Crohn's disease (CD) is characterized by intestinal inflammation and tissue destruction that are mediated in part by the inappropriate release of inflammatory cytokines. During the last decade, the inhibition of tumor necrosis factor (TNF-α) and other inflammatory cytokines has emerged as a therapeutic strategy for CD and other disorders that are associated with elevated TNF-α production. Monoclonal antibodies against TNF-α (eg, infliximab, adalimumab) have been shown to be effective for the induction and long-term maintenance of remission in CD. Antibody Fab fragments have also been shown to improve the symptoms of CD, and an anti-TNF-α Fab conjugated with polyethylene glycol (certolizumab pegol) may soon become available for the treatment of CD. Other potential therapeutic strategies include the use of recombinant soluble TNF-α receptor, which binds to and inactivates circulating TNF-α; agents that block leukocyte adhesion and transendothelial migration; and antibodies that target other inflammatory cytokines. Recent analyses of clinical trial data have suggested that elevated levels of the inflammatory marker C-reactive protein (CRP) may help to identify patients who are most likely to benefit from TNF-based therapies. Additional research is required to develop algorithms to incorporate information about CRP concentration or other biomarkers into clinical decision making for CD.

This article reviews a number of potential therapeutic strategies that have been developed to improve the symptoms of CD by targeting the biological effects of TNF-α. Other possible approaches to the inhibition of T-cell–mediated tissue injury are also briefly described. A companion article by Dr Stephen B. Hanauer discusses the integration of anti–TNF-α therapies into clinical practice for the treatment of patients with CD.

**STRATEGIES TO INHIBIT TNF-α FUNCTION**

TNF-α produces several physiological effects that promote inflammation: it stimulates the production of other proinflammatory cytokines, such as IL-1, IL-6, and IFN-γ; it stimulates further TNF-α production; it disrupts the intestinal endothelial cell boundary, increasing the permeability of the endothelial layer to antigens; and it increases the activity of antigen-presenting cells. One strategy to block these proinflammatory effects of TNF-α that has been extensively evaluated in CD is the administration of monoclonal antibodies against TNF-α. Infliximab, which is approved by the US Food and Drug Administration (FDA) for the treatment of CD, is a chimeric IgG1 monoclonal antibody to TNF-α that includes human constant domains and murine variable domains. Controlled clinical trials have shown that infliximab is effective for both the induction and maintenance of remission in patients with CD. A second anti–TNF-α monoclonal antibody, adalimumab, is FDA-approved for the treatment of rheumatoid arthritis. Adalimumab is a fully human recombinant IgG1 antibody to TNF-α, in which human heavy and light chains with binding affinity for TNF-α were independently identified and then combined, resulting in the removal of all murine sequences. Adalimumab has been shown to be effective for the induction of remission in patients with CD. Preliminary data also indicate that adalimumab is effective as maintenance therapy. An investigational agent, CDP-571, is a humanized IgG4 monoclonal antibody to TNF-α. In initial clinical trials, CDP-571 produced short-term improvement in CD symptoms after 2 to 4 weeks of treatment, but did not induce remission and was not associated with improvement in clinical outcomes after 6 months.

An alternative to the use of antibodies is the removal of the Fc antibody portion and the use of only the Fab fragment (the variable domains and hinge region). One example of this approach is certolizumab pegol (CDP-870), a humanized TNF-α Fab fragment in which the murine framework that surrounds the antigen-binding portion of the antibody has been replaced by human sequences and the Fc portion of the antibody has been removed (Figure 1). The Fab fragment is linked to polyethylene glycol (PEG) to increase its half-life. CDP-870 can be administered by intravenous or subcutaneous administration, and it is being developed as a subcutaneous treatment for CD. The removal of the Fc antibody portion has the

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Figure 1. Protein-Engineered Antibodies and Fusion Proteins

<table>
<thead>
<tr>
<th>Chimeric monoclonal antibody</th>
<th>Humanized monoclonal antibody</th>
<th>Human recombinant antibody</th>
<th>Humanized Fab fragment</th>
<th>Human recombinant receptor/Fc fusion protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>75% Human Mouse CH1 CDR</td>
<td>95% Human Mouse VL VH CH1</td>
<td>100% Human Mouse PEG PEG</td>
<td>100% Human Receptor Constant 2 Constant 3</td>
<td></td>
</tr>
</tbody>
</table>

CDR = complementary determining region; CH1 = complementary heavy chain; PEG = polyethylene glycol; VH = immunoglobulin heavy chain; VL = variable light chain.

