Treatment of Crohn's disease with colony-stimulating factors: An overview

Luisa Guidi[1]
Giammarco Mocci[1]
Manuela Marzo[1]
Sergio Rutella[2]

[1]Department of Internal Medicine, Operative Unit of Gastroenterology, and [2]Department of Hematology, Laboratory of Immunology, Università Cattolica del Sacro Cuore, Rome, Italy

Abstract: Current treatments for Crohn's disease are aimed at suppressing excessive immune activation in the bowel walls. However, alternative strategies can be drawn. These involve the augmentation of the innate immune response, in the hypothesis that patients affected with Crohn's disease are characterized by a relative immunodeficiency, with failure of the defensive barrier to luminal microbes and microbial products, resulting in a chronic inflammatory process sustained by T-cells. Alternatively, therapy could act by enhancing the number or the activity of subpopulations of T regulatory cells, able to reduce T-cell activation. Colony-stimulating factors are substances that could be efficacious in these settings. In fact, besides in vitro and animal studies, some human studies have been conducted in recent years with both granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor, the results of which are reported here.

Keywords: inflammatory bowel disease, Crohn's disease treatment, G-CSF, GM-CSF

Introduction

Crohn's disease (CD) is a chronic inflammatory, granulomatous disorder occurring throughout the gastrointestinal tract. The cause remains unknown, although various genetic (Satsangi et al 2003) and environmental (Ekbom and Montgomery 2004) factors have been postulated.

While the fundamental etiology of CD remains unknown, the prevailing hypothesis focuses on an excessive immune reaction as the underlying problem. Defects in the interaction between innate and adaptive immune response may play a crucial role in the development of inflammation in inflammatory bowel disease (IBD).

Furthermore, a variety of T-cell defects have been observed in IBD. These include an excess of Th1 and Th17-type responses with excess of interleukin (IL)-12/IL-23 and interferon (IFN) γ/IL-17 production in CD, and defects in T-cell programmed cell death (apoptosis) (Brown and Mayer 2007). Furthermore, defects in regulatory T-cell function have been hypothesized in IBD. All of this modifies immune tolerance and predisposes to intestinal inflammation. In IBD there is a breakdown in mucosal tolerance.

The aim of this review is to describe the results of experimental and clinical trials of treatments with granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF), directed at augmenting the intestinal innate immune defense rather than suppressing a secondary inflammatory response (Korzenik and Dieckgraefe 2000; Podolsky 2002; Wilk and Viney 2002) and possibly to improve T regulatory cell activity which may be effective in CD.

Medical treatment of IBD

The aims of medical treatment in IBD are: modification of the microbial environment to remove the antigenic drive; modification of the immune response, including inhibition

Correspondence: Luisa Guidi
Università Cattolica del Sacro Cuore, Istituto di Medicina Interna e Geriatria, Largo A. Gemelli, 8, 00168, Roma, Italia
Tel +39 6350 3239
Fax +39 6305 4641
Email lguidi@rm.unicatt.it

of the expression, synthesis or function of proinflammatory cytokines; increasing the activity of anti-inflammatory cytokines; inhibiting the proliferation of inflammatory cells and their recruitment into the intestine.

In facts, current treatments for CD are aimed to suppress the immune system to restore health. Established medical strategies for the treatment of CD include the use of immunosuppressive agents such as corticosteroids, azathioprine or 6-mercaptopurine, methotrexate.

Recent therapeutic innovations, such as anti-TNF-α antibodies like infliximab and adalimumab, have been designed to be more selective, interfering with specific elements of the inflammatory cascade.

Several other so called "biologic therapies" are currently under study, among which are monoclonal antibodies directed against several cytokines and receptors like IL-12/IL-23, IFNγ, IL-6R, CD3, or blocking leucocyte adhesion related molecules (α4-integrin, α4β7-integrin, ICAM-1). Some proposed biologic therapy are aimed to stimulate anti-inflammatory pathways (rhuIL-10, rhuIL-11, CTLA4-Ig, CD40L) or to inhibit signal transduction through MAP-kinase.

