamina propria T-lymphocytes from patients with Crohn's disease (but not ulcerative colitis) are resistant to apoptosis induction, and this seems to be an important pathogenic mechanism (23-24).

- Regulatory T-lymphocytes : Specific subpopulations of CD4+ T lymphocytes act as regulatory cells through secretion of antiproliferative and anti-inflammatory cytokines. To date two such populations have been described, i.e. Tr1 cells (producing mainly IL-10) and Th3 cells (secreting TGFβ) (25-27). Specific defects in regulatory cell populations cause colitis in experimental animals, and recent evidence suggests that similar defects may occur in inflammatory bowel disease (28).

Chemokine-mediated epithelium-dependent inflammation

Primary activation of the mucosal epithelium may result in the production of small chemoattractive cytokines, known as chemokines. This may occur following the interaction of intestinal bacteria with the apical (luminal) membrane of epithelial cells, and has been observed in Helicobacter pylori infection and in colitis induced by enteropathogenic bacteria (29-32). The exact molecular mechanisms underlying this route of activation have not been elucidated, but some evidence suggests that this pathway may be of importance in ulcerative colitis. Experimental colitis occurs after activation of the intestinal epithelium (for example by administration of dextran sulphate), which has several characteristics of ulcerative colitis and is independent of the presence of T-lymphocytes (33). The intestinal epithelium of patients with ulcerative colitis is a well-known source of chemokines, even in patients that are in a complete clinical remission (34,35). Interestingly, butyrate has been shown to be able to switch the pattern of epithelial chemokine production

Implications for therapeutic interventions

Based on the knowledge that is summarized above, several logical specific therapeutic interventions can be designed :

- Inhibition of activation of T-lymphocytes : T-lymphocyte subpopulations can be targeted by monoclonal antibodies, and a small phase II study has suggested potential benefit of anti-CD4 antibody treatment of patients with steroid-refractory Crohn's disease (36,37). However, the antibody used in this trial was found to cause long-term depression of the peripheral blood CD4 count, and this line of research was discontinued. More recent studies have focussed on the cytokine-mediated pathways of activation of T-lymphocytes by antigen-presenting cells (APC). IL-12 and IL-18 are produced by APC's and cause a Th1-differentiation of activated T-lymphocytes. Importantly, both IL-12 and IL-18 are produced in the inflamed intestinal mucosa from patients with Crohn's disease, but not in ulcerative colitis (38-41). In animal models (for example TNBS-induced colitis), neutralization of either IL-12 or IL-18, or interference with the CD40-CD40L interaction that is important for IL-12 induction, has important therapeutic benefit (42-44). Clinical studies targeting IL-12 are planned in Crohn's disease, and CD40 or IL-18 neutralizing reagents are available for clinical studies.
- Induction of T-lymphocyte apoptosis : Apoptosis of lamina propria T-lymphocytes can be induced by treatment with antibodies that bind to the cell surface of the cytokine-producing cells, and has been reported to occur following anti-IL-12 and anti-IL-6 treatment of animals with experimental colitis (45). A similar mechanism may underlie the efficacy of anti-TNF antibodies (46). However, this issue is complex, because neutralization of TNF also blocks activation

of NFκB, a transcription factor with important anti-apoptotic properties in T-lymphocytes (47-49). It is expected that small molecular inhibitors of NFκB, and possibly also of MAP kinases may, apart from their anti-inflammatory effects, also act through apoptosis induction.

- Regulatory T-lymphocytes : The importance of regulatory T-lymphocytes in humans has not been fully elucidated, but there is some preliminary evidence to suggest that defects in regulatory T-lymphocyte subsets may occur in Crohn's disease. Systemic administration of recombinant human IL-10 was generally well tolerated in Crohn's disease, but the clinical efficacy of this approach was limited, and at higher doses IL-10 treatment unexpectedly induced IFNg (50-53). In an animal model of transfer colitis, transgenic T-lymphocytes that expressed IL-10 under control of the IL-2 promoter were shown to effectively protect against the development of colitis (54). This finding suggested that IL-10 should be preferably delivered to the intestinal mucosa by T-lymphocytes, and expressed in the context and micro-environment of T-T cell or APC-T-cell interaction. For these reasons we have explored the possibility to generate T-lymphocytes with a Tr-1-like phenotype by ex vivo genetic engineering. Indeed, such cells can be produced, and preclinical development of this strategy is underway.
- Inhibition of T-lymphocyte trafficking : In order to cause inflammation, white blood cells, including T-lymphocytes need to move about. Chronic intestinal inflammation is dependent on the continuous recruitment of inflammatory cells into the intestinal mucosa, and the responsible mechanisms have in part been elucidated. Apart from general selectin and integrin-mediated interactions of lymphocytes with the inflamed endothelium, recruitment to the intestinal mucosa is dependent on binding of endothelium-expressed MadCAM to the a4β7 integrin on T- and B-lymphocytes (55-57). Blockade of this interaction, generally by administration of anti-α4 antibodies has beneficial effects in experimental colitis, including the spontaneous colitis that occurs in cottontop tamarins, and preliminary results of clinical trials have been reported (58). Leukocyte trafficking is also regulated by interactions of chemokines with chemokine receptors and small molecular weight chemokine receptor inhibitors have been synthesized.

