

Lecture

Newer biological agents for IBD

Nicoletta Mathou

SUMMARY

While traditional medical treatment has focused on non-specific suppression of the inflammatory process, advances in our knowledge of the immunopathogenesis of IBD have opened up a new array of potential biologically targeted treatments, allowing the management of IBD to enter a new era at the beginning of the 1990s with the development of new biological therapies selectively blocking the inflammatory cascade. Although mouse models of IBD have been useful to identify appropriate candidates, it is only through further trials in human disease that these targets can be validated.

It is increasingly becoming accepted that immunogenetics plays an important role in the predisposition, modulation and perpetuation of IBD. The major mission of the systemic immune system is to recognize foreign molecules and neutralize them. However, the mucosal immune system, being in constant proximity with the intestinal luminal contents, must constantly discriminate between those antigens that are potentially harmful from those that are not. The initial immune response against an invading pathogen is inflammation, with the recruitment of the appropriate inflammatory cytokines and cells, which after serving their purpose, are rapidly "down-regulated". In IBD, this initial immune response may be appropriate, but it is not down-regulated, leading to chronic, uncontrolled intestinal inflammation.¹

Under normal situations, the intestinal mucosa is in a state of "controlled" inflammation regulated by a delicate balance between proinflammatory (TNF- α , INF- γ ,

IL-1, IL-6, IL-12) and anti-inflammatory cytokines (IL-4, IL-10, IL-11). In individuals who are genetically predisposed to IBD, the immune response to environmental agents that are usually innocuous, causes either an excessive inflammatory (Thelper-1) response, or a lack of an appropriate anti-inflammatory T regulatory response. Not surprisingly though, and having in mind that the conventional treatment for IBD has focused on non-specific suppression of the inflammatory process and in parallel has its limitations in both efficacy and safety, the inflammatory and anti-inflammatory pathways have been thoroughly explored as specific targets for biological therapies in IBD that can favorably alter this inappropriate balance in the intestinal immune system.

The **biological therapies** being investigated for the treatment of IBD are predominantly proteins usually delivered intravenously or subcutaneously and encompass agents with diverse modes of action, including:

- a) native biologic preparations and isolates such as blood products and vaccines containing live, attenuated or killed microorganisms.
- b) recombinant peptides or proteins such as growth hormone, erythropoietin, etc.
- c) antibody-based therapies.
- d) nucleic acid-based therapies.
- e) cell and genes therapies.

Therapeutic proteins under study include recombinant human proteins, such as cytokines and growth factors, and monoclonal antibodies. Monoclonal antibodies are classified based on their target molecule of inhibition and the murine content that comprises the antibody structure.² The original biologic products were fully murine antibodies, but using genetic engineering technology the mouse component of the antibody structure has been reduced significantly. Chimeric antibodies (eg, infliximab) contain murine components only in the anti-

Gastroenterology Department, "Agia Olga" Hospital, Athens

Authors for correspondence:

Nicoletta Mathou, Agia Olga Hospital, 3-5 Agias Olgas str.,
142 33 Athens, Greece, Tel.: +210-2779293,
e-mail: nicolettamathou@hotmail.com

gen binding variable region of the antibody (25%), with the constant region being fully human (75%). A humanized antibody (eg, CDP571) is 95% human protein. Finally, a fully human monoclonal antibody (eg, adalimumab) can now be generated in transgenic mice.

In addition to monoclonal antibody technology, another molecular approach to neutralize an inflammatory protein is through the creation of a receptor fusion protein. With this approach the actual cell surface receptor that binds to a protein, such as TNF, is fused to the constant region of an immunoglobulin molecule (eg, etanercept).

The currently available and under investigation biological agents for IBD are summarized in *table 1*.¹⁴⁻¹⁸

Table 1. Biological agents for IBD

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| 1. Proinflammatory cytokines inhibitors |
| Anti-TNF monoclonal antibodies: infliximab, CDP571, CDP870, adalimumab |
| Soluble TNF receptors: etanercept, oncept |
| Anti-IL-6 receptor antibody |
| Anti-IL-12 monoclonal antibody |
| Anti-IFN- γ monoclonal antibody: HuZAF |
| 2. Anti-inflammatory cytokine mediators |
| IL-10, IL-11 |
| Recombinant IL-10: rHuIL-10 |
| Ex vivo IL-10 gene transfer to vectors |
| 3. Adhesion molecule inhibitors |
| Anti- $\alpha 4$ integrin monoclonal antibody: natalizumab |
| Anti- $\alpha 4\beta 7$ integrin monoclonal antibody: LDP-02 |
| Antisense oligonucleotide to intercellular adhesion molecule -1: ISIS 2302 |
| 4. T-cell inhibitors |
| Cyclosporine A |
| Anti-CD3 monoclonal antibody: visilizumab |
| Anti-CD25 monoclonal antibodies: daclizumab, basiliximab |
| 5. Cell-based therapies |
| Extracorporeal photoimmunotherapy |
| Adsorption apheresis |
| Autologous stem cell bone marrow transplantation |
| 6. Signal transduction inhibitors |
| P38 mitogen-activated protein kinase inhibitor: CNI-1493 |
| 7. Transcription factor inhibitors |
| Antisense oligonucleotide to P65 nuclear factor-K β |
| 8. Hematopoietic growth factors |
| Granulocyte-macrophage colony-stimulating factor: sargramostim |
| Granulocyte colony-stimulating factor: filgrastim |

