Journal of Gastroenterology and Hepatology

JGHF

doi:10.1111/j.1440-1746.2009.06074.x

REVIEW

New and emerging therapies for inflammatory bowel diseases

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Key words
inflammatory bowel disease, therapies, biological therapy, cell movement.

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Conflict of interest
The author has received speaker's fees from Abbott Australasia and from Ferring Pharmaceuticals, in addition to research support from Schering-Plough.

Abstract

The inflammatory bowel diseases have undergone an explosion of discovery in the last 10 years. The overwhelming focus of this has been in genetics and immune mechanisms of disease. While the former has provided critical information on predisposing factors, the latter has resulted in a panoply of novel immune-based therapies and technologies. These range from an improved approach to the use of conventional immunomodulators, such as azathioprine and 6-mercaptopurine, to commonplace availability of anti-tumor necrosis factor agents such as infliximab and adalimumab, through to small molecule inhibition of immune mediators. Unusual treatments, such as helminth infestation, stem cell transplantation, and leucocytapheresis, all derive from the burgeoning understanding of pathogenesis. Most important to our successful use of these therapies will be a fundamental understanding of the patient phenotypes and genotypes that will dictate particular treatment approaches in the future.

Introduction

The inflammatory bowel diseases (IBD) are chronic, relapsing, and remitting inflammatory disorders of the gastrointestinal tract. They typically result in diarrhea, abdominal pain, bleeding, and fever. Both ulcerative colitis and Crohn's disease are diagnosed using standard clinical, endoscopic, radiological, and histopathological features. Ulcerative colitis involves the rectum and a variable but continuous proximal portion of the colon, with inflammation confined to the mucosa and occasionally submucosa. Crohn's disease, on the other hand, can involve any part of the gastrointestinal tract, but it involves the distal ileum and proximal colon in about 60% of patients. The inflammatory process in Crohn's disease typically is transmural. Both diseases are associated with a number of extra-intestinal manifestations, many of which parallel the intestinal disease in severity. Some, such as ankylosing spondylitis and primary sclerosing cholangitis, may run an independent course.[1]

Inflammatory bowel diseases have become increasingly common in developed countries and now progressively have made their appearance in developing countries such as India and China.[2,3] Current estimates are that approximately 61 000 people suffer from ulcerative colitis and Crohn's disease in Australia, with a total community cost of the order of AUD 2.7 billion each year.[4] The diseases are problematic because of their unpredictable, relapsing nature, and the relatively young population afflicted.

The etiology remains enigmatic, but there is a consensus that IBD originates in a complex interplay between genetic contributions, bacterial (probably commensal) intestinal flora, and the immune system. Indeed, recently identified genetic mutations associated with IBD highlight the critical role of responses to bacteria (genetic defects in bacterial recognition factors such as CARD15, or in autophagy genes such as ATG16L1) as well as immune function (mutations in interleukin (IL)-23 receptor) (see Table 1).[7] However, identified genetic changes appear to be neither necessary, nor sufficient, for development of or protection from disease—genetic studies are no closer to providing us with new diagnostic tests or novel therapeutics. On the other hand, there have been enormous leaps forward in our understanding of disease pathogenesis. Studies over the last 15 years have focused on perturbations in the immune system, and an enormous number of cellular and protein mediators have been implicated in the perpetuation of the disease process. These have led to fundamental change in our approach to managing these diseases, with substantial shifts in approaches to immunomodulation through to use of anti-tumor necrosis factor (TNF) therapies and, soon, newer biologic agents. The horizon is dotted with new therapeutics predominantly directed toward the immune system, currently undergoing trials.