Colony-stimulating factors

Growth factors have recently emerged as potential tools for the modulation of intestinal inflammation and repair. At least 30 different growth factors are relevant for the maintenance of gut mucosal integrity, including transforming growth factor beta (TGF-beta), insulin-like growth factor (IGF), keratinocyte-like growth factor (KGF), epidermal growth factor (EGF), growth hormone (GH), and the colony-stimulating factors (GM-CSF, G-CSF, and macrophage colony-stimulating factor [M-CSF]) (Dignass and Sturm 2001; Playford and Ghosh 2005). Each of these regulatory peptide families plays an important role in the modulation of cellular proliferation, differentiation, angiogenesis, and inflammation; moreover, they serve an important function as messengers between the intestinal mucosa, enteric nervous system, and immune system. Although the hematopoietic tissues are the predominant site of action for these peptides, they are produced throughout the body, including the intestine, and are also produced by constituents of the lamina propria (primarily macrophages or monocytes) (Dignass and Sturm 2001).

The greatest body of evidence supporting the use of colony-stimulating factors in intestinal inflammation comes from studies conducted with GM-CSF and G-CSF in patients with CD.

In this framework, two possible pathogenetic mechanisms support the therapeutic use of agents like growth factors in IBD: the "innate immunodeficiency" hypothesis and the "T regulatory cell defect" hypothesis.

Innate immunodeficiency in IBD

The innate immune network represents the fist line response to microbial infections, and has a crucial relevance in the gut, where the lining mucosa has to face the enormous load of the intestinal microbiota. Several cell types respond to bacteria in a way independent from prior antigen exposure: these are cells coming from the bloodstream (neutrophils, monocytes) or resident in the gut (dendritic and Paneth cells, intestinal epithelial cells with their range of secreted antimicrobic peptides). The hypothesis that has recently been focused is that CD patients may possess a diminished initial inflammatory response (Korzenik and Dieckgraefe 2000). Specifically, CD may result from defective functioning of intestinal innate immune defense. Breakdown of this defensive barrier may permit persistent exposure of lamina propria cells to luminal microbes and microbial products, resulting in an aberrant, chronic inflammatory process mediated by T-cells.

Defects of several components of the innate immune network have been described in CD, which may drive the response towards an excessive inflammation sustained by the adaptive immune response network. Polymorphisms have been described in CD patients for some toll-like receptors (TLR) expressed on monocytes/macrophages, dendritic and epithelial cells and for NOD molecules, both implied in recognizing pathogen-associated molecular patterns. The role of the innate immune system in the pathogenesis of IBD has been recently emphasized after the identification of the NOD2/CARD15 gene, expressed in macrophages and Paneth cells as a disease susceptibility gene (Hugot et al 2001; Ogura et al 2001). CARD-15 variants in CD are associated with modifications in nuclear factor-κB activation, leading to an inappropriate immune response to the muramyl dipeptide component of bacteria. This, and other unknown mechanisms, may lead to activation of the adaptive immune response. The interpretation of these genetic defects, however, is not fully understood. NOD2 mutations have been interpreted both as being of "gain of function" type, leading to uncontrolled inflammation, as well as of "loss of function" type, in that case allowing the penetration of microbes in the gut mucosa, ie, lowering the threshold of defence from infection.

Interestingly, Crohn's-like intestinal disease has been identified in patients with genetic disease, such as chronic

granulomatous disease (GCD), glycogen storage disease 1b (GSD-1b), and cyclic neutropenia, in which a variety of qualitative or quantitative neutrophil deficiencies or other defects in innate immunity have been well characterized, have a therapeutic benefit of recombinant human granulocyte colony-stimulating factor (rhuG-CSF) and recombinant human granulocyte/macrophage colony-stimulating factor (rhuGM-CSF). Several cases of GSD-1b patients with an IBD indistinguishable from idiopathic CD have been described. These patients have severe neutropenia and impairment of neutrophils function. Yamaguchi and colleagues (2001) reported a case of GSD-1b with IBD-like colitis, who improved after G-CSF treatment, and reviewed ten cases from the literature, five of which were treated with G-CSF or GM-CSF and went on clinical remission of their IBD. Successively a series of 36 patients with GSD-1b was described (Dieckgraefe et al 2002). In this series, 75% of patients had gastrointestinal symptoms, 28% documented IBD and 22% had highly suggestive diagnosis of IBD, although diagnostics was not completed. Again, gastrointestinal symptoms improved in the patients treated with G-CSF. Similarly, in another series 57 cases of GSD-1b (Visser et al 2002), 18 were treated with G-CSF, showing a reduction in the number of infections and an improvement in the severity of their IBD, when present. In the nine patients treated for more than one year, an increase in neutrophil count was described.