PROINFLAMMATORY CYTOKINE INHIBITORS

Anti TNF therapies

TNF has received considerable attention as a therapeutic target in IBD. TNF plays a central role in the innate immune system's response against viral and bacterial infections and it represents an early and pivotal mediator of inflammation.

Infliximab

Among the various biologic agents, infliximab has received the most attention and is the only biologic agent approved in the US and Europe for the treatment and maintenance of the remission of patients with moderate to severe Crohn's disease unresponsive to conventional therapy, and for patients with actively draining fistulas.

Infliximab is a chimeric IgG1 monoclonal antibody, binding with high affinity to soluble and transmembrane TNF.

The first randomized placebo-controlled trial of infliximab focused on response rates after a single-blind infusion. A decrease in the CDAI of >70 points was observed in 65% of the patients treated with infliximab, compared with a 17% response in the placebo group, with the highest response rate at the dose of 5mg/kg (81%)(3).

Patients who maintained their response 8 weeks after being treated were re-randomized at week 12 to placebo or infliximab every 8 weeks for four additional infusions. A significant difference was seen in the rate of remission at week 44 (52,9% infliximab vs 20% placebo; $p=0.013$), suggesting that repeated doses of infliximab may provide useful maintenance therapy.

These results were confirmed in a large study (AC-CENT I) in which 573 patients with a CDAI score of at least 220 were enrolled.⁴

Infliximab has been shown additionally to heal endoscopic lesions in Crohn's disease patients, unlike earlier studies on corticosteroids.⁵

To establish whether infliximab is an effective maintenance therapy for patients with fistulae, a double-blind, randomized, placebo-controlled, multicentre trial was performed in 306 Crohn's patients with actively draining fistulae of at least 3 months duration. At the end of the study, the time to loss of response was significantly longer for patients received infliximab than those who received placebo (>40 vs 14 weeks; $p<0,001$).⁶

Initial studies on the use of infliximab in ulcerative colitis were scarce with conflicting data. Definite evidence for the role of infliximab in the treatment of ulcerative colitis has been recently offered by the two large placebo-controlled clinical trials ACT 1 and 2.

In the ACT 1 study, 364 patients with active ulcerative colitis despite use of steroids/azathioprine/mercaptopurine were randomized to receive placebo or infliximab 5 or 10mg/kg. Overall, infliximab was shown to be effective in treating active ulcerative colitis by reducing signs and symptoms, inducing remission, attaining mucosal healing and facilitating corticosteroid withdrawal compared with placebo.

In the ACT 2 study, 364 patients with ulcerative colitis, refractory to at least one standard therapy including mesalazine, corticosteroids or immunosuppressants were also randomized to receive infliximab 5 or 10 mg/kg or placebo at weeks 0, 2, 6, 14, 22. As a conclusion, in patients with moderate-to-severe ulcerative colitis, infliximab induces and maintains clinical response, clinical remission and mucosal healing, and permits the tapering of corticosteroids while maintaining remission.

Although well tolerated, serious adverse effects may rarely occur, including serious infections, drug-induced lupus acute infusion reactions, delayed hypersensitivity reactions, demyelination, and possibly an increased risk of lymphoma, cardiac failure and death. As far as tuberculosis is concerned, reactivation of latent tuberculosis is a severe complication with all anti-TNF strategies. Due to murine elements inducing immunogenicity, in a prospective study 61% of 125 infliximab-treated patients, developed antibodies to infliximab (ATI) after the fifth infusion. It is therefore recommended that infliximab-treated patients should receive a concomitant immunosuppressive agent to reduce the risk of ATI formation.^{7,8}

Finally, there have been several attempts to explain the lack of response observed in about 30% of patients. In a study involving 226 Crohn's patients, the response rate was significantly higher in patients with elevated rather than with normal CRP before-treatment values (76% vs 46%; $p=0.004$), suggesting that response was associated with higher systemic inflammation. To date, the only replicated predictor of response has been the concomitant use of an immunosuppressive.^{9,10}

CDP 571

CDP 571 is a humanized IgG4 anti-TNF α antibody. Four placebo-controlled trials have been reported in CD. Although initial studies suggested clinical responses and

steroid-sparing effects, significant differences in remission and maintenance of remission compared with placebo were not documented. Anti-idiotypic antibodies against the humanized antibody were detected in 5,3% of the patients receiving CDP 571, illustrating the concept that immunogenicity is not completely eliminated with newer-generation monoclonal antibodies. Further clinical development of CDP 571 for the treatment of Crohn's disease has been discontinued.