Immunomodulation—the old and the new

Broad suppression of the immune system has been a tactical approach to IBD therapy since corticosteroids were first used widely as treatment in the 1960s. The majority of Crohn's disease patients treated with corticosteroids for remission induction will achieve remission, but fewer than one-third remain in remission at 12 months, with another quarter becoming steroid 'dependent' and

Table 1 Identified or candidate genes associated with Crohn's disease and ulcerative colitis (modified from [5,6])

Chromosomal localization	Gene	Function
Confirmed or highly probable contribution to Crohn's disease risk		
16q12	CARD15	Detects bacterial muramyl dipeptide
2q37	ATG16L1	Autophagy
5q33	IRGM	Autophagy
22q12	XBP1	ER stress response
18p11	PTPN2	Tyrosine kinase growth factor receptor
12q12	MUC19	Mucus protein
5p13	PTGER4	Prostaglandin receptor
9q32	TNFSF15	Induces endothelial cell apoptosis
10q21	ZNF365	Zinc finger protein, unknown function
1p13	PTPN22	Tyrosine kinase receptor
1q23	ITLN1	Bacterial galactose-binding lectin
6p22	CDKAL1	Regulation of cyclin-dependent kinase
6q27	CCR6	Chemokine receptor, T_{reg} and Th17 cells
9p24	JAK2	Cytokine signaling
11q13	C11orf30	Oncogene, unknown function
17q21	ORMDL3	Unknown
17q21	STAT3	Cytokine signaling
21q22	ICOSLG	Costimulatory molecule, ICOS ligand
Confirmed or highly probable contribution to Crohn's disease and ulcerative colitis risk		
1p31	IL23R	IL-23 receptor
5q33	IL12p40	Common component of IL-12 and IL-23
6p21	MHC	Major histocompatibility complex
10q24	Nkx2-3	Lymphocyte and epithelial cell development
3p21	MST1	Macrophage stimulatory protein
Likely contribution to Crohn's disease risk		
2p23	GCKR	Growth/proliferation signaling pathway
2p16	PUS10	tRNA pseudouridine synthesis, unknown function
17q12	CCL2, CCL7	Chemokines, T cell, and monocyte recruitment
6p25	LYRM4	Protein folding, unknown function
6p25	SLC22A23	Organic ion transporter
2q11	IL18RAP	IL-18 receptor component

ER, endoplasmic reticulum; ICOS, inducible co-stimulator; IL, interleukin.

a substantial proportion requiring surgery.[8] Results are more favorable in ulcerative colitis although a significant number of patients are steroid-resistant. Salicylate agents such as sulfasalazine and mesalazine also act, at least partly, by selective inhibition of immune function, including reducing production of cytokines and chemokines,[9,10] inhibiting cyclooxygenase and lipoxygenase,[11] and inhibiting inflammation mediated by nuclear factor-κB by acting as an agonist of peroxisome proliferator-activated receptor-γ.[12]

Immunomodulatory agents such as azathioprine, 6-mercaptopurine, and methotrexate are well established as effective drugs in the management of Crohn's disease, while the thiopurines are also effective in ulcerative colitis. 6-mercaptopurine and its pro-drug, azathioprine, appear to mediate their effects predominantly by induction of apoptosis of T lymphocytes,[13] thereby reducing the effector compartment of the ongoing intestinal immune response. Moreover, a potential molecular mechanism underlying the delayed onset of activity of these agents has also been described. The initial response to thiopurines appears to be inhibition of proliferation of activated T lymphocytes, but these cells retain their effector functions. Only with repeated antigenic challenge in the continued presence of thiopurines was a pro-apoptotic effect seen.[14]

There are several key newer approaches to management of thiopurines in IBD. These revolve around an understanding of the metabolism of azathioprine to its effective and toxic metabolites (Fig. 1). Azathioprine is metabolized to 6-mercaptopurine, and thence to 6-thioguanine nucleotides (6-TGN), which are the mediators of the anti-proliferative and pro-apoptosis functions. An alternative pathway of metabolism, to 6-methylmercaptopurine (6-MMP), is mediated by *S-methylation* catalyzed by thiopurine methyltransferase (TPMT). 6-MMP is felt to be responsible for hepatotoxicity in a subset of patients who suffer this side-effect of thiopurines. Levels of TPMT activity are controlled by a common genetic polymorphism, and patients with low TPMT activity are at greatly increased risk of myelotoxicity with standard doses of these drugs. Indeed, this polymorphism may predispose to development of secondary leukemias.[15] Thus, there is now widespread agreement that therapy with thiopurines should be preceded by testing for TPMT activity (or genotype, if available). However, this does not obviate the requirement for careful monitoring for toxicity, as most patients who develop myelotoxicity have normal TPMT activity.[16]

