Evaluation of new therapies for inflammatory bowel disease

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The pathophysiology of inflammatory bowel disease (IBD) is gradually being unravelled and new therapies are being developed to target the disturbed biological processes. This article outlines the clinical features of IBD, its current therapy and pathogenesis. The difficulties for clinical pharmacologists and gastroenterologists associated with designing, executing and interpreting clinical trials in IBD are then discussed. The final section reviews methods that can be used to demonstrate the pharmacological actions of new treatments in patients with IBD. It is emphasized that proof of the therapeutic efficacy of a novel agent with a specific mechanism of action yields not only clinical benefit to patients with IBD, but also indicates the importance of the targeted biochemical pathway in the pathogenesis of the disease.

Keywords: Crohn's disease, inflammatory bowel disease, pharmacology clinical trials, treatment, ulcerative colitis

What is inflammatory bowel disease?

The inflammatory bowel diseases (IBD), ulcerative colitis and Crohn's disease, are characterized by chronic idiopathic intestinal inflammation [1]. Crohn's disease is a transmural inflammatory process that affects any part of the digestive tract from mouth to anus. In ulcerative colitis, inflammation is superficial and continuous and limited to the colon and rectum. In some patients, IBD is associated with extra-intestinal manifestations involving the liver, skin, eyes and/or joints.

The natural history of both ulcerative colitis and Crohn's disease consists typically of episodic relapses between longer spontaneous or treatment-induced remissions. When their disease is active, patients usually present with diarrhoea, abdominal pain and when the colon is affected, the passage of blood per rectum. Depending on disease severity and site, there may also be weight loss, anorexia and fatigue. The incidence of IBD in northern Europe is about 200/million/year [2]. Although both diseases have little impact on mortality, they have a substantial negative impact on the quality of life of affected individuals.

The mainstays of conventional medical treatment for both types of IBD are corticosteroids and aminosalicy-

lates. Primary nutritional therapy has a role for some patients with Crohn's disease, particularly children. Immunosuppression with a thiopurine (azathioprine or 6-mercaptopurine) is a widely used second-line therapy for resistant disease. Alternative immunomodulatory medication, such as methotrexate, and more specifically targeted cytokine therapies, such as infliximab, a monoclonal antibody to tumour necrosis factor alpha (TNF α), are increasingly used in patients with refractory disease, particularly Crohn's.

Unfortunately, none of these drugs is universally effective and all can be associated with adverse effects. The poor performance of existing therapy for IBD is revealed by the cumulative 1 year relapse rate of about 50% for both ulcerative colitis and Crohn's disease [3]. With the exception of colectomy for ulcerative colitis, itself a procedure with long-term morbidity in many patients, no therapy offers a cure. The limitations of currently available treatment for IBD have led to investigation of a range of new therapeutic strategies, most of them aimed at specific pathophysiological targets.

Pathophysiology of IBD

Although the aetiology of IBD remains obscure, its pathogenesis is gradually being unravelled [4–6]. The interaction of environmental factors with genetic susceptibility [7] leads to inflammation in the gut mucosa (Figure 1). This inflammation is immune-mediated and driven by gut bacterial flora. Crohn's disease is associated

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Figure 1 Abbreviations: IL = interleukin, IFN γ = interferon gamma, TNF α = tumour necrosis factor alpha, ROM = reactive oxygen metabolites, CD = Crohn's disease, UC = ulcerative colitis, ENS = enteric nervous system.

with a Th₁-type immune response with excessive production of TNF α , interferon-gamma and interleukin-12 (IL-12), whereas ulcerative colitis is the result of a mainly Th₂ response with abundant IL-5 and IL-10 production [8]. Consequent excessive production of inflammatory mediators including reactive oxygen metabolites, eicosanoids and proteases, together with defective repair mechanisms, perpetuate the inflammatory reaction and result in tissue damage.

Investigating new therapies for IBD: principles

It is beyond the scope of this review to discuss the preclinical development of new therapies for IBD and in particular how the pharmacological effects of proposed novel agents are modelled and tested *in vitro*. Animal models of IBD, whether chemically induced, immune-mediated, genetically engineered or spontaneous, have played a major role in elucidating the aetio-pathogenesis of the human disease, and have been widely used to test potential new therapeutic agents [4, 9]. No animal model of gut inflammation, however, perfectly mimics human ulcerative colitis or Crohn's disease. It is unsurprising,

therefore that conclusions gained from the investigation of the effects of conventional or novel drugs in experimental colitis have not always been replicated in human studies. For example, the 5-lipoxygenase inhibitor zileuton ameliorated animal colitis [10], but was disappointing in humans with inflammatory bowel disease [11].

As in other areas of medicine, the key test of the therapeutic usefulness of a new agent for IBD is the randomized clinical trial: the design and interpretation of such trials in IBD, however, is unusually difficult, for reasons which will be discussed below.

