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# Biological Therapy in the Management of Recent-Onset Crohn's Disease

## Why, When and How?

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## Abstract

Crohn's disease is a chronic inflammatory bowel disease that may involve any part of the gastrointestinal tract. Conventional therapy consists of corticosteroids, azathioprine or methotrexate, but the clinical management of Crohn's disease is significantly hampered by adverse effects. With the introduction of biological agents (such as infliximab), the goals of therapy have advanced, including induction of remission with bowel healing as well as reduction in the rate of complications, surgeries and mortality. Current therapy for moderate to severe Crohn's disease is based on 'step-up' algorithms, which initiate treatment with corticosteroids followed by immunomodulatory agents, and defer therapy with biological agents until patients become refractory to conventional therapeutics. Recently, it has been shown that induction therapy with infliximab and azathioprine in recent-onset Crohn's disease (i.e. 'top-down' approach) is superior to current step-up algorithms to induce clinical remission. The underlying molecular mechanisms responsible for these differences in clinical outcome remain to be defined. Experimental studies have demonstrated that corticosteroids are able to induce impaired apoptosis of immune cells, including T cells and dendritic cells, resulting in loss of tolerance and subsequent autoimmunity. Further research will have to determine whether corticosteroid therapy augments the mechanism of loss of tolerance in Crohn's disease, which could complicate future clinical management.

Crohn's disease is a chronic inflammatory disease of the gastrointestinal tract characterised by relapses alternating with episodes of quiescent disease.[1] The prevalence and incidence of this disorder continue to increase worldwide. Crohn's disease is a multifactorial disease caused by the interplay of genetic, environmental and immunological factors, but the exact aetiology is still unknown. Because of the limited efficacy and significant toxicity of conventional therapies, there is a continuous search for new therapies and better treatment regimens for this disabling disorder.

## 1. Conventional Therapies

Conventional treatment of Crohn's disease consists of corticosteroids and immunomodulators, including azathioprine and methotrexate (figure 1).[2] Moderate to severe attacks of Crohn's disease are generally treated with corticosteroids, and this treatment leads to an improvement of symptoms in the majority of patients (remission rates: 60–83%).[3,4] Although corticosteroids suppress active inflammation in the acute setting, they are ineffective maintenance agents, and long-term use is associated with high relapse rates and unacceptable toxicities, such as osteoporosis, diabetes mellitus and hypertension. A significant number of patients become resistant to or dependent on corticosteroids and need additional immunomodulatory therapy with azathioprine or methotrexate.[5-10] Azathioprine and its metabolite 6-mercaptopurine (6-MP) are useful for the treatment of chronic active disease and for maintaining remission, but their use is limited by a slow onset of action and potentially serious adverse events.[11-13] Methotrexate, a folic acid antagonist, acts more rapidly and has been established as an induction agent for corticosteroid-dependent Crohn's disease (remission rate: 40%) and for maintenance of remission after successful induction (65% relapse free after 40 weeks).[7,14] Nevertheless, methotrexate-induced myelosuppression and hepatotoxicity complicate the clinical use of this immunomodulator. The limitations in efficacy and safety encountered with current medical approaches for Crohn's disease continue to drive the search for better therapeutic agents.

## 2. Biological Therapies

Conventional therapies have focused on non-specific suppression of the inflammatory process, which is characterised by overproduction of immune cells, inflammatory cytokines and tissue-destructive enzymes. Over the last decade, advances in our knowledge of the immunopathogenesis of autoimmune diseases have opened a new era with the development of biological therapies selectively interfering within inflammatory cascades. Blockade of inflammatory pathways through targeted immunological approaches, most notably tumour necrosis factor (TNF) inhibition, has revolutionised our approach to treating Crohn's disease. TNF, an extra cellular mediator of inflammation, plays a central role in the initiation and amplification of the inflammatory reaction leading to tissue destruction observed in Crohn's disease. Antibodies against TNFα, such as infliximab, have been proven efficacious in inducing both clinical remission and muco-