While there is a narrow therapeutic window for thiopurines, the use of standard weight-based dosing does not guarantee achievement of efficacy or, alternatively, avoidance of toxicity. Patients

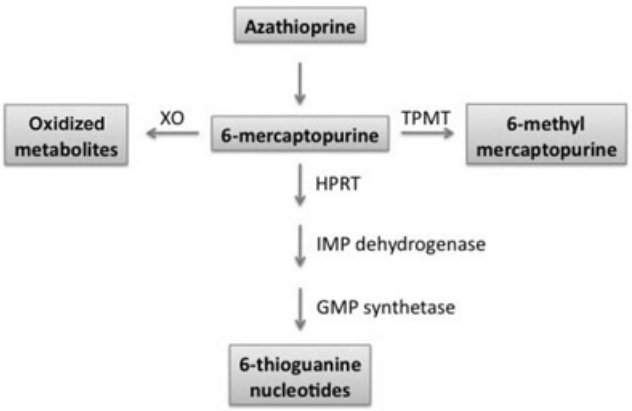

Figure 1 Metabolism of azathioprine and 6-mercaptopurine to yield 6-thioguanine nucleotides. GMP, guanosine monophosphate; HPRT, hypoxanthine-guanine phosphoribosyltransferase; IMP, inosine monophosphate; TPMT, thiopurine methyltransferase; XO, xanthine oxidase. (modified from [15]).

who do not achieve remission using standard dosing may have failed to achieve therapeutic levels of 6-TGN. In a proportion of patients, measurement of the levels of 6-TGN and the 'toxic' 6-MMP can be used to guide increased doses of thiopurines beyond the standard weight-based doses, while avoiding hepatotoxicity.[17] More intriguingly, and paradoxically given the metabolic pathways highlighted in Fig. 1, patients who do develop toxic levels of 6-MMP with inadequate levels of 6-TGN may be 'rescued' by the use of allopurinol administered together with 25–50% of the standard weight-based dose of thiopurine.[18]

The immunomodulator agent, mycophenolate mofetil, has been used in thiopurine-refractory IBD (both ulcerative colitis and Crohn's disease).[19–21] It may be of benefit in a subgroup of patients, and it is easy to administer, although there are no convincing controlled trial data to support its use. Evidence that it can cause an inflammatory colitis[22,23] gives pause for thought. Tacrolimus is another agent that has well-defined anti-T lymphocyte activities. A small, open-label study in ulcerative colitis suggested high-level efficacy.[24] Similarly small studies in luminal Crohn's disease have been largely negative, but a larger, randomized study of its use for perineal fistulas suggested efficacy in improving symptoms but not in fistula healing.[25] While the data on these agents are tantalizing, they are expensive and accompanied by significant risks of side-effects; their use should therefore probably be restricted to clinical trial or specialty centre settings.

The first of the new breed—anti-TNFα agents

Tumor necrosis factor-α is a central cytokine in a large number of inflammatory processes and has long been recognized in the tissues[26] and serum[27] of patients with active IBD. It is fundamental to most inflammatory processes by its direct effects in stimulating effector cells, and because of its ability to produce a host of downstream inflammatory events through its induction of the nuclear factor-κB pathway. TNFα also mediates effects in a membrane-bound form, on the surface of activated T lymphocytes.

