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Inflammatory bowel diseases: emerging therapies and promising molecular targets

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1. ABSTRACT

An enormous amount of pathoetiologic information continues to accrue from animal models of inflammatory bowel disease and study of the gut microbiome that is providing expanded insight into the causes and mechanisms of inflammatory bowel diseases. This knowledge is being translated into new therapeutics that are being tested in Crohn's and ulcerative colitis patients with an aim to enhance treatment responses by moving away from immunosuppression and toward immunomodulation. In the last decade, the frontier of emerging IBD therapy has been dominated by biological agents that specifically target pro-inflammatory cytokines most notably tumor necrosis factor-alpha. However, it is clear that the gaps in therapy (primary and secondary non-response and the potential for drug side effects and intolerances) continue. To fill these gaps, various approaches are being employed to develop novel strategies, from inhibiting additional pro-inflammatory cytokines to focusing on blocking inflammatory cell trafficking, decreasing inflammatory cell mass, enhancing regulatory cell function and reinforcing epithelial barrier function. To these ends, aggressive and innovative research is being pursued to develop more robust treatment strategies and identify key molecular targets.

2. INTRODUCTION

Inflammatory bowel diseases (IBD) include a spectrum of gut inflammation that results from dysregulated immune responses driven primarily by microbial antigens and pathogen-associated molecular patterns (1, 2). The majority of IBD consists of two well-established entities, Crohn's disease (CD) and ulcerative colitis (UC). It is estimated that approximately one and a half million people in the United States have IBD, and there is no significant difference in the incidence and prevalence of the condition between males and females, unlike many autoimmune inflammatory diseases. The peak age of onset is 15-25 years of age however, making the duration of disease, complications, and high rates of surgical treatment that much more burdensome for patients and their families (1, 3, 4).

The approach to IBD treatment has continued to evolve, influenced by the emerging knowledge of the pathophysiology of these inflammatory conditions. New information is leading to development and testing of novel agents and strategies and modification of existing ones (5, 6). The historic goal of treatment of IBD was to induce and maintain clinical (i.e. symptomatic) remission. However, it is becoming clear that the natural progression of the

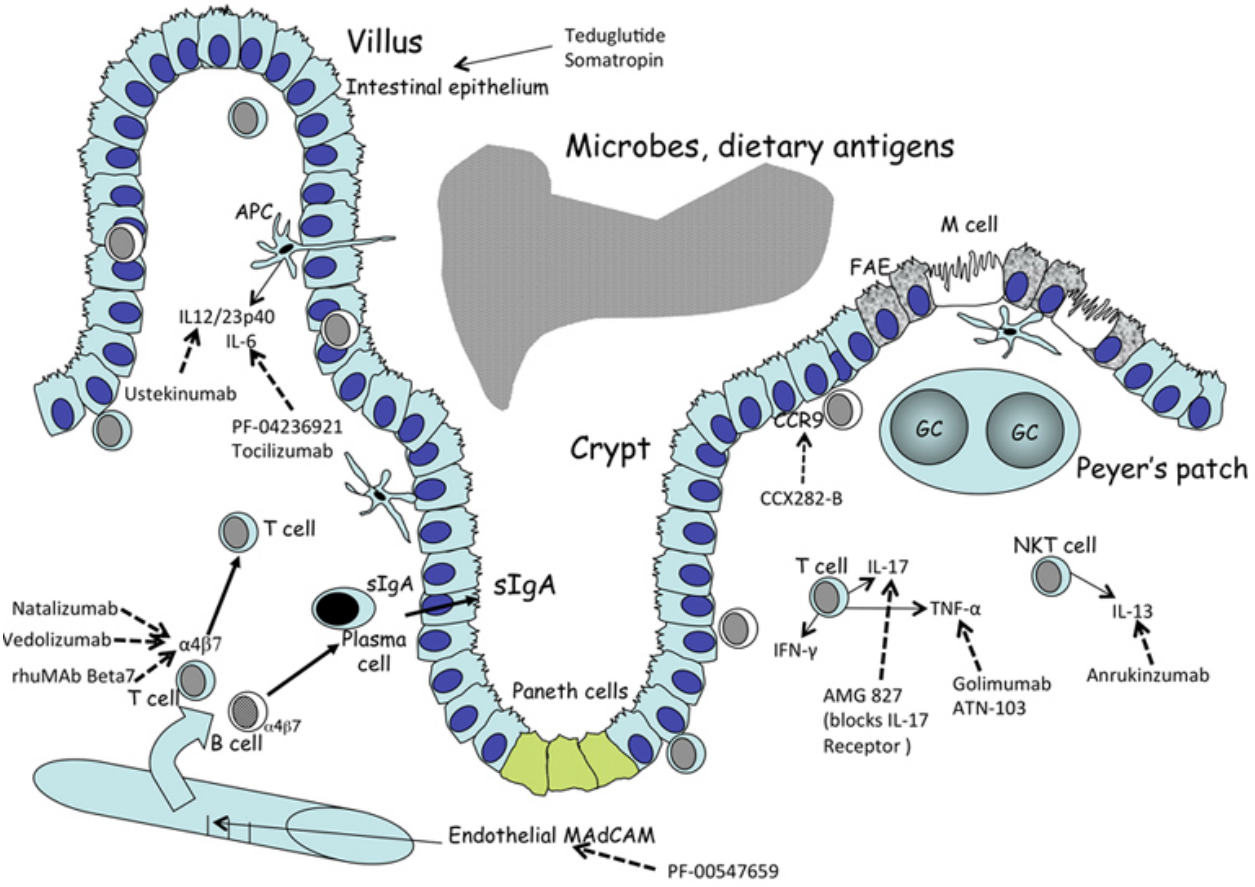

Figure 1. Lamina propria targets for selected novel therapies.

inflammatory process may not be beneficially altered if mucosal healing is also not achieved, a treatment goal that continues to be debated. Therefore the outcomes of treatment of IBD may be changing and along with them a higher standard of efficacy for emerging therapies (7). Additional goals of IBD treatment include decreasing rates of complications, hospitalizations, surgical interventions, infection, steroid use, cancer and overall mortality. Moreover, improving compliance and raising health-related quality of life are also important goals that can be expected to translate promising novel approaches into successful conventional therapy (7).