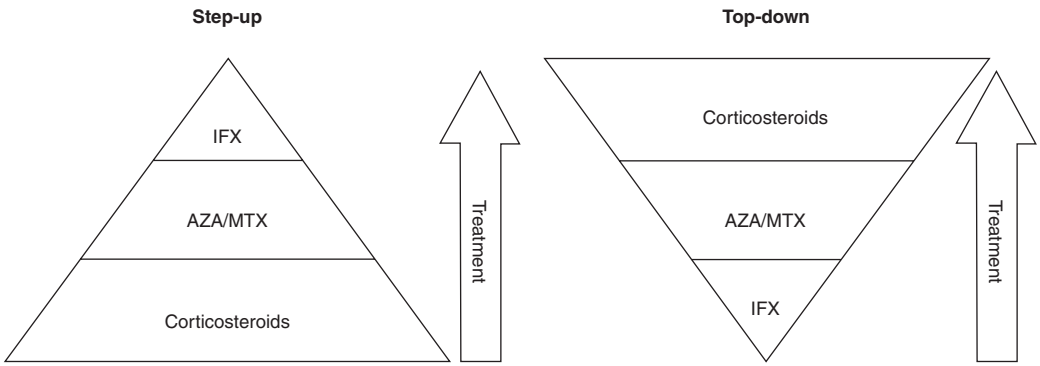


**Fig. 1.** Step-up versus top-down therapy in recent-onset Crohn's disease. Conventional therapy for Crohn's disease is based on 'step-up' algorithms, which initiate treatment with conventional immunosuppressive and immunomodulatory agents (i.e. corticosteroids, azathioprine [AZA], or methotrexate [MTX]) and defer therapy with biological agents until patients become refractory to conventional therapies. Recently, it has been shown that induction therapy with infliximab (IFX) and AZA in recent-onset Crohn's disease patients ('top-down') is superior to the conventional step-up approach in order to induce clinical remission.

sal healing in Crohn's disease,[15-17] and this success has become a therapeutic milestone that has opened the door for future biological therapeutics. Since the approval of infliximab, other anti-TNF compounds, such as adalimumab and certolizumab, have become available for patients with Crohn's disease who do not respond to conventional therapies.[18] Despite potent clinical effects, several factors limit the use of infliximab, such as toxicity, infectious complications (i.e. opportunistic infections and reactivation of tuberculosis), cardiovascular and autoimmune disorders, as well as a 1–2% mortality rate.[19,20] High costs and immunogenicity further complicate the clinical use of infliximab and other anti-TNF compounds.[21] In addition, it has been shown that the percentage of patients with Crohn's disease in medical remission rapidly decreases to approximately 30% by 5 years after diagnosis.[22] As can be concluded from this study, aggressive therapy is unnecessary to induce clinical remission in a significant proportion (i.e. >50%) of patients with Crohn's disease.[22]

Other biological agents that have proved effective in active Crohn's disease are aimed at blocking leukocyte adhesion (such as natalizumab)[23,24] or inhibiting several proinflammatory cytokines.[18] More clinical trials are currently being conducted, exploring the safety and efficacy of biological therapies, and the search will certainly open new and exciting perspectives on the development of therapies for Crohn's disease.

## 3. Loss of Immune Tolerance

The pathogenesis of Crohn's disease is still largely unclear but immune-mediated phenomena are obviously involved.[25] Crohn's disease arises from a pathological intestinal antigen-driven inflammatory response within a genetically susceptible individual. The complex interplay of genetic, microbial and environmental factors culminates in a sustained activation of the mucosal immune system of the gastrointestinal tract in which immune cells, cytokines and tissue-destructive enzymes eventually induce tissue destruction. In the intestine reside numerous different types of immune cells, including T or B lymphocytes, monocytes, macrophages, dendritic cells, eosinophils, neutrophils, granulocytes and epithelial cells.[26,27] The intestinal lumen contains huge quantities of non-pathogenic bacteria, which constantly interact with the host. The immune system is able to discriminate between commensal (harmless) and potentially pathogenic micro-organisms. On one hand, the mucosal immune system has to respond effectively to pathogens, such as bacteria and viruses, while on the other hand, the presence of non-pathogenic microbes has to be ignored by immune cells. Thus, the intestinal immune system must tolerate the commensal flora which are constantly present in the lumen and maintain mucosal homeostasis by controlled inflammatory responses, generally referred to as 'immune tolerance'. This is accomplished by the innate immune system through cell surface structures that function as mammalian pattern-recognition receptors, such as Toll-like receptors (TLRs).[28] TLRs specifically recognise microbes leading to adequate pathogen elimination. In order to regulate these TLR-mediated processes, several molecular mechanisms that ensure tolerance have been elucidated, such as decreased ligand recognition or inhibition of intracellular signaling.[29]

A fundamental aspect of commensal host-bacterial relationships in the gut is the development and maintenance of immune tolerance to the enteric flora.[30] Accumulating evidence derived from genetic, microbial and immunological observations, suggests that the normal indigenous flora of the intestine plays a crucial role in the pathogenesis of Crohn's disease. It is currently believed that 'loss of tolerance' against luminal commensals is a central event in Crohn's disease pathogenesis, which is due to altered pattern recognition.[31-34] Several complementary factors probably contribute to this loss of tolerance to enteric flora in Crohn's disease, such as inadequate regulatory T-cell function or excessive mucosal dendritic cell stimulation by the gut flora.[35]