Infliximab is a chimeric (75% murine, 25% human) monoclonal IgG1 antibody directed against TNFα. It requires administration by intravenous infusion, with weight-based dosing each 8 weeks after a three dose, 6-week induction period. Adalimumab is a fully human anti-TNFα antibody that is self-administered subcutaneously every 2 weeks in a standard dose format. These drugs have fundamentally changed the clinical approach to previously unresponsive IBD because of their dramatic efficacy, especially in Crohn's disease. The effects of the drugs are mediated by neutralization of soluble TNFα, as well as by induction of apoptosis of TNFα-bearing activated T lymphocytes[28] and monocytes.[29] There are now multiple randomized clinical trials that have demonstrated that these agents are useful therapeutic options in patients not responding to conventional steroid- and immunomodulator-based treatments; activity is seen in severe luminal disease as well as for fistulizing disease.[30] Long-term response rates, however, are less impressive than the initial remission-inducing effects of anti-TNF agents, with about 50% of initial responders losing response over 12 months.[30] The durability of response is at least partly related to the ability of these agents to induce mucosal healing,[31] which increasingly is being used as a therapeutic target because of its association with reduced surgery rates and hospitalization.[32] If administered in a planned fashion, rather than 'as required', there appears to be no advantage to simultaneous use of immunomodulators such as azathioprine where these agents alone have previously failed to induce remission.[33] Use of the agents, though, is complicated by development of specific anti-immunoglobulin antibodies, which over time, limit efficacy, as well as increase susceptibility to opportunistic infections including tuberculosis, reactivated viral and fungal infections.[34]

Newer antibodies directed against TNFα include certolizumab pegol, a pegylated Fab' fragment, which has been shown to induce durable remission in similar proportions of patients as infliximab and adalimumab.[35] Like adalimumab, it is administered subcutaneously. Unlike the earlier antibodies against TNFα, the absence of the Fc fragment of the antibody may lessen its immunogenicity and thus its response may be more durable, although head-to-head comparisons of anti-TNF agents have not been performed to determine this formally. Golimumab is another fully humanized monoclonal antibody against TNFα; it is currently in phase III clinical trials in Crohn's disease.

Anti-TNF therapy, in particular with infliximab, has also been demonstrated to be efficacious in ulcerative colitis, where it significantly reduces the requirement for colectomy and results in both clinical and endoscopic remission.[36] Trials using adalimumab in ulcerative colitis are in progress. Overall, case studies[37] and anecdotal experience suggest that the anti-TNF agents are less effective at inducing remission in ulcerative colitis than they are in Crohn's disease, and probably less effective than intravenous cyclosporine.

The 'Christmas Island' approach—blocking ingress of inflammatory cells

Trafficking of leukocytes into the intestinal mucosa is a highly dynamic and perpetual process. It allows continuous sampling of antigenic stimuli and maintains homeostatic control of intestinal flora, as well as tolerance to bacterial and food antigens. The

process by which this trafficking occurs is illustrated in Fig. 2. It is dependent upon the secretion of chemokines by endothelial cells (among others), which, in a concentration gradient-dependent fashion, bind to and activate chemokine receptors on the leukocyte cell surface. This outside-in signaling results in activation of leukocyte integrins, which adhere firmly to endothelial addressins, followed by transendothelial migration. Specific chemokines, chemokine receptors, integrins, and addressins are responsible for constitutive leukocyte migration in the intestine, including the chemokines CCL25 and CCL20, the chemokine receptors CCR9 and CCR6, and the integrin $\alpha_4\beta_7$. Several of these molecules have become therapeutic targets in IBD.

The α_4 component of the integrin heterodimers, $\alpha_4\beta_1$ and $\alpha_4\beta_7$, has been targeted by a recombinant humanized IgG4 monoclonal antibody, natalizumab. Studies of this antibody in Crohn's disease showed that migration into the intestine was effectively inhibited, with increased lymphocytes and monocytes in the peripheral blood. More importantly, large, randomized trials showed that in patients with clinically active disease and raised CRP, there was a statistically increased rate of remission induction.[38] However, natalizumab was voluntarily withdrawn by the manufacturer following several reports of fatal progressive multifocal leucoencephalopathy associated with it use. This devastating complication is related to opportunistic infection and also is seen with other profound immune suppressing therapies, and it has resulted in this potential IBD therapy being restricted in its use, including in further trials.

