Biological therapy in Crohn's disease

JN Gordon, TT MacDonald

The discovery of the central role of tumour necrosis factor-α in Crohn's disease and the subsequent introduction of infliximab into routine clinical practice has transformed the treatment of refractory disease. Advances in understanding of the immunopathological basis of Crohn's disease are leading to the development of new biological therapies which are likely to play an increasing role in future.

Crohn's disease is an idiopathic, chronic inflammatory disorder that primarily affects the gastrointestinal tract, and is associated with considerable morbidity. For the last 40 years corticosteroids have formed the mainstay of therapy and are effective at inducing remission in the majority of patients. However, they are not effective in maintaining remission, do not promote mucosal healing or influence the natural history of Crohn's disease, and have significant side effects. Furthermore, approximately 30% of patients become steroid dependant within 1 year with a further 15% being steroid refractory.

Azathioprine and methotrexate are often used for the induction and maintenance of remission in these patients. However, their value is limited because of their slow onset of action, significant side-effect profile, and efficacy rate of approximately 40% (Biancone et al, 2003). Over 60% of patients require surgery at some stage, following which, over 70% of patients have endoscopic evidence of recurrent disease at 1 year. There is an urgent need for alternative therapeutic strategies to treat patients with resistant disease.

In response to this, considerable resources have been directed at elucidating the underlying immunological basis of Crohn's disease over the last 10 years, resulting in the discovery of many new potential therapeutic targets. Progress in molecular immunology has simultaneously allowed the development of immunological therapies directed against these targets. We are now entering a period where it is possible to modulate any immunological pathway. In the coming years it is likely that, as a direct result of this, the treatment of Crohn's disease will shift from the empirical to a more direct, immunophysiological approach.

Dr JN Gordon is Clinical Research Fellow and **Professor TT MacDonald** is Professor of Immunology and Head of Division, Division of Infection, Inflammation, and Repair, University of Southampton, Southampton General Hospital, Southampton SO16 6YD

Correspondence to: Dr JN Gordon

IMMUNOLOGICAL BASIS OF CROHN'S DISEASE

In total, the gastrointestinal tract comprises the single largest collection of lymphoid tissue in the body, and is an area of intense immunological activity. In normal individuals the gut exists in a permanent state of controlled, low-grade 'physiological' inflammation (MacDonald et al, 2000).

In Crohn's disease it is now clear that dysregulation of this normal physiological response results in a strongly polarized mucosal CD4+ T-helper cell 1 (Th1) response, characterized by increased production of the pro-inflammatory cytokines interleukin (IL)-2, interferon (INF)-γ, and tumour necrosis factor (TNF)-α. This appears to be the result of a combination of events. High levels of IL-12 and IL-18 are present in the diseased mucosa. They are produced following bacterial activation of macrophages, which control the differentiation and maturation of Th1 cells.

The normal intestinal mucosa is an immunosuppressive site owing to the non-specific downregulatory effects of prostaglandin E_2 (PGE_2) and transforming growth factor (TGF)-β. When CD4+ T-cells sensitized to luminal antigens in the Peyer's patches migrate to the lamina propria, they die by apoptosis. In Crohn's disease, however, the cells do not die, but accumulate and secrete Th1 cytokines. The survival of these cells is a result of many factors. Antigen from the lumen can cross the damaged epithelium. Cytokines such as IL-6, IL-12, IL-18, IL-2 and IL-15, produced in excess in diseased mucosa, deliver anti-apoptotic signals to the T-cells. Additionally, suppressor cytokines produced by the Th3 subset of regulatory CD4+ cells appear unable to effectively downregulate the inflammatory response.

It appears that the normal negative regulatory function of TGFβ1 in the gut is inoperative in Crohn's disease (Monteleone et al, 2001). Excess Th1 cytokines in Crohn's disease have three main targets. By upregulating adhesion molecules on endothelial cells, there is a continuing migration of blood-borne inflammatory cells into the tissues. Cytokines also affect the epithelial barrier, reducing barrier function. Finally cytokines activate

mucosal fibroblasts to secrete large amounts of matrix metalloproteinases (MMPs), which degrade the mucosa and lead to ulcer formation. However, it is uncertain whether these phenomena are caused by a defect in the epithelial barrier leading to increased antigenic stimulation, or from an intrinsic defect in immune handling in the gut.

Further evidence to support the importance of these pathways comes from the identification of the NOD2 susceptibility gene found in 20% of cases of Crohn's disease (Hugot et al, 2001; Ogura et al, 2001). Although it is currently unclear exactly what the role of this gene is, it has domains that are involved in microbial recognition and apoptosis, both of which are crucial to control of the immune response (*Figure 1*).