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potential therapeutic advantage that it avoids complement fixation, and thereby reduces antibody-dependent cytotoxicity and T-cell apoptosis. However, it is also possible that antibody-mediated T-cell apoptosis is one mechanism by which the anti–TNF-α antibodies produce their therapeutic effects. This is an important issue that has very recently evolved with respect to the results of clinical trials. CDP-870 significantly improved the symptoms of CD in 1 recent clinical trial, but only in patients with elevated C-reactive protein (CRP). However, 2 subsequent, large clinical trials that prospectively evaluated CDP-870 in patients with elevated CRP and in those without elevated CRP demonstrated efficacy in both subgroups of patients (S. Schreiber et al, unpublished data, 2005).

TNF-α produces its effects by binding to 2 cell membrane receptors, p55 and p75. A third potential therapeutic strategy for CD is the administration of soluble TNF-α receptors, which bind to TNF-α and prevent it from activating its normal biological targets. Two different approaches to this strategy have been developed. The first is represented by onercept, a recombinant soluble TNF-α p55 receptor. Onercept has a relatively short half-life and must be administered at least 3 times a week by subcutaneous injection. One clinical trial that examined onercept for the treatment of CD found that it did not produce a significant beneficial effect. The second approach is represented by etanercept, a fusion protein that combines the extracellular portion of the p75 receptor with the Fc portion of human IgG1 (Figure 2).


One potential limitation with all of the antibody-based therapies is immunogenicity and the development of neutralizing antibodies. An important determinant of immunogenicity is the degree to which the antibody incorporates nonhuman components. In general, humanized antibodies (which contain approximately 5% murine sequences) or fully human antibodies (which contain no murine sequences) are less immunogenic than chimeric monoclonal antibodies (which contain approximately 25% murine sequences). However, it is possible for antibodies to develop in response to humanized or fully human antibodies, or even to endogenous antibodies. Lower antibody doses tend to be more immunogenic, and higher doses are more likely to produce tolerance.

Loading doses and the use of constant therapy (dosing based on the drug half-life to ensure that the patient is always exposed to the antibody) can also reduce the likelihood of immunogenicity. Immunogenic reactions can also be reduced by the coadministration of an immunosuppressant such as azathioprine, 6-mercaptopurine, or methotrexate. There may be a modest reduction in immunogenicity by pretreatment with a dose of intravenous corticosteroids before administering monoclonal antibodies. The risk of immunogenic reactions may also be greater.
when antibodies are administered by subcutaneous administration than when administered intravenously. It is likely that combining the various strategies to reduce antibody development may produce additive reductions in the risk of clinically significant immunogenicity, although the optimal strategies to prevent immunogenic reactions have not been well defined by clinical trials.

**The Importance of CRP in Predicting Response to Therapy**

Although all of the anti–TNF-α therapies block the effects of TNF-α, the efficacy of these agents in clinical trials of patients with CD has been quite variable. The reason for this variability is not well understood at present. One possibility is that only IgG1 antibodies are effective. However, as noted previously, the IgG4 antibody CDP-870 also improved the symptoms of CD during the first few weeks of treatment. In addition, the 2 soluble receptor agents were not at all effective for CD.

It has long been recognized that there is an association between circulating levels of the acute-phase reactant CRP and the symptoms of active CD. This relationship is not strong however, and it was initially believed by most clinicians that CRP and CD severity were not causally linked. As a result, treatment decisions have generally been made on the basis of clinical symptoms alone, without regard to CRP findings. More recently, post hoc subgroup analyses in a number of clinical trials of patients with CD have suggested that CRP may be more directly associated with the inflammation of CD, and that patients with elevated CRP levels are more likely to respond to some treatments than patients who do not have elevated CRP levels. However, this effect is largely due to differences in the response of patients to placebo between those with and without elevated CRP levels.

During the induction of response or remission treatment, the placebo response rate is often quite pronounced in patients who have normal CRP levels at baseline. In contrast, the placebo response rate in some studies was lower among patients who have symptoms and elevated CRP levels. It has been hypothesized that some patients who have symptoms of CD may actually have other conditions, such as short-bowel syndrome, bile-salt diarrhea, irritable bowel syndrome, partial mechanical obstructions caused by fibrosis of CD or from previous operations, bacterial overgrowth, or other causes of symptoms. When these patients are entered in clinical trials, a substantial number may improve spontaneously, whereas patients with biologically driven inflammation tend to have a less benign clinical course and less likelihood of a significant improvement with placebo. However, although it has been observed in post hoc subgroup analyses from several studies that the placebo response rate is greater in patients with low CRP levels, the explanation for this phenomenon remains speculative at present. The results from 2 large clinical trials, which prospectively evaluated CDP-870 in patients with and without elevated CRP, did not demonstrate an effect of elevated CRP on either the placebo response rate or efficacy (S. Schreiber et al, unpublished data, 2005). It is also possible that there are groups of patients with similar clinical manifestations but distinct pathophysiological processes that produce CD, and that therapies that block TNF-α are beneficial in some subgroups but not in others.