CDP 870 (Certolizumab Pegol)

CDP 870 is a Fab fragment of a humanized anti-TNF monoclonal antibody linked to polyethylene glycol molecule that increases its biological half-life. A placebo-controlled phase II trial of its subcutaneous use at doses of 100, 200 and 400mg showed significant short-term benefits at 2 weeks in patients with active Crohn's disease, but the difference was not sustained at 12 weeks. These preliminary results revealed only modest short-term effects, with a more pronounced effect in those with elevated CRP. Recently published data (PRECISE 2) showed efficacy and satisfactory tolerability of the drug at 26 weeks of follow up, independently of the CRP levels.¹¹ Further studies are under way.

Adalimumab

Adalimumab is a fully human IgG1 anti-TNF monoclonal antibody, which was recently approved by the FDA for the treatment of rheumatoid arthritis. Phase II/III trials of adalimumab for the induction and maintenance of remission in CD patients are ongoing and the preliminary results demonstrate efficacy at four weeks with loading doses of 160mg followed by 80mg, subcutaneously similar to those of infliximab. In addition, adalimumab was tolerated well in patients who lost their response to infliximab. Larger, phase III trials are underway to assess the long-term response to this agent in active CD.^{12,13}

Etanercept

TNF exerts proinflammatory effects by binding to two specific transmembrane receptors, p55 and p75. Etanercept is a human fusion protein formed by linking an Fc portion of an IgG1 human antibody to two human soluble p75 TNF receptors. It neutralizes the biologic activity of TNF by binding to soluble and transmembrane-bound TNF and prevent TNF from binding to the native TNF receptors. In contrast to its efficacy for rheumatoid arthritis, etanercept has not been found to be effective for the induction of remission in patients with active CD, although well tolerated at a dose of 25mg twice weekly subcutaneously.

Onercept

Onercept is a recombinant form of human soluble p55 TNF-receptor, neutralizing the biologic activity of TNF. A pilot study of onercept in patients with active CD showed a benefit at higher doses but final results of larger clinical trials were disappointing and the agent is no longer under development for CD.

Inhibitors of Proinflammatory Cytokine Receptors

Anti IL-6 receptor (IL-6R)

IL-6 is a cytokine with a central role in immune regulation, inflammation, and hematopoiesis. Increased serum concentrations of IL-6 and soluble IL-6 receptor (IL-6R) have been correlated to clinical activity of CD. A humanized monoclonal antibody against IL-6R (tocilizumab, MRA) has been developed and its efficacy in the treatment of active CD was investigated in a randomized to placebo trial. The response and the remission rates in the MRA biweekly infusion group were significantly higher than in the placebo group. These preliminary data need further confirmation from larger trials.

Inhibitors of Th 1 Polarisation

The biologic agents inhibiting Th1 polarisation in patients with IBD include monoclonal antibodies to IL-2, IFN γ , IL-18 and IL-12.

Daclizumab, a recombinant humanized IgG1 monoclonal antibody to IL-2R was investigated in an open-label pilot study in patients with refractory ulcerative colitis with beneficial results. **Basiliximab**, a chimeric monoclonal anti IL-2R antibody, was also evaluated, as a steroid-sensitizing agent in steroid-resistant UC, in a small study with encouraging results. Further placebo controlled trials are ongoing.

IL-12 is the key cytokine driving Th1 differentiation from precursor T-helper peripheral blood lymphocytes during the treatment period. A recently reported study was significant in active CD.

IFN γ is a key cytokine that enhances the Th1-mediated immune response while suppressing the proliferation of Th2 cells. Further clinical studies are needed to confirm the safety and efficacy of the humanized anti-IFN monoclonal antibody (HuZAF) in the treatment of CD.

IL-18 plays a role in the development of Th1 immune response by promoting Th1 differentiation. A monoclonal antibody has been developed, and human studies are awaited.

ANTI-INFLAMMATORY CYTOKINE MEDIATORS

IL-10 is an anti-inflammatory cytokine that down-regulates the production of proinflammatory cytokines and is elevated in the serum and intestinal mucosa of patients with active CD and UC. The results of three phase III trials of parenteral use were disappointing. However rectal administration improves colitis in animals and further research in humans is needed.