Small molecules that inhibit signal transduction pathways

Major disadvantages of biological therapies are the significant costs associated with production of proteins and antibodies and their immunogenicity. A new development is the generation of small molecular weight inhibitors of signal transduction pathways as anti-inflammatory therapies. Of specific importance for inflammatory reactions are the MAP-kinase pathway and activation of NFκB, and potent inhibitors of both pathways have been characterized (59-62). Clinical development of these reagents is complicated by the fact that several signal transduction pathways (including MAP kinase and NFκB activation) are linked, and that these systems are characterized by significant redundancies. Moreover, therapeutic interference with activation of these pathways may, apart from inflammation, also modulate induction of apoptosis. Nonetheless, both NFκB and MAP kinase inhibitors are now being developed as therapeutic strategies in inflammatory bowel disease (63-66).

A major advantage of NFκB inhibitors is the wide spectrum of potential applications and the availability of orally active compounds. NFκB inhibition may be useful in Crohn's disease (where the target would be monocyte and lymphocyte NFκB) and ulcerative colitis (targeting epithelial and monocyte NFκB). MAP kinases form several cascades of signal transduction that finally converge on nuclear translocation of AP-1 and NFκB. The p38 MAP kinase is importantly involved in inflammatory signalling, and several (orally available) compounds are in phase I and phase II trials. In a small pilot trial in patients with therapy-refractory Crohn's disease, intravenous administration of one of these compounds was shown to have therapeutic potential, and mucosal healing was observed in several patients.

Conclusions

Biological therapies of inflammatory bowel disease will significantly change the therapeutic landscape in inflammatory bowel disease. Several of these compounds enable treatment of patients that were refractory to standard therapy, and some have potent mucosal healing effects. Long-term administration of biological therapies also has disadvantages, in particular the generally high costs, potential immunogenicity, and although most compounds are remarkably well tolerated in the short term, long term toxicity remains uncertain. Uncontrolled observations in patients with inflammatory bowel disease and controlled clinical trials in patients with rheumatoid arthritis have indicated that anti-TNF therapies are synergistic with immunosuppressive therapies, in particular azathioprine and methotrexate, but further studies are clearly needed. The availability of biological therapies has also prompted reconsideration of the therapeutic strategies and goals of treatment of inflammatory bowel disease. For example, mucosal healing traditionally has not been a primary goal of the treatment of Crohn's disease, but now seems to be feasible in many patients. This would suggest that follow-up endoscopy should become the standard in the management of patients with Crohn's disease. Although no controlled data are available, using potent immunomodulatory reagents it may be possible to change the natural course of ulcerative colitis and Crohn's disease. No doubt, such strategies will require careful planning of

the time of introduction of immunosuppressives of biologicals, as well as identification of synergistic or antagonistic strategies. These activities should be considered against the background of immunologic mechanisms that lead to therapeutic refractoriness, such as epitope spreading and apoptosis resistance. In this respect, it would also be important to have more knowledge about the precise mechanisms of action of commonly used immunosuppressive drugs, such as azathioprine and methotrexate.

In conclusion, the availability of several "biologicals" for therapeutic use in inflammatory bowel disease has greatly increased our knowledge of critical pathogenic mechanisms. At present only one of these reagents (the anti-TNF antibody infliximab) has been registered for therapeutic use in Crohn's disease, but several clinical trials with other antibodies, peptides, and proteins are ongoing. The introduction of biological therapies may importantly alter the goals as well as the therapeutic strategies in inflammatory bowel disease.

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