Additional clinical trials are in progress to clarify the relationship between CD symptoms, biological markers such as CRP, and endoscopy findings. These studies may eventually lead to the development of algorithms to guide clinical decision making for patients with CD. Until such guidelines have been developed, a reasonable approach is to assess CRP values for patients with symptoms of CD. If CRP is elevated and there are no symptoms that suggest that emergency treatment is required (eg, obstructive symptoms), therapy may be initiated with an immunosuppressive or biologic agent without the need for a structural evaluation with endoscopy or radiographic studies. For a patient who is symptomatic but who has a normal CRP value, a structural evaluation should be considered. However, there is no single approach to the integration of CRP findings into the management of CD at present.

**The Role of T-cell Apoptosis in CD Treatment**

The first 2 anti–TNF-α medications that were examined for the treatment of CD were infliximab and etanercept. Infliximab was effective in patients with CD, whereas etanercept was not. It has been hypothesized that infliximab may induce apoptosis of T cells. In vivo experiments found that infliximab, but not etanercept, induced T-cell apoptosis.
Subsequent research found that adalimumab and infliximab both induced T-cell apoptosis, and that etanercept did not. These findings provide an appealing explanation for the differences in clinical efficacy of these agents that were observed in patients with CD, and suggest that apoptosis was linked to IgG1 monoclonal antibody therapeutic agents. However, the CDP-870 (humanized Fab antibody fragment) is also effective for CD. Therefore, more research is needed to further define the role of T-cell apoptosis in the treatment response with these agents.

**OTHER INFLAMMATORY CYTOKINES IN CD: IL-12, IFN-γ, IL-6**

As noted previously, CD is generally thought to develop as a consequence of the abnormal production of Th1 proinflammatory cytokines. Therefore, it has been hypothesized that other Th1 cytokines may be important in the pathogenesis of CD, and that therapeutic interventions to block these cytokines might improve symptoms in these patients. Antibodies have been developed against IL-6, IL-12, and IFN-γ, and initial clinical trials have evaluated the effectiveness of these antibodies for patients with CD. The effects of an anti–IL-12 antibody were examined in a phase 2 clinical trial, which found that 7 weeks of treatment reduced the expression of inflammatory cytokines within the colonic lamina propria, and resulted in clinical improvement in more patients than with placebo administration. Studies of anti–IFN-γ antibody failed to demonstrate significant clinical benefit in the study primary endpoint for patients in general, but for the subgroup of patients with elevated CRP, repeated dosing did appear to produce some benefit. IL-6 is a major stimulant of the production of CRP by the liver, and a single small study suggested that an antibody against the IL-6 receptor (MRA) produced some clinical benefit in patients with elevated CRP at baseline. Thus, the results of these preliminary studies suggest that treatments that target any of several Th1 cytokines may improve symptoms in patients with CD.

**CELL ADHESION MOLECULES**

Activated T lymphocytes migrate from the circulation to peripheral sites of inflammation by means of a carefully coordinated series of interactions between cell adhesion molecules on lymphocytes and receptor molecules on endothelial cells. Agents that interrupt these adhesion molecule interactions and prevent the transendothelial migration of T lymphocytes have been evaluated for the treatment of CD. One approach is an antisense oligonucleotide against the ribonucleic acid for intercellular adhesion molecule-1. This antisense oligonucleotide (alicafosren; ISIS-2302) improved symptoms of CD in some preliminary clinical trials, but was not effective in a larger phase 3 study. Post hoc subgroup analyses suggested that the dose of alicaforsen used may have been too low, and a high-dose trial was initiated, which also failed to demonstrate significant improvement in patients with CD. Topical alicaforsen is being tested as therapy for pouchitis and for proctitis.