Working within the current paradigm of IBD pathogenesis, namely the dysregulated immune response to gut luminal contents, emerging therapies are targeting all aspects of the innate and adaptive immune apparatus. These therapeutic approaches will be refined over time as adjustments are made for the influence of specific genetic defects, environmental exposures, and discoveries of redundancy and plasticity within the inflammatory pathways. Development of newer biological agents specifically targeting effector cytokines is focused on disrupting development and maintenance of effector T cells and not just their inflammatory products (8-10). Effector cell depletion has been another focus of attention of current research efforts, in an effort to "reset" the immune system (11-14). Inhibition of cell trafficking is also recognized as an effective therapeutic strategy in IBD, particularly when egress of cells to an intestinal site of inflammation can be specifically blocked (15-19). On the other hand, enhancing or restoring regulatory factors in the inflammatory response are being pursued whether by counter-regulating a polarized immune response or stimulating T regulatory cell function especially using cell-based therapies. Even restoration of the epithelial barrier in support of innate immune responses is being tested in for its effectiveness in IBD therapy (20, 21). Finally, altering the luminal contents to affect the immune response has been advocated for clinical trial testing based on preclinical data from probiotic and prebiotic studies (22-27) (Figure 1).

3. TARGETS OF EMERGING THERAPIES

3.1. Targeting effector cytokines

Animal models of colitis have demonstrated the effectiveness of targeting specific cytokines that mediate gut inflammation. In this regard, the effectiveness of antibodies against TNF-alpha in counteracting cell mediated inflammatory damage has been well established for over a decade, supporting the importance of this "down-stream" inflammatory mediator (1, 28, 29). Other effector

Table 1. Novel agents currently in active trials or analysis

Agent	Formulation	Target	Disease	Trial Phase
Golimumab	MAb	TNF-alpha inhibition	UC	II, III
AMG 827	MAb	IL-17 receptor blockade	CD	II
AIN 457	MAb	IL-17A inhibition	CD	II (Completed)
PF-04236921	MAb	IL-6 inhibition	RA, CD	I, II
Ustekinumab	MAb	IL-12/23p40 inhibition	CD	III
Anrukinzumab	MAb	IL-13 inhibition	UC	II
ATN 103	Nanobody	TNF-alpha inhibition	RA	II
Vedolizumab	MAb	α4β7 integrin blockade	CD, UC	III
CCX282-B	oral small molecule	CCR9 blockade	CD	II
rhuMAb Beta7	MAb	β7 integrin blockade	UC	I
PF-00547659	MAb	MAdCAM blockade	CD, UC	I, II
Tofacitinib	oral small molecule	Janus kinase inhibition	CD, UC	II (Completed)
Prochymal®	Allogeneic stem cells	Immunomodulation	CD	III
Mesenchymal stem cells	Autologous adipose and bone marrow derived stem cells	Immunomodulation	CD	II
MultiStem®	Allogeneic stem cells	Immunomodulation	UC	II
Somatropin	Growth hormone	Mucosal barrier	CD	II (Completed)
Trichuris suis ova	Helminth eggs	Gut lumen	CD	II

MAb: monoclonal antibody, UC: ulcerative colitis, CD: Crohn's disease, RA: rheumatoid arthritis

cytokines such as IL-17 and IFN-gamma (the inflammatory products of activated T cells) and IL-12/23p40 and IL-6 (cytokines that can direct the development of inflammatory T cells) have also been identified as potential targets (6). The IL-23/IL-17 and IL-12/IFN-gamma axes have been targeted for novel therapies in CD because these cytokines mediate the colitis in animal models and are excessively produced in human disease. IL-13 is a novel target for UC for similar reasons (see below). However, this knowledge alone is not enough to predict how effective agents or strategies will be within a disease, or whether a novel therapy for CD will also work in UC. For instance, IL-17 is elevated in a subset of UC patients not responding to an anti-IL-13 strategy, so would simultaneously blocking IL-17 effects extend therapeutic efficacy in this setting (30)? Only with detailed immune monitoring during clinical trials of novel agents will clues be obtained to the actual mechanisms of primary response, primary non-response, the potential for combined therapies (two or more targeted agents at a time), and the differential effectiveness in CD versus UC.

3.1.1. Targeting TNF-alpha

Infliximab and Golimumab: TNF-alpha is an effector cytokine in many inflammatory conditions, and several studies clearly demonstrated high levels of this molecule in active IBD. Today, antibodies against TNF-alpha are cornerstones of conventional therapy for CD and UC. The major TNF-alpha blockers being used today to treat patients with IBD are infliximab, adalimumab, and certolizumab pegol. The emerging aspects of anti-TNF-alpha drug development include formulations to ease administration, potentially decrease immunogenicity, and to broaden application from CD to UC. In this regard, the murine-human chimeric monoclonal antibody infliximab has had major success in treating CD and its penetrating complications (31-34). Infiximab is also the only anti-TNF-alpha biologic currently approved to treat UC following demonstration of its efficacy (35-37). However, its need for intravenous infusion administration and induction of anti-drug antibodies are disadvantages to its use. Emerging as a replacement drug for infliximab is golimumab, a human monoclonal IgG_1 antibody that has very high affinity for TNF-alpha and can be administered subcutaneously. The constant region of golimumab is identical to that of infliximab, but its variable regions have fully human sequences (38). The potency of golimumab (measured as the *in vitro* concentration required to inhibit by 50% TNF-alpha-induced E-selectin expression on human endothelial cells) is higher than infliximab and adalimumab (39, 40) but whether this will translate into a therapeutic advantage is unknown. Golimumab is already approved for use in patients with rheumatoid arthritis and active psoriatic arthritis and ankylosing spondylitis (38, 41, 42). Results from a set of double blind placebo controlled clinical trials testing efficacy of golimumab for induction and maintenance of remission in moderately to severely active UC are pending (http://clinicaltrials.gov/show/NCT00488631 and NCT00487539) (Table 1). It may be tested separately for efficacy in Crohn's disease in the future.

Adalimumab: Adalimumab is a monoclonal IgG_1 antibody against TNF-alpha that is "humanized" (i.e. the amount of mouse DNA sequence used to generate the antibody protein is minimized to around 5%). It has the advantage of subcutaneous administration and is approved for the induction and maintenance of remission of CD following successful clinical trials (34, 43-45). While helpful in healing fistulae, it has not received approval for this indication. The emerging aspect of adalimumab in IBD therapy is whether it has activity in ulcerative colitis. After open-label trials suggested efficacy of adalimumab for UC (46, 47), results of a multicenter, randomized, double-blind, placebo-controlled clinical trial that assessed efficacy and safety of 8 weeks of adalimumab in inducing clinical remission in anti-TNF-alpha naïve patients with moderately to severely active ulcerative colitis were

published (48). The data showed a statistically significant but otherwise modest clinical benefit at week 8 with remission rates of 18.5% in patients who received adalimumab 160mg/80mg induction regimen (p=0.031 versus placebo) and 10% in patients receiving 80mg/40mg regimen (NS versus placebo, 9.2%). The rate of adverse drug events was not significantly different among the groups. This was a disappointing result given the similar mode of action it has with infliximab and its comparable efficacy in Crohn's disease. Nevertheless, efforts are proceeding for regulatory approval of this agent in UC.