BIOLOGICAL THERAPY IN CROHN'S DISEASE

Biological therapy refers to the use of an agent known to act on a specific immunological pathway to affect a disease process. The two major classes of agent are monoclonal antibodies and small molecules. Currently monoclonal antibodies are the largest group, although over the last few years there has been increasing interest in small molecules such as antisense oligonucleotides, as these may ultimately prove cheaper and safer.

STRATEGIES TARGETING CD4+ LYMPHOCYTES

The central role of CD4+ lymphocytes in Crohn's disease was first highlighted by reports of patients with active disease entering remission following the development of acquired immunodeficiency syndrome (AIDS), or following bone marrow transplantation. However, treatment with depleting anti-CD4 monoclonal antibodies, while effec-

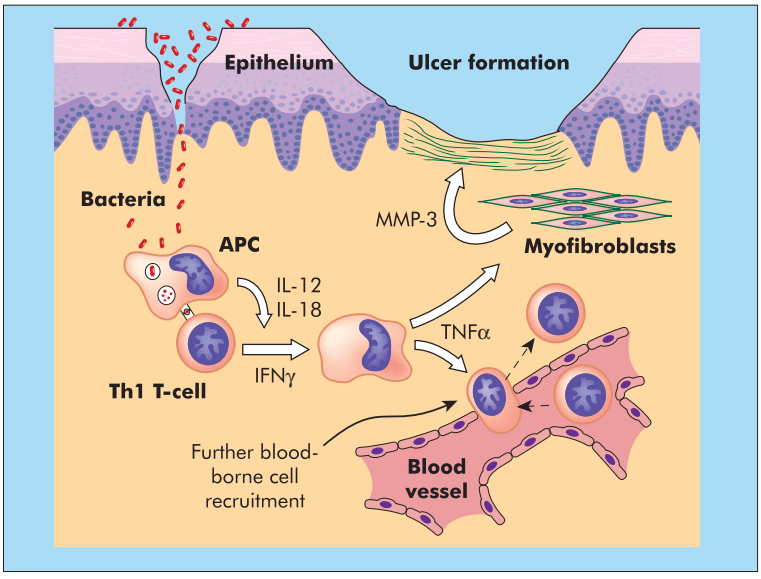

Figure 1. Immunological basis of Crohn's disease. APC = antigen presenting cell; IL = interleukin; IFN = interferon; MMP = matrix metalloproteinase; TNF = tumour necrosis factor.

tive in inducing remission, caused profound and long-lasting systemic immunosuppression precluding their clinical use (Stronkhorst et al, 1997).

ANTI-CYTOKINE THERAPY

TNFα was the first cytokine to be specifically targeted in the treatment of Crohn's disease and remains the most successful approach to date. It is an attractive target, as it is markedly elevated in Crohn's disease and has a wide spectrum of important biological effects. It can upregulate other inflammatory cytokines, activate macrophages, induce the expression of adhesion molecules, and stimulate the production of MMPs, enzymes intricately involved in epithelial destruction and ulcer formation. Biological agents developed to inhibit TNFα in vivo include neutralizing monoclonal antibodies, soluble TNF receptor fusion proteins linked to immunoglobulins (Ig), and small molecules.

Infliximab is a chimeric (75% human–25% mouse), monoclonal Ig-G1 antibody that binds both soluble and membrane-bound TNFα. Following a single infusion, 65% of patients with refractory disease will enter remission, with a subsequent median time to relapse of 8–12 weeks (Targan et al, 1997). In responders, repeated infusions every 8 weeks are significantly better than placebo in maintaining remission over 1 year (Hanauer et al, 2002). Perhaps most impressively, it is also effective in short-term healing of fistulating disease and promotes mucosal healing in Crohn's disease. However, its precise mode of action remains unclear.

In addition to neutralizing soluble TNFα, infliximab binds to membrane-bound TNFα inducing cell lysis through complement-dependent cell cytotoxicity. Furthermore it has been shown to activate the death domain of TNFR1 which activates caspase-8 leading to an increase in the ratio of BAX to Bcl-2, promoting apoptosis (ten Hove et al, 2002). It may exert its predominant effect not through neutralizing TNFα, but by causing clonal deletion of the activated mucosal T-cell population, accounting for its prolonged duration of action beyond the half-life of the antibody. One major drawback is reactivation of latent tuberculosis that can occur following treatment since TNFα is critical to the control of intracellular infections and granuloma formation. Additionally, human-antichimeric antibodies (HACA) may develop in up to 13% of subjects and reduce the efficacy of subsequent infusions. Pre-treatment with immunomodulatory drugs, e.g. azathioprine or methotrexate, may reduce this antibody response.