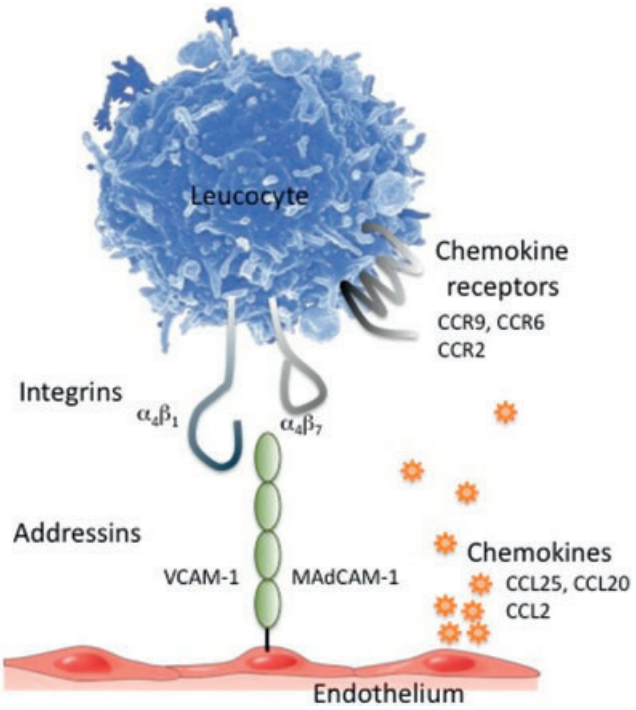

Figure 2 Attraction and adhesion of leukocytes in the gastrointestinal tract. Leukocytes migrate to the gut homeostatically when attracted by the intestine-specific endothelial cell-secreted chemokines, CCL25 and CCL20, which bind to the chemokine receptors, CCR9 and CCR6, respectively. This binding activates the leukocyte integrin, $\alpha_4\beta_7$, which then causes firm adhesion of the leukocyte to the addressin, MAdCAM-1, allowing transmigration into the tissues. During inflammation, homeostatic attraction and adhesion molecules are upregulated, but in addition, other molecules become more prominent in the process, such as CCL2, CCR2, $\alpha_4\beta_1$, and VCAM-1.

More gut-specific is a monoclonal antibody directed against $\alpha_4\beta_7$, vedolizumab, which is currently in phase III trials in both ulcerative colitis and Crohn's disease. An early publication suggested its efficacy in ulcerative colitis.[39] Other intestine-specific anti-migration strategies that are currently being trialed include: anti-β_7 monoclonal antibodies in ulcerative colitis; anti-MAdCAM-1 antibodies in ulcerative colitis; and two small molecule inhibitors of the chemokine receptor, CCR9, in Crohn's disease.[40]

A fundamental concern with these studies is the effect these agents might have on normal immune homeostasis in the intestine. All of the targeted molecules are central to migration of intestine-specific leukocytes in health, and while these cells contribute to the inflammation in active IBD, they are also critical to immune regulation in the uninflamed gut. Strategies targeting inflammation-specific leukocyte migration, such as antisense inhibition of the adhesion molecule, ICAM-1,[41] or inhibition of the ileal Crohn's disease-specific chemokine receptor, CCR2,[42] are inherently more attractive, although the former therapy was shown in a subsequent large trial to be ineffective.[43]

So many immune targets, so little time . . .