## 4. Corticosteroid-Induced Loss of Tolerance

Another critical mechanism for maintaining immune tolerance and preventing autoimmunity is programmed cell death or apoptosis of immune cells.[36] This has been demonstrated *in vitro* and *in vivo* by systemic autoimmune diseases that result from mutations in the pro-apoptotic Fas receptor or Fas ligand genes.[37] Previous studies revealed that loss of self-tolerance, as a result of defective apoptosis in the lymphocyte compartment, plays a major role in the onset of autoimmune diseases.[38,39] In addition, it has been shown that dendritic cells, antigen-presenting cells that are potent initiators of immune responses, are also important for maintaining immune tolerance,[40-44] but their exact role remains to be defined. It has been reported that dendritic cells accumulate in autoimmune patients harbouring a deficiency in apoptosis[45] and significant expansion of dendritic cells has been detected in Fas-deficient mice.[46] These findings suggested that accumulation of dendritic cells, based on a defect in dendritic cell apoptosis, results in chronic lymphocyte activation and subsequent autoimmunity. It has been recently confirmed that apoptosis in dendritic cells helps regulate self-tolerance.[47] This particular study demonstrated increased dendritic cell numbers in mice that displayed defective dendritic cell apoptosis leading to systemic autoimmune manifestations. These findings confirmed that apoptosis defects in dendritic cells can lead to loss of tolerance and autoimmunity. Thus, a defect in dendritic cell apoptosis can independently lead to autoimmunity, which is consistent with a central role for this cell type in maintaining immune tolerance.

Corticosteroids are effective as immunosuppressive therapy for a wide variety of inflammatory disorders and autoimmune pathology. Paradoxically, depending on their dose as well as on the activation state of target cells, corticosteroids can either induce or inhibit apoptosis of T lymphocytes.[48] It has been reported that corticosteroid treatment inhibits interleukin (IL)-2-dependent activation-induced cell death of leukocytes, resulting in increased systemic leukocyte numbers and autoimmunity.[49,50] Furthermore, corticosteroids may increase *de novo* synthesis of the protein leucine zipper, which has been demonstrated to confer resistance to activation-induced apoptosis in hybridoma T cells.[51] Importantly, Crohn's disease pathogenesis is characterised by apoptosis defects in T cells.[52,53] Altogether, corticosteroid-induced counterproductive effects on immune cell apoptosis may complicate the long-term course of Crohn's disease inflammatory pathology, and this might represent a clinical problem in the preferred first-line corticosteroid treatment of such patients.

The responsible mechanisms for these corticosteroid-dependent effects remain only partly understood. Previous experimental studies have revealed a relationship between corticosteroid treatment and loss of tolerance in liver transplant and airway hyperreactivity models leading to autoimmunity. First, it is known that immune tolerance in liver transplants is associated with apoptosis of infiltrating recipient leukocytes.[54] Importantly, peri-transplant corticosteroid administration reduced apoptosis of infiltrating immune cells and prevented development of systemic tolerance resulting in reduced graft survival.[54] Although these experimental findings demonstrated that corticosteroids reduce apoptosis of immune cells resulting in loss of tolerance, the underlying mechanism remains to be defined. Secondly, immune tolerance in respiratory organs has been described to be an important mechanism in preventing inflammation in the airway and this is mediated through the development of antigen-specific adaptive regulatory T cells.[55-57] Respiratory tolerance limits and controls immune responses against large quantities of innocuous antigens that enter the lungs. The effect of corticosteroid treatment on the development of immunological tolerance and airway hyperreactivity was studied in a murine model.[58] This study demonstrated that corticosteroid therapy prevents protective effects of respiratory tolerance on the development of airway hyperreactivity through reduced IL-10 synthesis by dendritic cells and by inhibiting the development of T-regulatory cells. These findings indicated that cor-

ticosteroids prevent the protective effects of respiratory tolerance on the development of airway hyperreactivity, resulting in an aggravated inflammatory response. Thus, experimental evidence demonstrated that corticosteroid treatment is able to induce loss of tolerance leading to autoimmunity (figure 2).

In Crohn's disease pathogenesis, corticosteroid-induced loss of tolerance is a controversial issue. However, there is emerging insight into the importance of immune cell apoptosis in controlling immunological processes in the gut. The problem with corticosteroids seems to lie in the sometimes negative effect on immune cell apoptosis and further research will have to clarify the responsible molecular mechanisms. It is possible that strategies which avoid corticosteroid use and directly positively target apoptotic mechanisms may be superior to conventional therapeutic protocols.