CDP571 is another neutralizing anti-TNFα monoclonal antibody, but with an IgG4 tail. It is fully humanized which should overcome problems of HACA formation. In clinical trials, although effective in improving Crohn's disease, it was not as efficacious as infliximab in inducing remission (Sandborn et al, 2001a). This may relate to the inability of IgG4 antibodies to fix complement and thus deplete T-cells in the lamina propria. The results of a large randomized placebo-controlled trial should be published early next year.

A different anti-TNFα strategy is the use of genetically engineered fusion proteins that combine a TNF receptor with an immunoglobulin tail. Etanercept is a fully humanized genetically engineered fusion protein that combines two chains of the human p75 TNF receptor with an IgG1 tail. It competes directly with cell-bound TNFα receptors to bind soluble TNFα. It does not neutralize membrane-bound TNFα, and does not promote apoptosis through complement activation or antibody-dependent cell cytotoxicity. Although effective in the treatment of rheumatoid arthritis, and in an initial pilot study in Crohn's disease, it failed to show any benefit over placebo in a large randomized controlled trial (Sandborn et al, 2001b). However, onercept, another similar fusion protein formed from the p55 human TNF receptor was effective in a phase I, proof of concept, pilot study, and a larger placebo-controlled trial is now underway.

In the last few years, interest has reawakened over the use of thalidomide in treatment of resistant Crohn's disease. Central to its mode of action appears to be its ability to downregulate IL-12 and TNFα production. However, it has other potentially important properties including anti-angiogenic effects and the ability to downregulate nuclear factor κB (NFκB). It has theoretical advantages over biological therapies, not least that it is cheap and non-immunogenic. In two initial uncontrolled studies it had impressive response rates of approximately 65% after 12 weeks of treatment (Ehrenpreis et al, 1999; Vasiliauskas et al, 1999). However, its teratogenic effects and high rate of adverse events, most notably the development of peripheral neuropathy, severely limit its usefulness. Newer thalidomide derivatives, reported to be non-toxic and non-teratogenic, are undergoing phase I and II clinical trials.

Finally, a pilot study using another small molecule to inhibit TNFα production has been reported. CNI-1493 inhibits the stress-induced mitogen activated protein (MAP)-kinase (p38, JNK; c-Jun activating kinase) pathway, a signalling pathway which links, among others, TNFα binding to its membrane receptor and transcription of pro-inflammatory molecules. In an uncontrolled study, 42% of patients with severe Crohn's disease

were in remission following 8 weeks of treatment and there was evidence of rapid mucosal and fistula healing (Hommes et al, 2002).

An alternative to inhibiting TNFα is targeting the cytokines that are critical to initiation of a Th1 response. Both macrophage-derived IL-12, and to a lesser extent IL-18, are important in polarizing CD4+ T-cells to produce Th1 cytokines. In murine models of colitis, both anti-IL-12 and anti-IL-18 therapies have resulted in a significant reduction in inflammation (Neurath et al, 1995; Wirtz et al, 2002). However, no human trials have been reported yet, although an anti-IL-12 trial is in progress (*Table 1*).

IMMUNOREGULATORY CYTOKINES

Downregulation of Th1 cytokine production by administering counter-regulatory Th2 cytokines appeared to be an attractive strategy to suppress inflammation. IL-10 is a Th2 cytokine that suppresses the major Th1 cytokines IL-2, INFγ and TNFα, and reduces antigen presentation. Despite being effective in animal studies, large scale placebo-controlled trials of rHuIL-10 (recombinant human IL-10) were not effective in treating severe Crohn's disease or preventing endoscopic recurrence after surgery. It was suggested that this may have been the result of an inability to attain high enough local levels of IL-10 with conventional delivery mechanisms. Alternative delivery methods including gene therapy and oral administration in lactobacilli are under investigation (Lindsay and Hodgson, 2001). However, a report indicating that high levels of IL-10 actually increased INFγ production means this may never be an effective therapy (Tilg et al, 2002).

IL-11 is another Th2 cytokine that antagonizes Th1 cytokine production, along with enhancing the function of the epithelial barrier. In a randomized controlled trial the higher dose proved significantly more effective (37% entering remission) than placebo (16% entering remission) (Sands et al, 2002). However, a more recent phase III trial was stopped early following interim analysis as it seemed ineffective.

INHIBITION OF LYMPHOCYTE TRAFFICKING

An alternative strategy to inhibiting cytokines is specific interruption of lymphocyte homing to the gut. α4-integrins are cell adhesion molecules that mediate migration of inflammatory cells from the blood stream to sites of inflammation by interacting with specific ligands such as ICAM-1 (α4β1) and MadCam1 (α4β7) on endothelial cells.