A large number of agents that address other immune pathways in Crohn's disease and ulcerative colitis are under assessment in clinical trials. The classical inflammatory profile of Crohn's disease is characterized by the so-called Th1 pathway, which involves elaboration of the cytokines, IL-12 and interferon-γ. Clinical trials of fontolizumab, a monoclonal antibody against interferon-γ, have been discontinued because of lack of efficacy, despite early promise.[44] Recent data suggest that a novel immune pathway, the so-called Th17 pathway, may be responsible for some of the earlier immune observations in Crohn's disease. A key mediator in this pathway is the cytokine, IL-23, and genetic data showing polymorphisms in the IL-23 receptor are associated with lowered risk of IBD, along with efficacy in Crohn's disease of an antibody directed against a common component (p40) of both IL-12 and IL-23,[45,46] strongly implicate elements of Th17 immunity in Crohn's disease pathogenesis. Indeed, anti-IL-17 monoclonal antibodies are currently under investigation in phase II trials in Crohn's disease,[47] although once again, caution needs to be exercised in targeting a molecule that has homeostatic roles in controlling intestinal pathogens.[48]

Other molecular targets include: the IL-2 receptor (targeted by daclizumab and basiliximab) in ulcerative colitis; the costimulatory molecule, CD28 (targeted by the CD152 fusion protein, abatacept) in Crohn's disease and ulcerative colitis; mitogen-activated protein kinases, important in TNFα signaling pathways (targeted by small molecule inhibitors[49]) in Crohn's disease; and the B cell marker CD20 (targeted by rituximab) in Crohn's disease and ulcerative colitis. While many of these drugs may be effective in subgroups of patients, the challenge in the future will be determin-

ing from patient genotype or disease phenotype, which agent is appropriate in which circumstance.

Do not try these at home

It is clear from multiple animal models, as well as the location in the intestine, the epidemiology of disease, and the response to antibiotics of some patients, that the pathogenesis of IBD is closely related to the bacterial content of the intestine. Specific pathogens have been sought for many years, but no conclusive evidence has been identified and, of course, the response to immune suppressing therapies mitigates against a primary bacterial pathogen. Rather, it is likely that an aberrant response to normal intestinal flora is the key to disease pathogenesis. Epidemiological data also point to a strong association between IBD and economic development,[50] along with a number of other diseases such as asthma, multiple sclerosis, and allergic disorders. Developed nation status, for example in South Korea, has been accompanied by a dramatic reduction in the burden of parasitic worm infestation and a concomitant increase in IBD incidence.[5] The altered balance between commensal bacteria and parasitic helminths may be responsible for a fundamental shift in the nature of gastrointestinal immune responses toward a type that results in chronic inflammation in those with other (e.g. genetic) predisposing factors.

This so-called 'hygiene hypothesis' has led to trials of self-limited parasitic infection, using *Trichuris suis*, a pig helminth, in both ulcerative colitis and Crohn's disease.[51,52] Although these initial data were promising, and *Trichuris suis* ova remain available through internet sources, continued human trials await more convincing safety and efficacy data in animal models.

Working on the assumption that circulating granulocytes and monocytes are major players in the immunopathogenesis of IBD, selective depletion of these cells has been attempted by extracorporeal adsorption, using technologies such as the Adacolumn and the Asahi filter. A number of small trials, some randomized, have been carried out in Japan and Europe, demonstrating the efficacy and safety of this treatment modality in refractory ulcerative colitis and Crohn's disease.[53] More widespread and larger trials are awaited.

Autologous hematopoietic stem cell transplantation has also been explored as a therapy in refractory IBD. This treatment option was based on the serendipitous observation of full remission of IBD in patients who had undergone hematopoietic stem cell transplantation for other indications. An initial uncontrolled study showed, remarkably, full remission off all anti-IBD medications at 18 months in 11 of 12 patients.[54] Clearly these dramatic and impressive results require larger randomized trials before such radical therapy can be considered acceptable.

Conclusion

The overwhelming data on disease pathogenesis and, to a lesser degree, genetics of IBD over recent years have yielded an abundance of modified old and highly novel therapies. These predominantly target the disturbed immune environment that characterizes IBD. Not all of the new treatments are completely rational, and some of them may have adverse effects on normal immune homeostasis and protection against pathogens. Indeed, with so many therapies likely to become available in the next 10 years, a much stronger emphasis on clinical phenotyping and subject genotyping will be required in order to target individual agents to those patients in whom they are most likely to be beneficial and safe.

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