Certolizumab pegol: Certolizumab pegol (Cimzia) is a humanized monoclonal antibody Fab fragment against TNF-alpha, pegylated by the addition of two polyethylene glycol molecules that increases the half-life as well as well its binding affinity to TNF-alpha (49, 50). Certolizumab was approved for use in inducing and maintaining reduction in symptoms of CD in concert with a series of clinical trials (51, 52). Its efficacy in the treatment of UC is currently being evaluated in a phase II trial (http://clinicaltrials.gov/show/NCT01090154).

Anti- TNF-alpha Nanobodies: Finally given that anti-TNF-alpha agents are among the oldest and most widely used biologics, it is no surprise that they are being subject to an upgrade in technology. "Nanobodies" are tiny molecules that were first described during the early part of this century (53). Morphologically, nanobodies are made up of the smallest functional fragment of a naturally occurring single-domain antibody. These are very stable and extremely soluble entities with very high tissue penetration. Nanobodies have been developed against a long list of molecules that include TNF-alpha. Nanobodies for treating thrombotic thrombocytopenic purpura and vonWillibrand disease were among the first described in literature (54). Since 2009, there has been a surge in development and testing of nanobodies targeting several molecules involved in pathogenesis of many conditions including infections and autoimmune disorders (55-59). Recently, a pre-clinical study describing secretion of anti-TNF-alpha nanobodies by orally administered Lactococcus lactis bacteria was published. It may prove to be an effective strategy for treatment of IBD in humans in the near future (60). There are currently 4 nanobodies in clinical trials and more trials are expected to begin in 2011. These agents may be administered intravenously or subcutaneously. ATN-103 is a nanobody that has specific affinity for TNF-alpha. ATN-103 is a bivalent nanobody with an extended half-life. It is already being tested in rheumatoid arthritis with trials being planned for Crohn's disease. (http://clinicaltrials.gov/show/NCT01063803).

Finally other strategies such as vaccination against TNF-alpha (producing autologous, polyclonal antibodies) and disrupting the soluble form of the TNF-alpha trimeric molecule by introducing a mutant subunit that abrogates the trimer biologic activity are being pursued in preclinical and clinical studies.

3.1.2. Targeting interferon-gamma (IFN-gamma) and IL-17

Fontolizumab (anti-IFN-gamma): Predominance of the interferon-gamma Th1 cytokine production by lamina propria T cells is a hallmark of animal models of CD as well as in Crohn's patients (61, 62). Fontolizumab is a humanized monoclonal antibody with potent binding and neutralizing activity against human interferon-gamma a prominent pro-inflammatory effector cytokine that activates macrophages, lymphocytes and facilitates antigen-presentation to drive immune responses (63).

Two safety and clinical efficacy trials assessing the ability of fontolizumab to induce remission in active CD were published in 2006. This early experience suggested that there was a biological response to fontolizumab (decreased CRP in the study drug group), and patients receiving multiple doses also had better outcomes compared to placebo (9, 64). A placebo-controlled Phase II trial assessing efficacy of fontolizumab in patients with CD was recently published (65). In this trial, a total of 201 patients with Crohn's Disease Activity Index (CDAI) scores between 250 and 450 were randomized to receive an initial intravenous dose of 1.0 or 4.0 mg/kg fontolizumab or placebo, followed by up to 3 subcutaneous doses of 0.1 or 1.0 mg/kg placebo every 4 weeks. At the end of the induction phase at 4 weeks, there was no significant improvement in patients who had received fontolizumab. However, during the maintenance period, the patient arm that received 1.0 mg/kg intravenous followed by 1.0 mg/kg subcutaneous fontolizumab did have significantly greater improvement in the CDAI score compared with patients who received placebo. But a similar improved response was not seen in the higher-dose fontolizumab group. However, all fontolizumab groups had significant improvement in C-reactive protein levels compared to placebo, again demonstrating a biological response that was somehow disconnected from a clinical response. Adverse event rates were similar in all groups.

The discovery of IL-23, along with its regulation of IL-17, another cytokine newly appreciated for its role in autoimmune inflammatory disease, forced a revision of the view that IL-12 and Th1 cells were solely responsible for Crohn's disease (and opening the door to other potential targets for novel therapies). With the realization that IL-17 was overlooked as an important cytokine in several animal models of autoimmune disease including colitis, IFN-gamma as a target has lost some of its primacy especially after the results of the clinical trials mentioned above. It is clear that IFN-gamma can inhibit Th17 cell development, so that blocking IFN-gamma could enhance IL-17 production and its mediated injury. On the other hand, in a mouse colitis model where activation of the innate immune system in the absence of lymphocytes leads to gut inflammation, the systemic disease (weight loss, increased serum inflammatory cytokines) was directly linked to IFN-gamma whereas the gut inflammation was directly linked to IL-17 (66). Therefore targeting IFN-gamma may still have a role in treating the symptoms of IBD, but until we understand the hierarchy of IFN- gamma and IL-17 effects in Crohn's disease in particular, it will be difficult to determine which patients may have higher likelihood of response to one or the other agent. In addition, combinations of targeted antibodies will likely be a future strategy to improve efficacy.

Secukinumab (anti-IL-17A) and AMG 827 (anti-IL-17 receptor A): While IL-17 has clear importance in some animal models of Crohn's colitis (IL-10 knockout mouse and $CD45RB^{high}$ SCID transfer model), its role in human disease is less clear. Increases in IL-17 have been recorded in human IBD (67, 68), and given the preclinical data in animal models where successful IL-17 blockade treats colitis, IL-17 has become a target in human clinical trials. The humanized monoclonal antibody secukinumab binds to the IL-17A isoform, and a Phase II trial testing its efficacy, safety and tolerability in moderate to severe Crohn's disease has recently been reported in abstract form (2011 ECCO meeting). There were no significant improvements in rates of clinical response or remission compared to control. Perhaps the specificity of the target, that is neutralizing IL-17A alone, could be responsible for the poor outcome. The IL-17 cytokine family is made up of 5 isoforms with IL-17A and IL-17F the most abundant and signaling through the same receptor, one made up of the IL-17 receptor A subunit. Therefore to get adequate benefit of IL-17 neutralization, it may be that both of these isoforms need to be targeted at the same time (preclinical data suggests as much (69)). In this respect a current trial using an antibody against the IL-17 receptor subunit A would block both IL-17A and F binding to their cell surface receptors, effectively knocking out both IL-17 isoform signaling (AMG 827 in Subjects With Moderate to Severe Crohn's Disease http://www.clinicaltrials.gov/show/NCT01150890).