IL-11 attenuates the inflammatory response by inhibiting the expression of NFK-B and in turn IL-1, TNF and other proinflammatory peptides. In trials a greater proportion of patients achieved remission compared to placebo but the appearance of thrombocytosis is of concern, given that CD is associated with a prothrombotic tendency. Oral and rectal administration is under investigation.

ADHESION MOLECULE INHIBITORS

The recruitment of leukocytes from the blood into the tissue is regulated by sequential engagement of adhesion molecules on leukocytes with signaling molecules on endothelial cells. The integrins are a family of cell-surface glycoproteins that act as adhesion molecules.

Natalizumab is an IgG4 humanized monoclonal antibody to $\alpha 4$ integrin. In a large phase II study natalizumab provided short-term efficacy in patients with moderate to severe CD with an optimal dose of two infusions at 3mg/kg four weeks apart. The results of a maintenance study with natalizumab demonstrated also a highly significant maintenance benefit. Recently, the ability to taper oral corticosteroids in patients treated with natalizumab was evaluated in a randomized double-control trial. Natalizumab seems to be associated with steroids sparing effects in patients with CD. Intravenous natalizumab showed also some evidence of clinical benefit at 2 weeks post infusion in patients with active ulcerative colitis. Clinical trials in IBD are on hold at the moment, because of a recent report of polymyeloleukodystrophy in two patients receiving concomitant natalizumab and INF β , the drug which was recently marketed for multiple sclerosis and has been taken off the market.

MLN-02 is a humanized IgG1 monoclonal antibody to $\alpha 4\beta 7$ integrin which selectively inhibits leukocyte adhesion in the gastrointestinal mucosa. The drug is investigated in phase II clinical trials in patients with active CD and moderately severe UC.

The interaction of lymphocyte-associated $\alpha 1\beta 2$ -integrin, also known as leukocyte function antigen (LFA)-

1, and its ligand, ICAM-1, is important for the recruitment of leukocytes to inflammatory sites. ISIS 2302 (alicaforfen) results in a reduction in ICAM-1 protein expression. Trials evaluating higher doses of alicaforsen are underway and it is likely that the eventual development of anti-LFA-1 antibodies will be evaluated for treatment of IBD. A recent trial of enema administration of ISIS 2302 was reported to have been positive.

INHIBITORS OF T CELL ACTIVATION

CD40 ligand (CD40L) is an important co-stimulatory molecule involved in T-cell activation. A humanized monoclonal antibody (toralizumab) has been developed but studies have been halted because of concerns about the risk of thromboembolism.

Anti-CD4 antibodies have been used in a variety of autoimmune diseases, and have been tested in CD and UC. In both cases patients achieved clinical and endoscopic improvement but because of concerns of CD4 lymphopenia no further studies have been performed.

Visilizumab is a humanized antibody to CD3 that has been shown to selectively induce apoptosis in activated T cells. Initial positive results were confirmed by preliminary results of an ongoing phase I/II study aiming to evaluate the efficacy and safety of visilizumab in patients with severe steroid-refractory UC.

GROWTH HORMONE AND GROWTH FACTORS

Human growth hormone and a variety of growth factors may play an important role in IBD because of their potential use to heal and restore mucosal integrity.

The rationale for the use of growth hormone in CD is to reverse the catabolic process associated with inflammation. Initial results were promising but more controlled trials using the more conventional endpoints of improvement and remission are required.

Keratinocyte Growth Factors (KGF) and Epidermal Growth Factors (EGF) have been used in UC patients with no definite results.

Sargramostatin (recombinant human granulocyte-macrophage colony-stimulating factor) and filgrastim (recombinant human granulocyte colony-stimulating factor) are haematopoietic growth factors that stimulate cells of the innate immune system and have been shown to be effective in the treatment of genetic syndromes resulting in neutrophil dysfunction and chronic granulo-

matous diseases. Phase II trials were performed in CD and it was suggested that both factors may be of benefit in patients with active and fistulising CD, possibly via an immunostimulant effect on neutrophils.

Conclusions

Infliximab is currently the only biologic agent approved for the treatment of inflammatory and fistulising Crohn's disease. However, ongoing research continues to generate new biologic agents targeted at specific pathogenic mechanisms involved in the inflammatory process. These will have to be tested in human disease to learn who are the likely candidates for this therapy, when is the best time for such interventions, and what combination of treatments and what schedule of administration is likely to produce the ultimate benefit with minimal toxicity. It is incumbent on clinicians to have a basic understanding of immunologic mechanisms and follow the clinical evidence on biologic therapies as it emerges.

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