Recent studies have reinforced the hypothesis of an innate immune deficit in patients with Crohn's disease: in a study concerning acute inflammation, Marks and colleagues (2006) have shown that in CD patients, trauma to rectum, ileum or skin led to abnormally low neutrophil accumulation, IL-8 and IL-1β production, as compared to normal subjects or other inflammatory conditions. Furthermore, Harbord and colleagues (2006) have examined the composition of exudate in patients with CD and ulcerative colitis (UC), and assessed the effect of G-CSF administration on tissue penetration of neutrophils in patients. In this study, neutrophil and monocyte/macrophage populations and inflammatory mediators were measured in cantharidin blisters at 24 h. Neutrophil chemotaxis was assessed in vitro using blister fluid as the chemoattractant. Significantly fewer neutrophils migrated into blisters in Crohn's patients. The production of neutrophil chemokines, but not other inflammatory mediators, was reduced. This correlated with reduced chemotaxis in vitro. The administration of two subcutaneous injections of G-CSF (5 μg/kg) significantly increased blister neutrophil concentrations in control subjects and Crohn's patients.

Immunodeficiency states are by definition associated with infections. Immune inadequacy and infections are the two sides of the same coin. Recently the interaction between IBD and infection has been reviewed (Irwing and Gibson 2008), both at the etiopathogenetic level and during the clinical course of the disease. These observations are the premises to the use of immune-enhancing drugs in IBD.

T regulatory cell defect in IBD

The immune system protects a host from pathogens, distinguishes self from non-self structures and prevents nonessential and self-destructive immune responses through mechanisms of central and peripheral tolerance (Danese and Rutella 2007). Tolerance can be operationally defined as absence of antigen (Ag)-specific pathogenic autoimmunity or the acceptance of an allograft, attributable to lack of Ag accessibility (ignorance), absence of T-cells (deletion) or lack of sufficient activation signals (unresponsiveness). A growing body of evidence indicates that specific T-cell populations with suppressive/regulatory properties are devoted to the maintenance of Ag-specific T-cell tolerance both in mice and in humans (Jonuleit and Schmitt 2003). The family of regulatory T-cells (Treg) encompasses T-cell populations with distinct suppressive mechanisms, eg, naturally occurring $CD4^+CD25^+$ Treg cells, T helper type 3 (Th3) cells, and Treg type 1 (Tr1) cells. Naturally occurring $CD4^+CD25^+$ Treg cells develop in the thymus during T-cell maturation and survive in the periphery to prevent harmful autoimmune reactions. Adaptive Treg cells develop from mature T-cell under specific conditions of sub-optimal costimulation or Ag exposure. Although earlier studies suggested that these might be two distinct subsets, it is currently believed that the adaptive Treg cells can either develop from classical naive T-cells or differentiate from the naturally occurring $CD4^+CD25^+$ Treg cells.

In recent years, emphasis has been placed on Treg defects in chronic intestinal inflammation. Although a state of physiological and controlled mucosal inflammation exists in the normal gut, tolerance to bacterial and dietary Ags is an essential feature to maintain intestinal homeostasis. To regulate such equilibrium, functionally distinct T-cell subsets expressing a regulatory phenotype exist in the intestine and down-regulate immune responses.

In a mouse model of IBD obtained by transfer of naïve T-cells in immunodeficient animals the onset of colitis can be prevented by co-transfer of $CD4^+CD25^+$ Treg cells (Read et al 2000) and these cells can also cure the intestinal

inflammation in these mice (Mottet et al 2003). Co-transfer of other Treg populations, among which Tr1, can prevent intestinal inflammation the SCID transfer model (Allez and Mayer 2004).

A previously unrecognised role of G-CSF in the in vivo generation of human Tr1 cells has been shown (Rutella et al 2002; Rutella 2007). These in vitro findings have been backed by in vivo studies demonstrating Tr1-mediated protection from graft-versus-host disease in a mouse model of G-CSF-mobilized allogeneic stem cell transplantation (Morris et al 2004).