In the development of new treatments directed at specific pathogenic targets, there is a range of techniques which can be used in clinical trials in patients with IBD to confirm that the target has been hit: these vary with the agent under evaluation and will be outlined later in this review. At this juncture, however, the importance of hypothesis testing is emphasized. Proof of therapeutic efficacy of a specially targeted agent yields not only clinical benefit, but also indicates the importance of the targeted cytokine, mediator or biochemical pathway in the pathogenesis of IBD. Conversely, a trial which fails to show clinical benefit, despite effective blockade of a

purportedly pathogenic compound or pathway, suggests that this pathway plays a minor role, at most, in the pathogenesis of the disease. Recent examples of the application of these principles in Crohn's disease include infliximab, the therapeutic efficacy of which confirms the central pathogenic importance of TNF α [12], and ridogrel, the clinical failure of which suggests that thromboxanes are relatively unimportant [13].

Clinical trials in IBD

Investigating the clinical efficacy of new drugs in IBD is not straightforward. Confounding factors common to other diseases, such as mistaken diagnosis, comorbidity, poor compliance and inadequate dosage or duration of therapy, will not be considered further here. Instead, we outline below some of the problems of trial design, execution and interpretation which are particular to IBD. These result primarily from the heterogeneity of IBD, in particular Crohn's disease, together with the difficulty in defining trial end points in both ulcerative colitis and Crohn's disease. Readers are referred also to recent reviews elsewhere [14–16].

Disease heterogeneity

Remission induction vs remission maintenance studies Both types of IBD are characterized by a waxing and waning natural history: clinical trials have to be aimed clearly therefore either at induction of remission in patients with active disease, or at remission maintenance in subjects whose disease is quiescent at the time of recruitment.

In trials of remission maintenance, factors which increase the likelihood of relapse need to be matched in the control and test patient groups. In Crohn's disease, for example, these include site of disease and its behaviour (penetrating or nonpenetrating) [17], time since previous relapse or surgery, smoking habit [18], and levels of inflammatory markers such as serum C-reactive protein [19] and faecal calprotectin, a neutrophil-specific protein [20].

Site and extent of disease In ulcerative colitis, the inflammation affects a varying extent of the colon, from a few centimetres of the rectum, to the entire colon. Moreover, the extent of inflammation in any individual patient can change with time [21]. These differences have important implications for the response to particular drug delivery systems. Topically active agents administered as suppositories or enemas will not benefit patients with extensive colitis. In contrast, for example, oral formulations of aminosalicylates such as Asacol, in which the active agent is released proximally in the colon, will be less effective

in patients with left-sided colitis than those whose entire colon is involved [22]. Such considerations are even more relevant in Crohn's disease, in which any part of the bowel from mouth to anus, can be affected.

Disease behaviour As indicated above, the natural history of Crohn's disease is influenced by whether it is penetrating or nonpenetrating in its behaviour [17], the former indicating a more aggressive form of disease less likely to respond to medical therapy. Furthermore, the symptoms of Crohn's disease may be due to a variety of different pathological processes, ranging from mucosal inflammation through stricturing to abscess and fistula formation. Again, patients with different pathological explanations for their presentation will show different responses to a single therapy.

Other factors Other factors influencing response to a trial drug include smoking habit, concurrent therapy and the time since discontinuation of previous treatments: the immunosuppressive effectiveness of thiopurines, for example, may take up to 4 months to be lost. Factors less easy to identify and define in planning recruitment are changes in gut physiology which may themselves differ between patients with either disease and influence a test drug's pharmacokinetics: these include malabsorption due to small intestinal disease and abnormalities of intraluminal pH and gut transit [23].

Genotype Recent research into the genetics of IBD is beginning to reveal genotypes which not only identify susceptibility to the disease [7] but also provide a clue to disease behaviour and response to therapy. The DR3 DQ2 haplotype, for example, appears to be associated with more extensive ulcerative colitis [24], while the NOD2 genotype in Crohn's disease is associated with fistulizing ileal and right colonic disease, stenosis and the need for resection [25].

The importance of thiopurine methyl transferase (TPMT) in the metabolism of the thiopurines, and of the multidrug resistance gene (MDR) in determining response to therapy with steroids, methotrexate and cyclosporin [26] is now recognized. Furthermore, failure of anti-TNF therapy with infliximab has been linked to polymorphisms in the TNF/lymphotoxin- α region [27] and the pANCA+/ASCA- antibody profile [28]. It is likely the response to other new agents is affected, in many instances, by the genotype of the patient treated. Pharmacogenomic studies will clearly have a major impact in the future in defining drug selection, dosage, efficacy and safety in individual patients. Conversely, unrecognized genotypic differences between patients are likely to make a substantial contribution to variability of clinical response in therapeutic trials.

