

## New Therapeutic Advances in Inflammatory Bowel Diseases

Daniel Rachmilewitz, M.D.

The biologic approach to IBD therapy has developed in recent years as a result of a better understanding of specific immunopathological processes in intestinal inflammation

Advances in the development of biologic drugs were the result of two major findings, in basic research.

1) The ability to dissect immunopathologies in the intestinal mucosa up to the level of single molecules. The best example of such progress is the generation of sophisticated experimental models of inflammatory bowel disease such as. Knock out laboratory and transgenic animals in which experimental colitis is exacerbated or ameliorated because of the lack or over expression of a single gene. Treatment strategies to decrease/neutralize or increase the concentration or effect of the protein encoded by that gene can be performed. 2) Advances in biotechnology now enable the insertion of genes into viral vectors so that targeted delivery of cytokines is possible, antisense oligonucleotide can be designed to hybridize with target RNA's thus the expression of specific molecules can be decreased, commercial amounts of growth factors generated and humanized antibodies creating less immunogenicity can be engineered.

There are several categories of biologic therapies that are relevant to IBD.

1) Monoclonal antibodies, receptor fusion proteins and soluble receptor antagonists. 2) Recombinant cytokines. 3) Nucleic acid based therapies. 4) Hormones and growth factors. 5) Gene therapy. 6) Cell neutralization strategies, leukocytapheresis.

### **Neutralizing proinflammatory cytokines:**

**Infliximab:** The most effective biologic therapy currently approved for commercial use is Infliximab, the chimeric monoclonal antibody targeting TNF- $\alpha$ . Infliximab is effective for induction of clinical response and remission in patients with active luminal inflammatory CD in patients with draining enterocutaneous and perianal fistulas and for subsequent maintenance of remission. Clinical remission rates at week 4 in patients with active CD after a single infusion were 4% placebo and 48% infliximab 5mg/kg. The complete fistula closure rates in patients with CD and draining fistulas after three induction infusions at weeks 0, 2, and 6 were 13% for placebo and 55% for infliximab 5mg/kg.. Maintenance of clinical remission rates at one year in patients with inflammatory CD who have responded to induction therapy with infliximab were 9% for placebo every eight weeks, 24% for infliximab 5mg/kg every eight weeks and 32% for infliximab 10mg/kg every eight weeks. Maintenance of complete fistula closure rates at one year were 58% for infliximab 5mg/kg every eight weeks and 38% for placebo every eight weeks.

Two pilot controlled trials of infliximab for severe steroid refractory UC have been performed, one with a positive result and one with a negative result. Two large phase 3 trials of infliximab in patients with active UC are underway.

Triggered by the success of anti-TNF- $\alpha$  neutralizing strategy for the treatment of active CD, various pro-inflammatory cytokines believed to be significant in intestinal inflammation were chosen as targets for neutralization by monoclonal antibodies, a relatively simple strategy where the potential major problem in general is the immunogenicity of the introduced antibodies.

**Monoclonal antibodies:****IFN- $\gamma$ - neutralizing antibodies:**

**Fontolizumab:** The rationale of treating CD with interferon- $\gamma$  neutralizing antibodies is its central role in TH1 polarization, its increased secretion by CD mucosa and the role that it may have in dysregulating the mucosal immune response, as well as the regression of experimental colitis when IFN- $\gamma$  is neutralized. Fontolizumab is an anti-IFN- $\gamma$  monoclonal Ab humanized through recombinant DNA technology. A preliminary report demonstrated an advantage over placebo in patients with high CRP.

**Boosting immunoregulatory cytokines:**

**IL-10:** This is a regulatory cytokine with anti-inflammatory properties, which down regulates the production of Th1 cytokines, mainly IL-2 and IFN $\gamma$ . Systemic administration of IL-10 seemed to be beneficial in early studies however, larger-scale studies failed to show such an effect. Recently, sophisticated local delivery systems enable high IL-10 concentration in the intestinal mucosa without high systemic exposure thus modulating inflammation locally and avoiding systemic side effects. Whether local IL-10 treatment will be effective in human disease remains to be determined.

Small trials were conducted with IL-11 (oprelvekin) IFN- $\alpha$ , IFN- $\beta$  and recombinant IFN- $\beta$ Ia and with antibodies against T lymphocytes, but none was found to have a significant effect.

**Anti-Adhesion molecules:** Selective adhesion-molecules blockade is a novel promising strategy in the therapy of IBD. It is aimed at interfering with the recruitment and migration of inflammatory cells to the intestinal mucosa. The relative specificity of the interference is possible due to the recognition of recruitment processes that are unique to the gut-associated-lymphoid-tissue

**Blocking the  $\alpha$ 4-integrin pathway:****Antibodies to  $\alpha$ 4-integrins:**

**Natalizumab:** This is a humanized monoclonal antibody to alpha 4-integrin, in which the variable region of a murine alpha 4-integrin antibody has been inserted into a human IgG4 molecule. The rationale of using alpha 4-integrin blockade in IBD is the importance of the interactions between these molecules, expressed on the surface of most leukocytes, and adhesion molecules that are up regulated at sites of chronic inflammation on vascular endothelium, e.g., VCAM-1 and MAdCAM-1, as do interactions between alpha 4-integrin and extracellular matrix molecules within the inflamed tissue. In a pilot study 30 patients with active Crohn's disease received a 3-mg/kg infusion of natalizumab (n = 18) or placebo (n = 12). Seven (39%) natalizumab-treated patients achieved remission at week 2, compared with 1 (8%) treated with placebo.