Preclinical studies of G-CSF and GM-CSF in CD

G-CSF has been used in experimental colitis in white New Zealand rabbits, where it reduced leukotriene B4 and thromboxane B2 in dialysis fluid (Hommes et al 1996). Furthermore, in another animal model of IBD (rats treated with 2,4,6-trinitrobenzene sulfonic acid) the pretreatment with G-CSF attenuated both loss of body weight and colonic wall thickening (Egi et al 2003), and this effect was associated with a significant inhibition of IFNγ and IL-12 p35 transcription.

Sainathan and collegues (2008) examined the effects of GM-CSF in the dextran sulfate sodium (DSS)-induced acute colitis models. In this study mice were treated with daily GM-CSF or phosphate-buffered saline (PBS), during colitis induction. The authors have shown that sargramostim ameliorates acute DDS-induced colitis, obtaining significant improvement of clinical and histological parameters. Furthermore, a reduction has been demonstrated in the expression of pro-inflammatory genes such TNF-α and IL-1β (Sainathan et al 2008).

Clinical studies of GM-CSF in CD

GM-CSF, a myeloid growth factor, plays a pivotal role in the development and function of phagocytic cells. GM-CSF is expressed by $CD4^+$ T-cells and Paneth cells in the intestine. Receptors for GM-CSF have been identified on intestinal epithelial cells, including Paneth cells (Fukuzawa et al 2003). Moreover, GM-CSF is expressed at high levels in those regions of the gastrointestinal tract that are associated with the greatest concentration of luminal microbial colonization. These findings suggest that GM-CSF may help maintain the function of the intestinal innate immune barrier and that exogenous GM-CSF may augment host defense and ameliorate inflammation associated with CD. Sargramostim is a recombinant version of the human GM-CSF, most commonly used for myeloid-cell recovery after chemotherapy.

In an initial pilot study, Dieckgraefe and Korzenick (2002) did an 8-week, open label, dose-escalating study of GM-CSF for treatment of active Crohn's disease. Patients with a Crohn's disease activity index (CDAI) score greater than 220 and lower than 475 were eligible for enrolment. Fifteen patients were screened and all were enrolled. The mean starting CDAI was 346 (range 228–471), which indicated a moderate-severe active CD. These patients were not receiving concomitant immunosuppressive therapy, whereas antibiotics and aminosalicylates were allowed. The primary end point was clinical response, defined as a decrease in CDAI, at the end of the treatment (day 56), of greater than 70 points from baseline, and remission, defined as an absolute CDAI of less than 150. Other end points included the health-related quality of life, evaluated with inflammatory bowel-disease questionnaire (IBDQ), and adverse events. Patients were enrolled into one of three groups, and were given sargramostim at 4, 6, or 8 μg/Kg per day. All patients completed 8 weeks of treatment. The daily self-administered injections were well tolerated. Twelve of 15 patients reported localized reactions at the site of injection, which varied from transient itching to a 2–3 cm area of induration and erythema. Reactions were generally larger at higher doses and diminished with continued administration of sargramostim. Ten patients had bone pain. A single oral dose of paracetamol before subcutaneous GM-CSF injection provided relief for most patients. Sargramostim had a dose-dependent effect on mean absolute neutrophil counts, which increased to 13.1, 18.5, and 20×10^9/L by the second week in the 4, 6, and 8 μg/kg per day groups, respectively. Mean absolute eosinophil counts increased to 4.1, 6.7, and 9.1×10^9/L by the second week in the 4, 6, and 8 μg/kg per day groups, respectively. Peak effects on absolute neutrophil count and absolute eosinophil count tended to take place between weeks 2 and 4, with slight decreases by week 8. Changes in absolute neutrophil and eosinophil counts were similar in the responder and nonresponder groups. After 8 weeks of GM-CSF treatment, 12 of 15 patients had a clinical response and 8 were in remission. Patients had a progressive weekly decrease in CDAI from a pretreatment mean score of 346 to 156 at week 8. The response rate was 75%, 85% and 75% in the 4, 6, and 8 μg/Kg per day dose groups, corresponding to a mean decrease in CDAI of 166, 216, and 169, respectively. Increased IBDQ score indicates an improved quality of life in terms of improved physical, social and emotional performance. After 8 weeks the treatment with GM-CSF

was stopped and patients were followed up clinically for evidence of increased disease activity. Of the 12 responders, one underwent elective surgical resection after cessation of treatment, one was offered retreatment at week 11, and two were enrolled at week 8 in a maintenance protocol. At week 12, the remaining 8 responders had a mean CDAI score of 205, which was significantly lower than their pretreatment baseline score of 358.