## 5. Step-Up versus Top-Down Therapy for Crohn's Disease

Current step-up algorithms to treat Crohn's disease are based on initiation of treatment with conventional immunosuppressives and immunomodulators, including corticosteroids, azathioprine or methotrexate (figure 1). Biological therapies are introduced when patients become refractory to conventional therapies. The advent of biological therapy, in particular neutralising antibodies against TNF, has dramatically changed the management of patients with more severe and/or refractory Crohn's disease. A single infusion of infliximab 5 mg/kg led to a response in >80% of patients with Crohn's disease.[59] This therapy has been shown to be relatively safe and is ideally combined with continued immunomodulator therapy in order to reduce immunogenicity.[60]

To date, anti-TNF therapy has been reserved for patients with refractory disease who have not re-

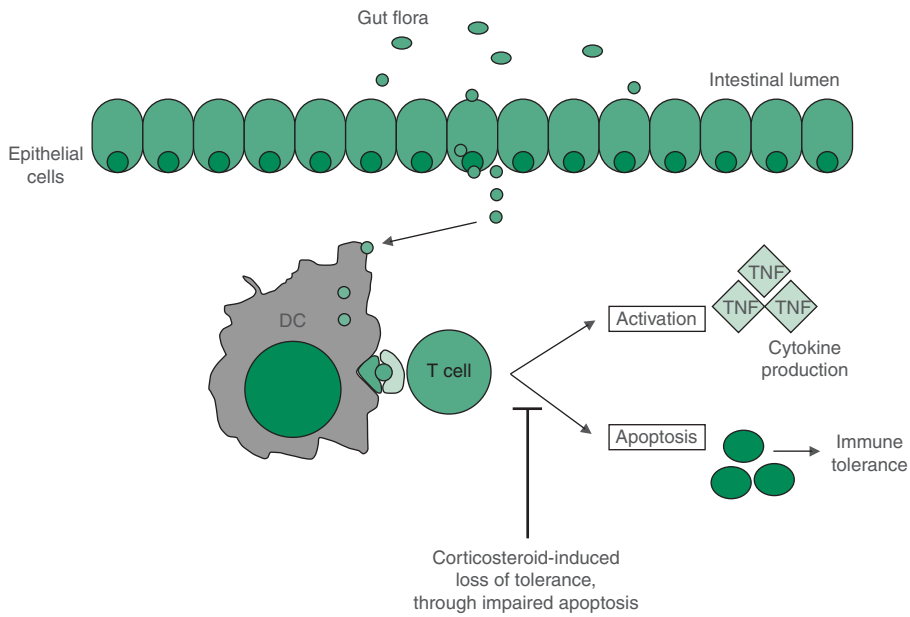


**Fig. 2.** Corticosteroid-induced loss of tolerance. Gut flora is internalised and processed by dendritic cells (DCs) and presented to immune cells, such as T cells. The intestinal immune system must tolerate the commensal flora and maintain mucosal homeostasis, referred to as immune tolerance. Immune cells undergoing apoptosis is an essential process in maintaining mucosal homeostasis and controlling the gut immune system. Loss of tolerance to commensal autologous flora results in enhanced reactivity against gut antigens and an inappropriate activation of the immune system. Evidence exists that corticosteroids can break immune tolerance through interfering with immune cell apoptosis. **TNF** = tumour necrosis factor.

sponded to corticosteroids and immunomodulators (step-up approach). Recently, a more aggressive (top-down) therapeutic strategy was used early on in the disease course in order to find out whether this would lead to better clinical outcomes (figure 1).[61] Crohn's disease patients (n = 130) with moderate to severe disease diagnosed within 4 years and never treated with corticosteroids, immunomodulators or biological agents were randomised to receive either step-up treatment with repetitive topical or systemic corticosteroids or top-down treatment with three infusions of infliximab (at weeks 0, 2 and 6) together with azathioprine (2–2.5 mg/kg/day). In the top-down group, relapsing patients were given repeated infliximab and corticosteroids when they did not respond to infliximab. In the step-up group, azathioprine was added in the case of repeated need for corticosteroids or dependency, and infliximab was given after failure of immunosuppression. This study demonstrated that the top-down approach in patients with recent onset Crohn's disease is superior to step-up treatment for inducing clinical remission, for avoiding corticosteroid therapy, and for inducing long-lasting endoscopic improvement and mucosal healing. Patients in the step-up arm needed prolonged exposure to corticosteroids to control disease (25% at 6 months and 12.5% at 12 months). Interestingly, it was only because of the introduction of immunomodulatory therapy that patients achieved clinical remission in the step-up arm, e.g. 40% at 6 months and 62.5% at 12 months were treated with azathioprine or methotrexate after two courses of corticosteroids failed to control disease. In addition, two rather remarkable observations were made: (i) almost 100% of study patients, with recent-onset Crohn's disease and naive to previous therapy, responded to infliximab versus only 60–70% of patients who had been treated with previous courses of corticosteroids or immunomodulators; and (ii) the difference in clinical outcome of peri-anal fistulising disease was striking: in the top-down group, the development of fistulas was absent and in those patients presenting with fistulas at baseline, a long-lasting response was observed, as opposed to in the step-up arm where fistulising disease was not well controlled. These findings suggest that treatment with anti-TNF therapy in treatment-naive Crohn's disease patients is preferable to an approach in which patients are repeatedly treated with conventional immunosuppressives before initiating biological therapy.