3.1.3. Targeting IL-12/23p40

Interleukin-12 (IL-12) and Interleukin-23 (IL-23) are associated effector cytokines that play an active role in promoting intestinal inflammation in IBD. IL-12 (p35/p40) and IL-23 (p19/p40) are distinct heterodimeric molecules that share a common p40 subunit and bind to distinct cell surface receptors which also share a common subunit, the IL-12 receptor (IL-12Rbeta1/IL-12Rbeta2) and the IL-23 receptor (IL-23R/IL-12Rbeta2). IL-12 drives induction of inflammatory Th1 cells that secrete interferon-gamma, and IL-23 helps maintain and stimulate IL-17 secretion from Th17 cells (70).

Briakinumab: Briakinumab is a human monoclonal IgG_1lambda antibody directed against IL-12/23p40 subunit (8, 71). Results of a phase II dose ranging and safety efficacy study demonstrated a significantly higher response in patients on 3 mg/kg briakinumab (75%) compared to placebo (25%) at the end of 7 weekly injections (P=0.03) (72). With this data in hand, a follow-up briakinumab Phase IIb study was completed with a 12 week induction phase and a 12 week maintenance phase with the objectives of testing efficacy and safety in Crohn's disease. In this study, patients with active Crohn's disease received a dramatically different dose schedule with intravenous infusions of briakinumab (400mg, 700mg) or placebo every four weeks for 12 weeks. The primary endpoint of remission at week 6 was not met, with no difference among rates for placebo (9%), 400 mg (13%), or 700 mg (17%); however, at Week 12 there were differences among remission rates for briakinumab compared to placebo (11%) versus 400mg (29%, p=0.03) and 700mg (22%, p=0.087). Twelve week maintenance treatment provided no significant response or remission compared to placebo. There were no significant differences between rates of adverse events, but infectious adverse events in both groups during induction and maintenance dosing were rather high (25-34%). However, the rates of serious infectious adverse events and opportunisitic infections (Candidiasis) were low, less than 5% (73). This agent is on hiatus from further development for CD treatment in part due to circumstances related to its testing in other inflammatory diseases.

Ustekinumab: Ustekinumab is a fully human monoclonal IgG kappa antibody that can bind to IL-12, IL-23, and the p40 monomer (74). Binding of ustekinumab to IL-12 or IL-23 interrupts coupling of the cytokines to the cell surface IL12 receptor (via the IL-12Rbeta1 subunit) and thereby prevents intracellular signaling and T cell activation. While this agent is currently approved for use in plaque psoriasis, it has also been investigated for multiple sclerosis, psoriatic arthritis, and Crohn's disease. The first published trial of ustekinumab in Crohn's disease randomized 104 patients in a double-blinded, cross-over design, placebo-controlled Phase IIa induction study and showed that patients receiving a single intravenous dose of ustekinumab had a significantly higher response rate at 6 weeks compared to placebo (54% vs 22%, p=0.024) (75). Interestingly, when analyzing the subset of patients (n=49) who had previously been exposed to infliximab (regardless of outcome), the clinical response rates for ustekinumab were significantly higher at every timepoint compared to placebo, 55-59% versus 15-26%, respectively. Ustekinumab admisitration was associated with decreased CRP serum levels, higher baseline CRP was associated with higher ustekinumab response rates, and infliximab-experience may amplify these effects (76). More recently, a phase II study of ustekinumab in Crohn's disease was presented in abstract form (77). Subjects received a single intravenous induction dose (0, 1, 3 or 6 mg/kg), followed by 0 or 90 mg SC ustekinumab at 8 and 16 weeks for responders by week 6. The clinical response rates were significantly greater than placebo at week 6 (23.5% placebo vs 34.1-39.7% for ustekinumab) without differences in remission rates. However at the end of the maintenance phase the response and remission rates were significantly higher for ustekinumab compared to placebo, 64.9% vs 42.5%, and 41.7% vs 27.4% respectively. Impressively, almost half of the subjects reported prior failure to 2 or more anti-TNF agents. These data replicate the response to p40 targeting seen with the original briakimumab trial. It appears that targeting p40 will be a promising strategy going forward; perhaps the gap in treatment response relates to the relative abundance of p40 monomer, which could act as a sink for ustekinumab, but this will need to be built into future trials to test as a predictor of response.

STA-5326 (apilimod mesylate): STA-5326 mesylate (also called apilimod mesylate) is a small molecule that is a selective transcription inhibitor of c-Rel translocation. It was found to inhibit the gene transcription of p35 (the IL-12 subunit) and p40 (the subunit common to both IL-12 and IL-23) (6). In 2006, results of a safety and

dose-ranging study with an accrual of 73 patients that evaluated oral STA-5326 mesylate (apilimod mesylate) in CD were published. The data revealed a remission rate of between 15 and 36% at the end of 4 weeks. Approximately half of the patients demonstrated a decrease in mucosal disease activity (78). A subsequent phase II dose-ranging randomized placebo control clinical trial assessing the efficacy of oral STA-5326 mesylate (apilimod mesylate) in CD was not completed due to an interim analysis that showed no possible efficacy. There are also no studies that have assessed STA-5326 mesylate in UC.

3.1.4. Other strategies targeting cytokines

Several other strategies to inhibit inflammatory effector cytokines have involved use of agents that have been found to exert inhibitory influence over signaling pathways involved in cytokine activity and production. These effects are generally never as specific as a targeting antibody, but they have shown effectiveness in *in vitro* and animal models. Semapimod is a synthetic guanylhydrazone mitogen-activated protein kinase 38 blocker that can inhibit TNF-alpha production. While an early phase study that tested the safety and efficacy of semapimod in moderately to severely active CD showed a significant difference in response was demonstrated between CD patients and those who were on placebo, a subsequent phase II did not reveal a significant response in CD patients (79, 80). Similarly, doramapimod (BIRB 796), a p38 inhibitor of mitogen-activated protein kinase, did not show clinical or endoscopic healing efficacy in a large phase II study that assessed patients with moderate to severe CD (81).