Natalizumab is an IgG4 humanized monoclonal antibody to α4 integrin, a cellular adhesion molecule that is important in the homing of lymphocytes to the gut. Natalizumab was developed primarily for the treatment of multiple sclerosis (MS) and CD. It was approved by the FDA in December 2004, and withdrawn from the market in February 2005 after 2 patients who received natalizumab in combination with PEG-interferon developed progressive multifocal leukoencephalopathy (PML). In another clinical trial, a patient with CD treated with natalizumab in combination with azathioprine who was initially thought to have had a fatal astrocytoma was reclassified as having PML. Natalizumab was clearly effective for the treatment of MS, and was approved under an accelerated approval program on the basis of 1-year data in patients with MS that demonstrated that it was approximately twice as effective as IFN-beta. An intensive safety evaluation of all of the patients with MS who were treated with natalizumab has been completed, and no additional cases of PML were identified. Regulatory review of these safety data to determine whether natalizumab should return to clinical use for MS is anticipated in the latter half of 2005. Safety evaluation of patients with CD and rheumatoid arthritis who were treated with natalizumab is still ongoing. In patients with CD, a phase 3 clinical trial for induction of response and remission failed to demonstrate the efficacy of natalizumab in the overall patient population, but demonstrated efficacy in a subgroup of patients with elevated CRP. A second phase 3 trial for induction of response and remission in patients with elevated CRP also demonstrated efficacy with natalizumab. In addition, a phase 3 trial for maintenance of response and remission
with natalizumab demonstrated a significant maintenance benefit throughout 12 months.35

A second antibody, MLN-02 (also referred to as LDP-02), is a fully human IgG1 monoclonal antibody against α4β7, a cell adhesion molecule that interacts with mucosal addressin cell adhesion molecule 1 in the gut mucosa. MLN-02 is thought to affect lymphocyte homing specifically in the gut, with less effect on lymphocyte trafficking in other tissues.31 Thus, it is expected that MLN-02 should be associated with a lower risk of PML. A phase 2 clinical trial that examined this agent for ulcerative colitis demonstrated significant effects for induction of response, remission, and endoscopic healing.32 A second phase 2 trial in patients with CD has been completed, and the result for the primary study endpoint of clinical response was not significant, but the result for the secondary study endpoint of clinical remission was significant.33 The doses of MLN-02 that were used in the CD study did not completely block α4β7 receptors. In addition, although fully human antibodies are generally less immunogenic than chimeric or humanized antibodies, MLN-02 was highly immunogenic in the ulcerative colitis study in which approximately 40% of patients developed antibodies to MLN-02 after 2 doses, and approximately 25% of patients developed neutralizing antibodies.32

**GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR**

The treatment strategies described above are based on the hypothesis that CD reflects an abnormally high level of activation of immune cells within the intestinal tract. An alternative view of CD, which suggests that the disorder is actually an immune deficiency disease, has recently been proposed.34 This concept is based on the observation that certain aspects of CD resemble a number of relatively uncommon disorders that have in common neutrophil dysfunction. These include chronic granulomatous disease, Chediak Higashi syndrome, glycogen storage disease, and other disorders, which primarily affect children. As many as one third to one half of patients with these various neutrophil dysfunction syndromes also develop intestinal inflammation, diarrhea, and perianal fistulas that are indistinguishable from CD, with the exception that they are accompanied by neutrophil dysfunction. Therefore, it has been proposed that therapies to stimulate neutrophil function might be an effective therapy of CD. The efficacy of recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF; sargramostim) for the treatment of CD has been evaluated in a placebo-controlled study.35 In this study of 124 patients, treatment with daily injections of sargramostim for 8 weeks did not improve the primary study endpoint of treatment response, but did improve a secondary endpoint, remission.35 One patient in the GM-CSF treatment arm developed neutralizing antibodies to GM-CSF. This patient did not experience any clinically significant neutropenia during the study, although the long-term risk is not clear. Approximately 40% of the patients reported bone pain, and 90% had injection-site reactions. Although this study provides some evidence supporting the potential therapeutic role of GM-CSF in CD, the need for daily injections and high incidence of painful side effects may limit its clinical usefulness, especially in comparison with TNF-α treatments, which are generally well tolerated but have rare, potentially serious side effects.

**SUMMARY**

Considerable evidence suggests that the proinflammatory cytokine TNF-α is central to the pathogenesis of CD, and that interventions that target TNF-α significantly improve the severity of symptoms, induce remission, and maintain remission in patients with CD. Not all of the anti-TNF-α therapies have been equally effective for the treatment of CD symptoms in clinical trials, and it appears that inhibition of TNF-α alone may not be sufficient to improve symptoms. Some studies have suggested that the induction of T-cell apoptosis may also be required to effectively treat CD, although additional research is required to clarify this issue. Anti–TNF-α Fab fragments have also been shown to improve the symptoms of CD in some studies. Administration of soluble T-cell receptors or receptor fusion proteins has not been shown to be effective. Biological markers of inflammation such as CRP may help to identify patients who are most likely to benefit from anti–TNF-α or other immunosuppressive therapies.

**REFERENCES**