These clinical outcomes together with experimental data indicate that corticosteroid treatment is able to induce loss of tolerance, which might complicate the clinical management of Crohn's disease. Infliximab specifically targets one molecule (i.e. TNFα), a central player in the pathogenesis of Crohn's disease, leading to immune cell apoptosis.[62-64] Differences in mechanism of action between corticosteroids and infliximab could contribute to the observed discrepancy in clinical outcome between step-up and top-down treatment protocols in recent-onset Crohn's disease.

## 6. Small Molecules

As a consequence of the limited efficacy and toxicity of current therapies available for Crohn's disease, there is widespread interest in the development of novel drugs. Over the last decade, many signaling pathways that play a crucial role in the pathogenesis of Crohn's disease have been elucidated. As a result, attention has shifted towards modulation of intracellular signaling cascades and this has led to the development of novel therapeutics. Small molecules targeting signaling molecules are considered a promising strategy for the clinical management of Crohn's disease.[65] In particular, pharmaceutical intervention of mitogen-activated protein (MAP) kinase (MAPK) pathways has attracted widespread interest; i.e. extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 MAPK.[65,66]

Treatment for 12 days with the small molecule semapimod resulted in clinical remission and endoscopic healing in 12 patients with therapy resistant Crohn's disease.[67] The drug was well tolerated and adverse effects included local irritation at the infusion site (phlebitis) and mild increases in liver enzymes, both resolving spontaneously within weeks. Recently, c-Raf was identified as the cellular target of semapimod,[68] a potent initiator of ERK, JNK

and p38 MAPK signaling pathways. These results indicated that c-Raf is a critical mediator of disease progression in Crohn's disease and defined c-Raf as a novel therapeutic target for the clinical management of this disorder. Therefore, semapimod and probably other c-Raf inhibitors could constitute a novel therapeutic approach for severe Crohn's disease.

Similarly, recent findings provided insight into the immunosuppressive action of azathioprine. It was shown that azathioprine and its metabolite 6-MP inhibit activity of the proximal signaling molecule Rac through inhibition of Vav guanosine exchange activity in T cells. Azathioprine-induced inhibition of Rac activity resulted in reduced downstream activation of MEK (MAP-ERK kinase), nuclear factor (NF)κB and bcl-x(L), resulting in enhanced T-cell apoptosis.[69,70] Hence, therapeutic interventions that specifically target proximal signaling molecules such as c-Raf or Rac might be a powerful strategy for combating Crohn's disease.

## 7. Conclusions

Corticosteroids are powerful agents that effectively control acute and chronic inflammatory disorders. However, there is experimental evidence that corticosteroids may also limit beneficial immunological processes, such as immune tolerance (i.e. inactivation responses to commensal bacteria), by interfering with immune cell apoptosis. Crohn's disease has been treated for many decades using a step-up therapeutic approach, consisting of repeated corticosteroid treatments. Biological agents, such as infliximab, are introduced when patients do not respond to conventional therapies. Recently, the treatment paradigm for Crohn's disease was shifted, demonstrating that induction therapy with infliximab and azathioprine (top-down) is superior to the conventional step-up approach to induce clinical remissions in patients with recent-onset Crohn's disease. These observations might indicate that patients with newly diagnosed Crohn's disease may benefit from a top-down approach, rather than treating patients repeatedly with corticosteroids before initiating anti-TNF treatment. Further studies are needed to define the possible underlying mechanisms responsible for corticosteroid-induced loss of tolerance and to find out whether corticosteroids could complicate the long-term course of Crohn's disease. Well designed and sufficiently powered multicentre trials should further investigate the efficacy of this therapeutic top-down approach before recommending a reevaluation of clinical protocols for treating Crohn's disease.

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