Thalidomide is a glutamic acid derivative that has gained notoriety for its devastating teratogenic effects. There is growing evidence that thalidomide effectively inhibits transcription of TNF-alpha and other cytokines that may also be of value in combating the inflammation of IBD (82, 83). In a retrospective study published in 2007, thalidomide was shown to be effective in some patients with refractory luminal and fistulizing Crohn's disease (82). Randomized controlled trials (RCTs) testing efficacy of thalidomide in adults with active CD have not begun yet. It is important to note that an RCT conducted in children with CD, lenalidomide, an analogue of thalidomide, did not demonstrate any significant benefit over placebo (84). Similarly, despite pre-clinical evidence to support efficacy of thalidomide and lenalidomide in decreasing intestinal inflammation associated with IBD, no maintenance trials have yet been published to justify its safety for use in humans. However, clinical trials may be considered (85).

RDP58 is an oral *d*-amino acid decapeptide that showed immunosuppressive activity including inhibition of TNF-alpha synthesis as well inhibition of IFN-gamma, IL-2 and IL-12 (86). It has been demonstrated in animal models that RDP58 is effective in reducing intestinal inflammation including histological scores in colitis (87, 88). RDP58 has also demonstrated variable preclinical efficacy in treatment of several inflammatory and presumably autoimmune conditions that include IBD, interstitial cystitis and autoimmune encephalomyelitis (86, 89, 90). Recently, RDP58 was evaluated for its efficacy and safety in treatment of mild to moderate UC in a multicenter, double-blind, randomized proof of concept clinical trial. The results of the trial demonstrated that while there was significantly better clinical response at higher doses of the drug, no significant improvement was seen in endoscopic improvement although histologic scores of study drug patients showed improvement (86).

Janus kinase (JAK) represents a family of tyrosine kinases that function as important intracellular messengers transducing cell surface receptor activation (interferons, interleukins) to intranuclear signals (91-94). There is evidence that inhibitors of the JAK family of tyrosine kinases may be effective in treatment of inflammatory conditions that include both rheumatoid arthritis and IBD. INCB18424 (Ruxolitinib), a small molecule antagonist of Jak1/2, and CP690,550 (Tofacitinib) that targets Jak3 and Jak1 over Jak2 are potentially effective treatments for RA as well as IBD (94, 95). CP-690,550 has undergone phase II trials in RA as monotherapy and in combination with methotrexate demonstrating efficacy in alleviating pain and improving function of patients with RA. Phase III trials testing efficacy of CP-690,550 in RA underway (96-98). Inhibition of the JAK pathway was postulated to be a good target in IBD inflammation since increases in downstream STAT3 activation was measured in the gut tissue of active Crohn's and ulcerative colitis patients (99). Recently, a randomized, double-blind, placebo-control multi-center clinical trial investigating the safety and efficacy of CP-690,550 in patients with moderate to severe ulcerative colitis showed that compared to placebo (38.1% clinical response at week 8), patients taking 15 mg or 10 mg po BID had clinical response rates of 76.3% and 65% respectively (77). These data provide the possibility of add-on oral therapy to targeted anti-cytokine therapy to amplify inhibitory effects and perhaps improve maintenance of response.

3.2. Targeting immune cells directly

Because activated T cells are such a central part of the excessive immune response seen in IBD, they have themselves become targets for therapeutic intervention, whether by inhibiting their function or limiting their numbers through induced cell death (1, 5, 29, 100). B-cell mediated mechanisms may also play a role in IBD pathogenesis as there is increased secretion of IgM and IgG classes of antibodies by mucosal cells, and B cells have antigen-presenting capabilities (29). However, it is also important to note that in mice that lack T and B cells, colitis can still be induced by activation of the innate immune system (macrophages and dendritic cells) alone, so that strategies that target specific cells of the adaptive immune system may not fully restore homeostasis.

Visilizumab is a monoclonal antibody directed against $CD3^+$ T cells. The anti-CD3 antibody is thought to selectively induce apoptosis in these T cells when in the activated state. The beneficial action of visilizumab is that it binds to $CD3^+$ T cells via its non-FcR site and, in contrast to those antibodies that bind to $CD3^+$ T cells at the FcR site, visilizumab does not activate the CD3 T-cells resulting

in a "cytokine release" syndrome (101). Phase I clinical trials have assessed efficacy of visilizumab in CD as well as in UC (6). Due to lack of any meaningful response in patients with fistulizing CD, further trials testing efficacy of visilizumab in CD were not conducted. However, while encouraging results were demonstrated in early UC trials, a subsequent placebo controlled phase II trial in patients with steroid refractory UC was abandoned when an interim analysis in 2008 failed to show any efficacy and had safety concerns compared to placebo (102).

Daclizumab is a humanized monoclonal antibody designed to directly target effector T cells by binding to CD25, a T cell receptor for IL-2 and a marker of T cell activation (5). However, because CD25 expression is also a marker of a suppressor subset of regulatory T cells that naturally inhibit inflammatory responses, it is not clear whether there are differential effects on the effector/inflammatory versus suppressor/regulatory $CD25^+$ T cells. Daclizumab is currently approved for use in acute kidney transplant rejection. Two clinical trials studying the effectiveness of daclizumab in induction of remission in patients with active UC failed to show any significant benefit compared to placebo, therefore further trials were not planned. So far, there have been no clinical trials assessing the efficacy of daclizumab in CD (103, 104).

Basiliximab is a chimeric monoclonal antibody that is specifically directed against the alpha subunit of IL-2 T-cell surface receptor CD25. Two non-comparator early phase clinical trials suggested that basiliximab may be effective in inducing remission in patients with moderate-severe steroid refractory UC (12, 105). This led to a phase II trial evaluating the same category of patients with UC. This trial was recently completed but has not been published yet (Basiliximab in Moderate to Severe Ulcerative Colitis: http://clinicaltrials.gov/show/NCT 00430898). There are no reported clinical trials assessing the efficacy of basiliximab in CD; however there is anecdotal evidence suggesting that it may also be effective in inducing remission in patients with active CD who are resistant to steroids. In 2008, a report was published in which clinical researchers reported a case of a 24-year old woman with steroid-refractory CD and a first-degree family history of UC who attained remission after treatment with basiliximab. There were no adverse events reported in the patient (14).

Another strategy to stem the activation of effector T cells was pursued by blocking their co-stimulation using a CTLA4-fusion protein (abatacept, a drug approved for treatment of rheumatoid arthritis) to bind to the B7 co-stimulation molecule on antigen-presenting cells thus preventing the needed co-stimulation for T cell activation. However, a Phase III study testing this drug in Crohn's disease was terminated early after data suggested there was a lack of efficacy compared to placebo (http://clinicaltrials.gov/show/NCT00406653).