In the phase II study, Korzenik and colleagues (2005), using a 2:1 ratio, randomly assigned 124 patients with moderate to severe active CD to receive 6 μg/Kg of sargramostim per day or placebo subcutaneously for 56 days. Patients who had been taking stable doses of antibiotics or aminosalicylites for at least four weeks were eligible. Patients who had been taking azathioprine, mercaptopurine, methotrexate, or oral or rectal glucocorticoids within 4 weeks before the study began were not eligible, nor were those who had been receiving anti-TNF-α therapy within 12 weeks before the study began. Moreover, prior use of sargramostim or filgrastim (rhuG-CSF) was prohibited. Efficacy measures included changes from base line in disease severity (measured by CDAI score), mucosal healing (measured using the Crohn's Disease Endoscopic Index of Severity), and health-related quality of life (measured by IBDQ score), while safety was assessed according to the incidence of adverse events.

The primary end point was a clinical response defined by a decrease from baseline of at least 70 points in the CDAI score at the end of treatment (day 57). Prospectively defined secondary end points included a clinical response defined by a decrease of at least 100 points in the CDAI score, remission (defined by a CDAI score of 150 or less), and an increase in the IBDQ score. Of the 124 treated patients, 81 received sargramostim and 43 placebo. All demographic and disease characteristics, including prior use of therapy for CD, were similar in the two groups except for the median age and the duration of disease, which were younger and shorter, respectively, in the sargramostim group than in the placebo group. Ninety percent of patients had previously received glucocorticoids, and 69% had received immunosuppressive agents. There was no significant difference between groups in the rate of the primary end point of clinical response (54% in sargramostim group as compared with 44% in the placebo group; P = 0.28). However, there were positive secondary outcomes with regard to the end points of a decrease from baseline at least 100 points in the CDAI score and remission (48% in sargramostim group vs 26% in the placebo group, P = 0.01, and 40% vs. 19%, P = 0.01). Moreover, the time to response was significantly shorter in the sargramostim-treated group (P = 0.018), and the significant between-group differences in response and remission were maintained at the 30-day post-treatment follow-up visit. Sargramostim therapy was also associated with improvements in mucosal healing and particularly, in quality of life, and the same was for the improvements in the health-related quality of life. There was no significant difference in the overall incidence of adverse events between the sargramostim group and the placebo group (98% vs. 93%, P = 0.22). Two types of adverse advents were reported more frequently in the sargramostim group than in placebo group: injection-site reactions and bone pain.

Clinical studies of G-CSF in CD

A case report by Vaughan and Drumm (1999) described the successful treatment of fistulas with G-CSF in a CD patients.

Successively, recombinant human G-CSF (rhuG-CSF) has been administered to five patients with CD and severe endoscopic postoperative recurrence (Dejaco et al 2003). They received 300 μg rhuG-CSF subcutaneously three times weekly for 12 weeks. It was safe and well tolerated. All patients achieved clinical remission, while two of them showed also complete mucosal healing. Neutrophil counts, IL-1 receptor antagonist and soluble TNF receptors p55 and p75 plasma levels were increased during drug administration.