Two phase 2 studies of intravenous natalizumab at several doses showed significant short term benefit for Natalizumab in patients with active CD. A large phase 3 study in patients with active CD failed to show a benefit for natalizumab 300mg every four weeks x3 does, primarily due to an unexpectedly high placebo response rate. Maintenance therapy with natalizumab 300mg or placebo every 4 weeks through six months demonstrated a highly significant maintenance benefit with the difference between the treatment groups at six months exceeding 30%. A pilot study of natalizumab in patients with active UC suggested clinical benefit.

**Antibodies to  $\alpha 4\beta 7$  integrins:**  $\alpha 4\beta 7$  is expressed on activated peripheral blood T and B-lymphocytes. The rationale for blocking  $\alpha 4\beta 7$  interactions in CD is its role in gut homing of memory CD4+, CD8+ and B lymphocytes where they bind to MAdCAM-1 that is expressed on gut endothelium and on microvilli.

**MLN02 (LDP02)** is a humanized monoclonal IgG1 antibody against  $\alpha 4\beta 7$ , constructed by grafting the complementary determining region of mouse anti  $\alpha 4\beta 7$  into a human IgG framework. In contrast to UC where there was a moderate response to this preparation in CD there was no significant superiority when MLN02 was compared to placebo in 185 patients with mild to moderate disease. Further studies in CD are currently performed in order to assess the efficacy, and the appropriate dose of this drug.

**Blocking the  $\beta 2$ -integrin pathway:** The rationale for interfering with  $\beta 2$ -integrin pathways in IBD is that these molecules are involved in several leukocyte-vascular/leukocyte-epithelial cell adhesion processes, where  $\beta 2$  integrins (such as  $\alpha L\beta 2$ -integrin (LFA-1)) that are expressed on leukocytes as well as on intestinal epithelial cells of UC and CD patients interact with ICAM-1 that is differentially expressed in CD and in UC.

**ICAM-1 antisense oligonucleotide (Alicaforsen, ISIS-2302):** This is a 20-base pair complementary nucleotide chain that hybridizes with ICAM-1 mRNA that is thus degraded by RNase-H and the message and the expression of ICAM-1 is therefore decreased. The effect of Alicaforsen, was not significantly better than placebo in treating active or steroid dependent CD (75,76). In a later study patients treated with higher doses (300-350 mg infused 3/weekly for 4 weeks) seemed to have higher serum levels and to benefit from the drug, however there was no placebo group and further details are needed in order to evaluate the significance of LFA-1/ICAM-1 blockade for the treatment of active CD.

**Growth hormone:** The rationale to use GH as a therapeutic agent in Crohn's disease is based on the experimental data: Its stimulation of growth and differentiation of small bowel mucosa, as well as on the motivation to reverse the catabolic effects associated with inflammation. 37 patients with moderate to severe CD were treated with daily s.c. injections of growth hormone or placebo for 4 months, A significantly greater decrease in CDAI was demonstrated in the treatment vs. placebo group.

**Colony stimulating factors:** A major goal of treatment for CD is suppressing an over reactive mucosal immune response. However, recently an alternative theory has been suggested emphasizing several points of similarity between CD and immunodeficiency, specifically neutrophil dysfunction states. Several uncontrolled small studies demonstrated a significant decrease in CDAI in patients with inflammatory, as well as fistulous CD that were treated with G-CSF (filgrastim) and GM-CSF (sargamostim).

**Extracorporeal immunomodulation:** Early reports on the benefit of lymphocyte apheresis to treat CD were published at the late 1980's, describing selective extracorporeal T cell depletion by ultracentrifugation in 54 patients with active CD, the majority of whom went into long-lasting remission and decreased steroid use to zero. Two strategies that decrease specific lymphocyte subpopulations were developed in Japan. One technique is a leukocyte removal filter column-Cellsorba™ (Asahi Medical, Tokyo, Japan). All leukocyte subpopulations-99% of granulocytes and macrophages and 40-60% of lymphocytes as well as platelets are trapped within the filter made of polyester fibers. In preliminary studies in CD an intensive induction protocol-leukocytapheresis every week for 5 weeks induced remission in 50% of the

patients. An alternative leukocytapheresis method - Adacolumn<sup>®</sup> (Japan immunoresearch laboratories, Takasaki, Japan)- uses a column adsorbing granulocytes and monocytes/macrophages to 2-mm in diameter of cellulose acetate beads, without significantly adsorbing lymphocytes. Several trials demonstrated a positive effect of the column in active UC.

**Probiotics:** The rationale for using probiotics in IBD is based on evidence implicating enteric bacteria in the pathogenesis of various models of murine colitis and IBD in humans. Probiotic therapy has been effective for the attenuation of experimental colitis, prevention of pouchitis; maintenance of pouchitis; and maintenance of remission of pouchitis, Crohn's disease, and ulcerative colitis. Recently it was shown that the beneficial effect of probiotics is mediated by their DNA and that Toll R-9 signaling is essential in mediating their anti-inflammatory effect.

**Modulation of IBD with immunostimulatory DNA sequences:** Immunostimulatory DNA sequences - ISS-DNA, also known as CpG DNA, are unmethylated CpG dinucleotides within consensus sequences present in bacterial and viral genomes. ISS-DNA and their synthetic analogues activate innate immunity via Toll R-9 receptors. ISS-ODN was shown to prevent and ameliorate the severity of colitis in several different animal models and therefore may be effective also in the treatment of human IBD. Clinical trials to test their efficacy are underway.

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