Lastly the physical removal of inflammatory cells from the peripheral blood using a matrix that traps granulocytes and some monocytes has been tested in IBD patients. Only one blinded, sham-controlled study done in UC has been completed and no efficacy was shown (106) despite other unblinded studies showing efficacy (107).

3.3. Inhibition of cell trafficking

Another unique therapeutic mechanism for limiting inflammation in CD and UC is by inhibiting lymphocyte trafficking from the bloodstream into gut tissue. Most leukocytes have adhesion molecules called integrins on their cell surface that can be induced by mediators of inflammation. These integrin molecules are cell surface glycoproteins that promote binding to endothelial cells thus beginning the process of diapedesis. Relevant for gut vascular endothelium, $alpha_4beta_1$, and $alpha_4beta_7$ integrin molecules bind specifically to the endothelial cell ligands vascular cell adhesion molecule-1 (VCAM-1) and mucosal vascular addressin cell-adhesion molecule-1 (MAdCAM-1). In addition, once in the lamina propria, the $beta_7$ subunit can form a dimer with the $alpha_e$ subunit, $alpha_ebeta_7$, that binds to the e-cadherin integrin molecule on gut epithelial cells playing an important role in anchoring intraepithelial lymphocytes to enterocytes within the gut epithelium.

Natalizumab is a humanized monoclonal antibody directed against $alpha_4$ subunits, targeting the two integrin molecules $alpha_4beta_1$, and $alpha_4beta_7$. Natalizumab blocks integrin association with vascular receptors in many tissues besides the gut-specific $alpha_4beta_7$, limiting trafficking out of the bloodstream so much so that natalizumab-treated patients can show a peripheral lymphocytosis (108, 109). While approved for use in Crohn's disease and multiple sclerosis, safety issues related to the susceptibility to developing progressive multifocal leukoencephelopathy mandates that current use of natalizumab be discontinued in Crohn's disease if no therapeutic benefit is observed in the first 12 weeks of therapy (109-111). The investigational drug vedolizumab (MLN-0002/LDP-02) is a humanized monoclonal antibody also directed against an adhesion molecule. The important difference between natalizumab and vedolizumab is that vedolizumab specifically targets the whole $alpha_4beta_7$ integrin molecule with the intent to affect only the cell trafficking through the gut vasculature (16, 18). Vedolizumab is currently undergoing Phase II clinical trials to test its efficacy in treatment of UC as well as CD. (http://clinicaltrials.gov/show/NCT 01177228, NCT00619489, and NCT00655135). Data from these trials are still be analyzed. Another antibody, rhuMAb $Beta_7$, directed against the $beta_7$ integrin subunit alone is in early testing in ulcerative colitis.

Another agent being tested, CCX282-B targets chemokine receptor 9 (CCR9). CCR9 is located on the cell surface of T-cells that transmigrate from the intestinal tract during inflammation. CCR9 in turn specifically attaches to its ligand CCL25 that is expressed by intestinal (particularly small bowel) mucosal cells during intestinal inflammation suggesting a more important role of this mechanism in CD patients with small bowel involvement (112).

Yet another recent strategy aimed at inhibiting cell migration to areas of gut inflammation involves the molecule intercellular adhesion molecule-1 (ICAM-1). ICAM-1 mediates trafficking of effector leukocytes to the gut by binding to the beta2-integrin leukocyte function-associated antigen-1 (LFA-1). Alicaforsen, an anti-sense oligonucleotide has been developed to target ICAM-1. It has been specifically designed to deplete ICAM-1 level by inhibiting its sequencing messenger mRNA, and while systemic administration has not shown benefit in treatment of IBD, a topical formulation has shown some benefit in UC (114-116).

3.4. Immune modulation

Therapies are being tested that aim to modulate the immune response in IBD, mostly by inducing more regulation of the immune response, to "reset the immunostat" and even, in the case of sargramostim, to purportedly improve innate immune function. In terms of shoring up the regulatory response, suppressive cytokines such as IL-10 have been given by direct parenteral administration to patients as well as in the form of IL-10-secreting Lactobacillus, each with modest effects. Another strategy to induce actual T regulatory cells or enhance their activity has been tried through cell-based methods and growth factors that can exert immunomodulatory effects.

3.4.1. Mesenchymal stem cells

One cell-based strategy was tested by the use of allogeneic mesenchymal stem cells (MSCs) (remestemcel-L, PROCHYMAL®, Osiris) in an open label study for Crohn's disease. Ten adult patients with active Crohn's disease receiving 2 infusions one week apart (2-8 x 10^6 cells/kg) all experienced a drop in the CDAI at day 28 with clinical response seen in 3 of 9 evaluable patients and remission in one patient (117). Based on these findings, a larger, randomized, placebo-controlled, double-blind Phase III study of Prochymal in Crohn's disease was begun in 2007 (Osiris Protocol 603, NCT00482092 clinicaltrials.gov). At one point enrollment was stopped when the final scheduled interim analysis showed that one of the PROCHYMAL® treatment arms would not meet the primary endpoint due to unexpectedly high placebo response rates (118). However, after additional analysis showed that in one treatment arm the endpoint of remission had been met in the treatment-per-protocol group and was approaching significance in the intent-to-treat group, the study was re-opened for enrollment only into this better-performing dose arm or placebo. No further data have been presented as to the outcome of this trial.

Given the beneficial results of MSC effects on experimental models of colitis in animals, pilot studies of MSC (both bone marrow- and adipose tissue-derived) have been reported in human Crohn's disease. One report used Crohn's patients as the source of autologous bone marrow-derived MSCs (119). This Phase I study enrolled 10 Crohn's patients with active disease refractory to corticosteroids, immunosupressants or biologics. Patients received two doses of MSCs (1-2 million cells/kg) one week apart and were assessed 5 weeks later for a clinical response (drop in CDAI $\geq$70 points) and by colonoscopy for improvement in endoscopic score. Seven patients remained for endpoint assessment and three reported a clinical improvement (two of these patients were also the only ones to have endoscopic improvement). One of the obvious drawbacks of this approach is that MSCs need to be newly made for each instance of therapy and whether MSCs from the same patient with a genetic defect predisposing to Crohn's disease in the first place will also have altered immune function is not clear. Since MSCs do not have to be HLA-matched donor to recipient, the advantages of autologous MSCs are not clear.