Korzenik and Dieckgraefe (2005) have evaluated the use of filgrastim (rhuG-CSF) with an open-labeled, 12 week trial for the treatment of active CD. In this study, twenty patients with active luminal CD and CDAI $>$ 220 and $<$ 450 were enrolled (three had perianal fistulas as well, one had enterocutaneous fistulas). Concomitant immunosuppressant were prohibited, whereas mesalazine compounds and antibiotics were permitted if used for at least 8 weeks and at stable dose for 4 weeks. Steroids were permitted if on for at least 8 weeks and with a stable dose of prednisone of $<$ 20 mg/day for at least 4 weeks. The primary end-point was a decrease in the CDAI of $>$ 70 points, whereas remission was considered to be a CDAI $<$ 150. The mean CDAI at initiation was 307 ± 54. All patients received rhuG-CSF daily for 12 weeks at an initial dose of 300 μg self-administered subcutaneously. The absolute neutrophil count (ANC) was targeted between 25 and 35×10^9/L and the dose was adjusted if patients' ANC exceeded this range. Dose reduction because of a greater than the target range was necessary in all but three patients. However, responders and non responders did not differ significantly with regard to their μg/Kg dose. Thirteen patients

completed the 12 weeks of the study; seven patients withdrew, three for worsening disease, one for an intercurrent illness, one because needed surgery, one discontinued due to a lack of improvement, and one due to a small perianal abscess at the site of a fistula. The mean CDAI at week 4 was 229 ± 98 which represent a statistically significant change compared with week 0. The mean CDAI for those completing the 12-week study was 162 ± 82. Eleven patients had a decrease of at least 70 points and eight decreased more than 100 points. Five patients were in remission. Patients were seen in follow-up 4 weeks after completion of therapy. Among the 11 responders at week 12, four maintained a response lasting for additional 4 weeks while the others had an increase in disease activity although still below baseline (mean CDAI 81 ± 64 below week 0). A response, defined as closure of more than 50% of fistulae, was demonstrated in three of four patients with fistulous disease. Adverse events were limited and transient. Most patients experienced bone pain that was minimized by a single oral dose of acetaminophen administered before injections. However, all bone pain resolved within a few weeks and with continued rhuG-CSF therapy. Interpretation of the results of this study is complicated by the issue of dosing. Ten patients had a reduction of the dose during the study. However, the dose of most individuals was decreased early on and the duration and extent of benefit may have been limited by the dose administered.

Discussion and conclusions

Improvement in gastrointestinal symptoms has been noted in patients with immunodeficiency diseases when treated with G-CSF and GM-CSF (Yamaguchi et al 2001; Dieckgraefe et al 2002; Visser et al 2002).

The results of clinical trials conducted in CD are summarized in Table 1.

GM-CSF (Dieckgraefe and Korzenik 2002) has been suggested to be beneficial in an open-labeled pilot trial in CD.

The randomized, placebo-controlled trial with sargramostim (GM-CSF) was negative as designed (Korzenik et al 2005) with no significant difference between groups in the rate of the primary end point of a clinical response defined by a decrease from baseline of at least 70 points in the CDAI score on day 57 (54% in sargramostim group, as compared with 44% in the placebo group; P = 0.28). These results might have been affected by the high rate of response in the placebo group. However, there were positive secondary outcomes with regard to the end points of a decrease from baseline of at least 100 points in the CDAI score and remission on day 57 as well as a decrease of at least 70 points in the CDAI score at other times. Clinical responses were achieved without concomitant immunosuppressive therapy, were rapid and sustained, and were associated with significant improvements in disease specific quality of life. Improvements were observed in mucosal healing in sargramostim-treated group.

For what concerns G-CSF the open-label trials have shown efficacy: 5/5 remissions and 2/5 mucosal healing in the small study on postoperative recurrence (Dejaco et al 2003); in the successive study on active CD (Korzenik and Dieckgraefe 2005), of the thirteen patients which have completed the study, eleven achieved the primary end points (decrease in the CDAI of at least 70 points from baseline) and five were in remission.

Both drugs were safe in these reports, showing more frequently minor adverse events related to the injection site and transient bone pain. However, dose reduction was necessary for most patients treated with G-CSF (Korzenik and Dieckgraefe 2005), because of excessive ANC.

Experimental and clinical studies have shown differences between filgrastim and sargramostim. The available data have suggested that GM-CSF is associated with a more wide and potent action on multiple cells, such as neutrophils, monocytes, and intestinal epithelial cells which expressed receptors for GM-CSF. On the other hand G-CSF has a more selective effect on the innate immune system, acting on neutrophils, but its action could be exerted by a modulation of T regulatory cell.

Further studies are warranted, both to establish the real efficacy of these drugs and to elucidate the modifications produced in the immune system of IBD patients by the treatment.