Natalizumab is a chimeric IgG4 monoclonal antibody directed against the human α4-integrin. In a multicentre randomized placebo controlled trial of 248 patients with moderate to severely active Crohn's disease it induced remission in 44% of patients who received two infusions of 3 mg/kg, compared with 29% in the placebo group. A significant response to treatment was seen in 71% of patients as defined by a decrease in Crohn's disease activity index of >70 points compared with 38% in the placebo group. Although statistically significantly better than placebo, remission rates 15% greater than placebo are not as good as would have been hoped (Ghosh et al, 2003). LDP-02 has been developed, an antibody specific to the α4β7-integrin, which interacts with MadCAM-1, an adhesion molecule only expressed on mucosal endothelial tissue. Trials of this are ongoing.

The alternative approach of inhibiting endothelial adhesion molecules has not proven successful. Two large placebo controlled trials using an ICAM-1 antisense oligonucleotide given systemically failed to show efficacy except for in a small subgroup of obese women who achieved higher serum concentrations. A further higher dose study is underway (van Assche and Rutgeerts, 2002).

ALTERNATIVE STRATEGIES

Several other strategies have been tried in animal models and small human pilot studies. One possibility is the use of peroxisome proliferator activated receptor (PPAR)-γ agonists, e.g. the antidiabetic agent troglitazone. PPARγ activation

TABLE 1.
Immunotherapy in Crohn's disease

Anti-TNFα therapy	Monoclonal antibodies	Infliximab – chimeric IgG1 antibody
		CDP571 – humanized IgG4 antibody
	TNFα-receptor fusion proteins	Etanercept – p75 fusion protein
		Onercept – p55 fusion protein
	Small molecules	Thalidomide
		CNI-1493 – MAP-kinase inhibitor
Alternative cytokine inhibitory strategies	Anti-IL-12 antibody	
	Anti-IL-18 antibody	
	Anti-IL-2 antibody	
Immunomodulatory cytokines	rHu-IL-10	
	rHu-IL-11	
Inhibition of lymphocyte trafficking	Natalizumab – anti-α4 integrin	
	LDP-02 – anti-α4β7 integrin	
Other strategies	Antisense to nuclear factor κB	
	Anti-CD40L antibody	
	Anti-CD40 antibody	
	Growth hormone	
	Peroxisome proliferator activated receptor γ agonists	

IgG = immunoglobulin G; MAP = mitogen activated protein; TNFα = tumour necrosis factor α

inhibits the signal transducer and activator of transcription (STAT) signalling pathway, reduces Th1 cytokine and MMP release, and has proven effective in the trinitrobenzene sulphonic acid mouse model (van Deventer, 2002). Other techniques include antisense oligonucleotides to NFκB, anti-CD40L monoclonal antibodies, anti-IL-2 therapy and use of recombinant growth hormone therapy. At least some of these are likely to progress to larger scale trials over the next few years.

CONCLUSION

Biological therapies are an important advance in the treatment of refractory Crohn's disease and their use is likely to increase considerably over the next few years. However, initial enthusiasm must be tempered by realism. Following treatment with infliximab, approximately 50% of patients enter remission, and despite maintenance therapy only a third of these remain in remission at 1 year (Hanauer et al, 2002). There is therefore a large number of patients for whom current biological therapy is ineffective, with factors that influence response rates currently undetermined.

Furthermore, as Crohn's disease appears to represent a heterogeneous group of disorders, witnessed by the identification of the NOD2 susceptibility gene predominantly in patients with ileal disease, it is likely that no one treatment will be suitable for all. Second, the relative ineffectiveness of specific anti-TNF strategies highlights the fact that infliximab's efficacy is not simply a result of TNFα neutralization, emphasizing the complexity of the immune response. Third, it is still not clear when biological therapies should be introduced. They are currently reserved for patients with disease refractory to conventional therapy, although if truly disease modifying, earlier introduction may be of greater benefit. This has proved particularly successful in rheumatoid arthritis. Considerations such as cost, immunogenicity, risk of infection and the need for potentially long-term therapy are all serious disadvantages.

KEY POINTS

- New therapies are needed for Crohn's disease as approximately 30% of cases fail to respond to standard therapy.
- Advances in our understanding of the immunological basis for Crohn's disease have lead to the development of multiple new promising biological therapies which are currently undergoing clinical trials.
- Tumour necrosis factor α (TNFα) plays a central role in disease pathogenesis in Crohn's disease.
- The anti-TNFα monoclonal antibody, infliximab, has been proven effective in inducing and maintaining remission in patients with resistant Crohn's disease.
- It is extremely likely that over the coming years new targeted biological therapies will play an important role in the treatment of Crohn's disease.

Despite this, we are in the vanguard of biological therapy, which, over the coming years, is likely to play an increasingly important role in the treatment of Crohn's disease. HM

Conflict of interest: Professor TT Macdonald has consulted on the role of biologicals in Crohn's disease for Centocor Inc, Genentech Inc and Celltech Ltd.

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