Adipose-derived MSCs (aMSCs) have also been used in fistulous Crohn's disease. Two reports of induced colitis in mice, chemically-induced DSS colitis and the hapten-induced TNBS colitis, demonstrate how intra-peritoneally administered human adipose-derived MSCs could ameliorate existing inflammatory bowel disease (120, 121). In a Phase II study of adipose MSCs in complex perianal fistulas (both Crohn's and non-Crohn's disease), autologous MSCs were harvested and processed for direct injection for treatment of complex perianal fistulas. Patients were treated with a fibrin glue plug with or without the injected aMSCs. Twenty million aMSCs were injected, half within the tract walls and half around the external opening. Evaluation 8 weeks later was followed by a second treatment with twice as many cells if healing was not observed (healing was defined as absence of drainage, spontaneous or expressed, and complete re-epithelialization of the external opening). Crohn's patients experienced a 71% healing rate with aMSCs compared to 14% using the fibrin glue alone. Overall, MSCs remain an interesting "black box" of a therapy; while it is likely that inhibitory effects are exerted on activated inflammatory cells, it has yet to be determined whether stem cells provide a regenerative response in inflamed tissue and even how long MSCs exist in the areas of inflammation (where they seem to home).

3.4.2. Autologous stem cell transplantation

The role of T cells in regulation of inflammation responsible for IBD is also being explored (122). The evidence for this strategy being of potential benefit in IBD derives from experiments conducted in other autoimmune diseases, most importantly rheumatoid arthritis, in which transplantation of autologous hematopoietic stem cells following a "lymphoablative" conditioning regimen demonstrated decreased production and propagation of non-regulatory T-cells eventually leading to lowering of inflammation (123, 124). In 2005, a study reported successful autologous stem cell transplantation in patients with CD unresponsive to conventional medical strategies. The investigators observed gradually improved health-related quality of life as well as mucosal healing following rapid clinical remission in most patients (125).

3.4.3. Sargramostim and filgrastim

Other agents that have potential to modulate the dysregulated immune response in IBD are granulocyte-macrophage colony stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF). Sargramostim is a granulocyte-macrophage colony

stimulating factor which specifically stimulates production of neutrophils, monocytes, and epithelial cells (126). The thought behind this application was that GM-CSF showed efficacy in treating the granulomatous colitis seen in chronic granulomatous disease, a primary immunodeficiency caused by a defect in polymorphonuclear function. A phase 1 study published in 2009 assessed efficacy of sargramostim in treatment of patients with moderately to severely active CD. The study demonstrated modest benefit with only a few patients experiencing clinical remission. There were no serious reported adverse drug events (127). Recently published results of a placebo-controled randomized trial that assessed the efficacy of sargramostim in corticosteroid-dependent patients with Crohn's disease demonstrated that it was more effective than placebo in inducing corticosteroid-free remission. It was also demonstrated that treatment with sargramostim was associated with significant improvements in health-related quality of life (128). Phase III studies in CD have been terminated but no results have been published.

There is evidence for granulocyte colony-stimulating factor's ability to inhibit effects of T helper 1 cells and its ability to induce IL-10-secreting regulatory T cells (129). Filgrastim, a synthetic granulocyte colony-stimulating factor that specifically stimulates production of neutrophils has also shown promise in early safety and efficacy trials (126). In a recently published early phase study, the clinical benefit of G-CSF therapy in active CD was tested. The results of the study were important as it was demonstrated that clinical benefit from G-CSF treatment was associated with induction of IL-10 secreting T cells as well as a rise in plasmacytoid dendritic cells in the lamina propria of the inflamed intestinal mucosa (129). These studies suggest that induction of regulatory cells and cytokines are associated with improvement of symptoms in CD.

3.5. Mucosal barrier enhancement/restoration

The intestinal epithelium is an important part of the innate immune system in that it regulates lamina propria exposure to microbial antigens and microbe-associated molecular pattern signals as well as functioning as a source of cytokine and chemokines. Any insult that results in damage to epithelial cells or failure of the tight junctions to keep the contents of the two environments separate can contribute to intestinal inflammation by activating the innate and adaptive immune responses. Some emerging therapies include agents that seek to enhance or restore this important physical barrier homeostatic system (20, 29, 130).

Teduglutide is a dipeptidyl peptidase IV-resistant glucagon-like peptide-2 analogue. This agent is being tested for its ability to restore the intestinal epithelial barrier due to its effects on maintaining gut permeability (131-134). Results of a randomized, placebo-controlled, double-blind trial evaluating efficacy of teduglutide in inducing clinical and mucosal remission in moderate to-severe CD demonstrated a significant benefit for clinical response and therefore more studies have been planned (131).

Epithelial growth factor (EGF) is a well-known inducer of epithelial cell proliferation and EGF receptor tyrosine kinase activaton. While EGF delivered as an enema to ulcerative colitis patients has shown good results in inducing mucosal healing (135), somatropin, human growth hormone, has demonstrated efficacy in repairing the intestinal epithelium in pre-clinical studies. Some early studies have demonstrated that somatropin enhances the innate immune system and repairs the intestinal epithelium (136, 137). A phase II study evaluating the efficacy of somatropin in induction of histological healing in children with Crohn's was recently completed and results are pending (http://clinicaltrials.gov/show/NCT00109473). These strategies aimed at epithelial restitution offer an interesting approach to enhancing mucosal healing, a goal of treatment that seems to predict longer lasting disease remission.

3.6. Intra-luminal targets

Not only is the regulated interaction between the host and the gut microbiome important for limiting inflammation in the gut, but patients with inflammatory bowel disease have an intestinal dysbiosis (138). What is not clear is whether this dysbiosis is a primary event, possibly predisposing to inflammation, or if it is secondary to the inflammatory state and/or any genetic factors that are linked to disease susceptibility. What is clear is that attempts to change the gut microbiome have not been shown to be consistently effective in ameliorating disease, whether through introduction of presumed probiotic organisms or through the induction of probiotic communities and a favorable metabolic environment through the use of prebiotics. Because a complete discussion of probiotic and prebiotic trials in IBD is beyond the scope of this review, it is worth mentioning work done using Trichuris suis ova, a porcine whipworm, to transiently infect the GI tract. It is known that certain microbes can induce polarized immune responses, and it was postulated that infection with Trichuris could induce cytokines to counter-act the inflammatory effector cytokines driving the disease or perhaps induce regulatory cells and their inhibitory effects. In a double-blind, placebo-controlled study in active ulcerative colitis, 43.3 % of patients treated for 12 weeks with ova reported clinical improvement versus 16.7% of placebo (p=0.04) (139). In fact, clinical improvement is associated with induction of IL-10 production and even the appearance of epithelial restitution factors (140). Future directions in probiotic and prebiotic use will need to include measures of changes in the gut microbiome along with resulting alterations in the luminal metabolic and mucosal immune responses that accompany clinical improvement and mucosal healing, testing whether components of microbes exert the same beneficial effects as the whole organism, and defining the differences in responders versus non-responders at the microbiomic, immunologic and genetic levels.