Points that need to be addressed by future research include the evaluation of the response to the treatment of patients with early onset of the disease, given the initial role of the innate immunity and of T regulatory cells in the models of immunopathogenesis of CD. Furthermore, these drugs could be useful for those patients having neutropenia, mainly as a consequence of treatments with azathioprine or other immunosuppressant drugs, and for those with infections and septic complications, which are particularly common in fistulizing CD. Finally, as these drugs can be considered as immune enhancer rather than suppressors, a theoretical possibility of combination with anti-TNF or other biologicals exists, and could be explored in animal models.

In conclusion, the results of the reported studies suggest that a treatment designed to modulate intestinal mucosa immune homeostasis (including innate immunity and the T regulatory network) with colony-stimulating factors such

Table 1 Clinical studies of GM-CSF and G-CSF in patients with Crohn's disease

Authors	Type of study	Drug	Number of patients	Primary end points: results	Secondary end points: results
Dieckgraefe and Korzenik 2002	Open-label	GM-CSF	15	• Clinical response (decrease in CDAI of greater than 70 points from baseline): 12 of 15 • Remission (absolute CDAI of less than 150): 8 of 15	
Korzenik et al 2005	Double-blind randomized	GM-CSF	124 (81 GM-CSF; 43 placebo)	• Clinical response (decrease in CDAI of greater than 70 points from baseline): 54% versus 44% (p = 0.28)	• Clinical response (decrease in CDAI of greater than 100 points from baseline): 48% versus 26% (p = 0.01) • Remission (absolute CDAI of less than 150): 40% versus 19% (p = 0.01)
Dejaco et al 2003	Open-label	G-CSF	5	• Clinical remission: 5 • Mucosal healing: 2 of 5	
Korzenik and Dieckgraefe 2005	Open-label	G-CSF	20 (13 completed)	• Clinical response (decrease in CDAI of greater than 70 points from baseline): 11 of 13 • Remission (CDAI < 150): 5 of 13	• Clinical response (decrease in CDAI of greater than 100 points from baseline): 8 of 13 • Closure of more than 50% of fistulas: 3 of 4

Abbreviations: CDAI, Crohn's disease activity index; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor.

as G-CSF and GM-CSF might have a role in patients with Crohn's disease.

Disclosure

The authors report no conflicts of interest in this work.

References

Allez M, Mayer L. 2004. Regulatory T-cells: peace-keepers in the gut. *Inflamm Bowel Dis*, 10:666–76.

Brown SJ, Mayer L. 2007. The immune response in inflammatory bowel disease. *Am J Gastroenterol*, 102:2058–69.

Danese S, Rutella S. 2007. The Janus face of $CD4^{+}CD25^{+}$ regulatory T-cells in cancer and autoimmunity. *Curr Med Chem*, 14:649–66.

Dejaco C, Lichtenberger C, Miehsler W, et al. 2003. An open-label pilot study of granulocyte colony-stimulating factor for the treatment of severe endoscopic postoperative recurrence in Crohn's disease. *Digestion*, 68:63–70.

Dieckgraefe BK, Korzenik JR. 2002. Treatment of active Crohn's disease with recombinant human granulocyte-macrophage colony-stimulating factor. *Lancet*, 360:1478–80.

Dieckgraefe BK, Korzenik JR, Husain A, et al. 2002. Association of glycogen storage disease 1b and Crohn disease: results of a North American survey. *Eur J Pediatr*, 161(Suppl 1):S88–92.

Dignass AU, Sturm A. 2001. Peptide growth factors in the intestine. *Eur J Gastroenterol Hepatol*, 13:763–70.

Egi H, Hayamizu K, Yoshimitzu M, et al. 2003. Regulation of T helper type-1 immunity in hapten-induced colitis by host pretreatment with granulocyte colony-stimulating factor. *Cytokine*, 23:23–30.

Ekbom A, Montgomery SM. 2004. Enviromental risk factors (excluding tobacco and microorganisms): critical analysis of old and new hypotheses. *Best Pract Res Clin Gastroenterol*, 18:497–508.

Fukuzawa H, Sawada M, Kayahara T, et al. 2003. Identification of GM-CSF in Paneth cells using single-cell RT-PCR. *Biochem Biophys Res Commun*, 312:897–902.

Harbord MW, Marks DJB, Forbes A, et al. 2006. Impaired neutrophil chemotaxis in Crohn's disease relates to reduced production of chemokines and can be augmented by granulocyte-colony-stimulating factor. *Aliment Pharmacol Ther*, 24:651–60.

Hommes DW, Meenan J, Dijkhuizen S, et al. 1996. Efficacy of recombinant granulocyte colony-stimulating factor (rhG-CSF) in experimental colitis. *Clin Exp Immunol*, 106:529–33.

Hugot JP, Chamaillard M, Zouali H, et al. 2001. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature*, 411:599–603.

Irving PM, Gibson PR. 2008. Infections and IBD. *Nat Clin Pract Gastroenterol Hepatol*, 5:18–27.

Jonuleit H, Schmitt E. 2003. The regulatory T-cell family: distinct subsets and their interrelations. *J Immunol*, 171:6323–7.

Korzenik JR, Dieckgraefe BK. 2000. Is Crohn's disease an immunodeficiency? A hypothesis suggesting possible early events in the pathogenesis of Crohn's disease. *Dig Dis Sci*, 45:1121–9.

Korzenik JR, Dieckgraefe BK. 2005. An open-labelled study of granulocyte colony-stimulating factor in the treatment of active Crohn's disease. *Aliment Pharmacol Ther*, 21:391–400.

Korzenik JR, Dieckgraefe BK, Valentine JF, et al. 2005. Sargramostim for active Crohn's disease. *N Engl J Med*, 352:2193–201.

Marks DJ, Harbord MW, MacAllister R, et al. 2006. Defective acute inflammation in Crohn's disease: a clinical investigation. *Lancet*, 367:668–78.

Morris ES, MacDonald KP, Rowe V, et al. 2004. Donor treatment with pegylated G-CSF augments the generation of IL-10 producing regulatory T-cells and promotes transplantation tolerance. *Blood*, 103:3573–81.

Mottet C, Uhlig HH, Powrie F. 2003. Cutting edge: cure of colitis by $CD4^+CD25^+$ regulatory T-cells. *J Immunol*, 170:3939–43.

Ogura Y, Bonen DK, Inohara N, et al. 2001. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature*, 411:603–6.

Playford RJ, Ghosh S. 2005. Cytokines and growth factor modulators in intestinal inflammation and repair. *J Pathol*, 205:417–25.

Podolsky DK. 2002. Inflammatory bowel disease. *N Engl J Med*, 347:417–29.

Read S, Malmstrom V, Powrie F. 2000. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of $CD25^+CD4^+$ regulatory cells that control intestinal inflammation. *J Exp Med*, 192:295–302.

Rutella S. 2007. Granulocyte colony-stimulating factor for the induction of T-cell tolerance. *Transplantation*, 84:S26–S30.

Rutella S, Pierelli L, Bonanno G, et al. 2002. Role for granulocyte colony-stimulating factor in the generation of human T regulatory type 1 cells. *Blood*, 100:2562–71.

Sainathan SK, Hanna EM, Gong Q, et al. 2008. Granulocyte macrophage colony-stimulating factor ameliorates DSS-induced experimental colitis. *Inflamm Bowel Dis*, 14:88–99.

Satsangi J, Morecroft J, Shah NB, et al. 2003. Genetics of inflammatory bowel disease: scientific and clinical implications. *Best Pract Res Clin Gastroenterol*, 17:3–18.

Vaughan D, Drumm B. 1999. Treatment of fistulas with granulocyte colony-stimulating factor in a patient with Crohn's disease. *N Engl J Med*, 340:239–40.

Visser G, Rake JP, Labrune P, et al. 2002. Granulocyte colony-stimulating factor in glycogen storage disease type 1b. Results of the European Study on Glycogen Storage Disease Type 1. *Eur J Pediatr*, 161(Suppl 1):S83–7.

Wilk JN, Viney JL. 2002. GM-CSF treatment for Crohn's disease: a stimulating new therapy? *Curr Opin Investig Drugs*, 3:1291–6.

Yamaguchi T, Ihara K, Matsumoto T, et al. 2001. Inflammatory bowel disease-like colitis in glycogen storage disease type 1b. *Inflamm Bowel Dis*, 7:128–32.