4. PROMISING MOLECULAR TARGETS

4.1. Interleukin-13

Among the most promising targets that are being tested as treatments for IBD are two directed against IL-13 and IL-6. In ulcerative colitis, preclinical data implicated excess IL-13 production in a mouse model of ulcerative

colitis, the oxazolone model (141). In this model mice developed histologically similar lesions to human disease (inflammation limited to the mucosa), and mucosal natural killer cells (NKT) cells were found to produce excessive amounts of IL-13. In fact the oxazolone-induced colitis could be reversed not only when anti-IL-13 antibody was administered to mice after the colitis had begun, but animals who were genetically engineered to be unable to support IL-13 signaling (IL-13 receptor alpha chain knockouts) were resistant to oxazolone colitis itself. These observations lead to investigations into human inflammatory bowel disease, and in fact patients with ulcerative colitis also had excess production of IL-13 by a unique subset of mucosal NKT cells, the so-called type II NKT cell (142). IL-13 is a credible candidate effector cytokine in ulcerative colitis because *in vitro* it has direct toxic effects on human gut epithelial cells (e.g. the colon cancer cell line HT-29) (143). It also has deleterious effects on the gut epithelial tight junction, causing disruption by up-regulating claudin-2, a tight junction protein associated with decreased epithelial resistance. In this way excess IL-13 could contribute to ulcerative colitis inflammation by disrupting the epithelial and tight junction contribution to the barrier component of innate immunity.

One study that used interferon-beta-1a for its anti-IL-13 effects has recently been reported (30). It is known that interferon-beta-1a blocks IL-13 production by human CD4 T cells and also up-regulates intracellular SOCS proteins that block IL-13/IL-4 receptor signaling (144, 145), making it a rational choice for UC treatment. In this open-label study where 16 patients received interferon-beta-1a weekly for 12 weeks, there was an 80% clinical response rate to the study drug. Interestingly, while all the patients had high baseline production of IL-13, only those experiencing a clinical response had significant decreases in IL-13 production post-treatment. No other inflammatory cytokines including INF-gamma, TNF-alpha, IL-6, or IL-17 and any significant change in either group of patients after treatment, suggesting IL-13 has an important role in the clinical manifestations of ulcerative colitis.

Several anti-IL-13 monoclonal antibodies that have been tested in allergic inflammations such as asthma, allergic rhinitis, and atopic dermatitis are being considered for trials in UC. Currently Anrukinzumab (IMA-638), an anti-IL-13 humanized monoclonal antibody that has been used in Phase II asthma studies, is being tested for efficacy in a phase II study in UC.

4.2. Interleukin-6

IL-6 represents another promising target for emerging therapies in IBD. Although elevated in a number of other inflammatory states (ranging from sepsis to rheumatoid arthritis), IL-6 levels in the serum and gut mucosa seem to reflect the activity of disease in IBD (146, 147). In fact, animal models deficient in IL-6 expression or function showed protection against chemical colitis (148), spontaneous colitis in a common gamma chain-knockout mouse (149), and in colitis induced by transfer of colitogenic T cells into an immunodeficient mouse (150). IL-6 has a unique mechanism of signaling in that it can bind to the IL-6 receptor (IL-6R) whether embedded in the cell membrane or to the soluble form of the receptor. The soluble IL6-IL-6R complex is then able to insert itself into cell membranes and commence active signaling, even in cells that previously did not express the IL-6R protein itself. In fact, IL-6 likely exerts its effects in IBD primarily through the soluble IL-6R because lamina propria T cells do not express cell surface IL-6R (151).

One agent that has been developed to treat IL-6-mediated diseases is tocilizumab, a monoclonal antibody directed against the IL-6R; it blocks IL-6 from binding to IL-6R protein (152). In the only published trial of tocilizumab in Crohn's disease, 36 patients received infusions of 8 mg/kg tocilizumab or placebo every 2 or 4 weeks. At the end of 12 weeks 8 of 10 patients in the every 2-week regimen had a clinical response (CDAI drop $\geq$70 points) versus 4 of 13 in the placebo group (p=0.019). However, while it was clear that there was a biologic response to the drug (CRP levels were decreased in the treatment group; IL-6 drives CRP production), there was no mucosal healing that accompanied the clinical response. These data were enough to plan a phase 2 study using a different anti-IL-6 monoclonal (PF-04236921) in Crohn's disease.

The IL-6-IL-6R pathway will likely remain an interesting target in IBD for another reason. Th17 cells have been implicated in the pathogenesis of colitis in animal models, and IL-6, together with TGF-beta, is a critical component for directing naive T cells to develop into IL-17-secreting cells. In the absence of IL-6, inducible T regulatory cells are produced instead. These natural suppressor cells exert anti-inflammatory effects, suggesting that one additional mechanism of anti-IL-6 or IL-6R targeting could be to enhance anti-inflammatory cells.

5. CONCLUSIONS

Since current biologic therapies continue to have substantial primary and secondary non-response rates, a compelling drive persists to develop and test novel therapies based on emerging data from basic immunologic pre-clinical and clinical studies. As reviewed above, many new therapies are being tested that inhibit or modulate specific components of the inflammatory response in order to improve IBD symptoms and signs. While these strategies are based on sound biologic assumptions, these studies are being conducted using genetically diverse subjects at various points in their disease process, factors that can add considerable variation to the clinical outcomes (a clinical trial is distinctly different from studies using genetically identical mice given a timed injury to induce short-lived colitis). Unfortunately, few clinical studies include meaningful monitoring of the pharmacologic or biologic effects of treatment that could provide insight into what features of the drug effect are coupled to clinical response and what factors predict non-response. Only in this way could we address the gaps in therapy, namely the unmet clinical need of non-response (or loss of response) and the unmet need to identify mechanisms of actions that are critically important for drug effects in successful IBD

treatment. It may be that two or three agents targeting non-overlapping components of the inflammatory response will need to be deployed in series or concurrently in order to synergize a maximum therapeutic effect. Finally, changes in clinical trial design, while incorporating outcomes generalizable to whole IBD populations, will need to be refined in order to identify subsets of patients who respond optimally to targeted therapies, fulfilling the promise of "personalized medicine." It is clear from the foregoing review of emerging therapies in IBD, that our community is well positioned to meet this challenge.

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Abbreviations: APC: antigen presenting cell, NKT: natural killer T, sIgA: secretory immunoglobulin A, M cell: microfold cell, FAE: follicle associated epithelium, GC: germinal center, MAdCAM: mucosal adressin cell adhesion molecule

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