Trial design

Stratification The heterogeneity of ulcerative colitis and Crohn's disease indicates that clinical trials should, ideally, be stratified prospectively to minimize differences between patients treated with novel and comparator drug (or placebo). Unfortunately, however, trials to date have generally been too small to match patient groups satisfactorily; retrospective subgroup analysis and Type II errors have each occurred too often.

Choice of comparator Argument persists as to whether a novel agent should be compared with an existing therapy or placebo. In severe active disease, use of a placebo is clearly unethical. Indeed, it has been argued that placebos should never be used when conventional therapy is known to be effective [29]. However, many clinicians will feel that use of a placebo group in patients with mild-moderately, rather than severely active, ulcerative colitis or Crohn's disease, allows, at least in the first instance, clearer assessment of the therapeutic potential of a new agent. This is particularly true in IBD, where the use of a placebo is ethically less problematic, since conventional drugs such as aminosalicylates are of only limited efficacy. A further potential advantage of placebo-controlled studies is that the overall size of the trial can usually be smaller than when standard comparator therapy is used, since the difference sought in power calculations will be larger: this results in exposure of fewer subjects to the novel agent, an important consideration in relation to the possibilities of failure of efficacy and toxicity of new drugs. Clearly, if placebos are to be used, withdrawal criteria must be drawn up which allow prompt removal of patients from the trial if they fail to respond or deteriorate.

Endpoints Objective measurement of disease activity and definition of outcome is contentious, particularly in Crohn's disease [14, 16]. There is a lack of widely accepted hard endpoints, death fortunately being too rare in either form of IBD to be a useful measure of the efficacy of a novel therapy. Outcome measures employed to date include clinical activity indices, quality of life scores, achievement of predetermined therapeutic goals, laboratory measures of inflammation, and macroscopic and microscopic mucosal appearances as well, of course, adverse drug effects.

Clinical activity indices There is a wide range of clinical activity indices for both ulcerative colitis and Crohn's disease [30]. Most combine varying numbers of subjective and objective measurements to produce a quantitative activity score; these range from symptoms recorded in patients' daily diaries to blood results and mucosal

appearances at endoscopy. The multiplicity of such scores indicates their inadequacy. Difficulties include the unreliability of symptom-recording by patients, inappropriate weighting of certain variables in some scoring systems, the poor correlation between clinical scores and disease activity assessed biochemically and endoscopically [31], and the consequent difficulty in defining remission and/or clinically significant improvement on the basis of such scores [32]. Despite these problems, activity indices such as the Crohn's disease activity index (CDAI) [33] and the simpler Harvey-Bradshaw index [34] for Crohn's disease, and Powell-Tuck index [35] and simple colitis clinical activity index (SCCAI) [36] for ulcerative colitis, are widely used to evaluate clinical outcome in therapeutic trials in IBD.

Quality of life scores There is increasing interest in the use of quality of life scores to assess response in clinical trials in IBD [37]. To date, however, there is insufficient experience of such scoring systems to suggest that they should be used as a primary rather than secondary outcome measure in clinical trials in IBD [16].

Therapeutic goals In an attempt to obviate some of the problems associated with using clinical scoring systems to assess response to therapy, specified goals have been proposed as alternative outcome measures. In trials in Crohn's disease, these have included reduction of steroid dosage [38], and avoidance of surgery, closure of fistulae [39], and prevention of endoscopic recurrence after surgery [40]. Such goals have the advantages of being well defined and usually of clinical relevance.

Laboratory measures of inflammation Unfortunately, although serum C-reactive protein, orosomucoid, ESR and platelet count correlate directly and haemoglobin and albumin indirectly with disease activity in IBD, no single simple blood test or set of tests is sufficiently reliable or clinically relevant to be used as a measure of response to novel therapies [41]. Four day faecal excretion of indium-labelled leucocytes in patients with IBD may provide a better guide to therapeutic response [42], but is cumbersome for the patient and involves exposure to radio-isotopes. Whether measurement of faecal excretion of calprotectin [20] will fulfil its early promise, remains to be seen.

Mucosal appearance and histology The mucosal appearances at colonoscopy can be assessed and scored at least semi-objectively in ulcerative and Crohn's colitis [43, 44], and at the ileo-colonic anastomosis after surgery [40]. Indeed, it has been proposed that the primary goal of therapy in IBD should be mucosal healing, confirmed endoscopically; this aim has been achieved in some patients with

Crohn's disease treated with azathioprine in combination with steroids [45] and with infliximab [46].

Scores to assess inflammation microscopically have also been devised but none has achieved widespread usage. In Crohn's disease at least, the patchy distribution of inflammation leads to a risk of sampling error, whether or not as a result of conscious investigator bias.

Both macroscopic and microscopic assessment of response to novel therapies requires repeated endoscopy, a procedure which is neither pleasant nor entirely safe, particularly in patients with active colitis. Furthermore, in trials of patients with small bowel Crohn's disease proximal to the terminal ileum, the mucosa is inaccessible to endoscopic examination, at least until further experience with the wireless capsule endoscope has been gained [47].

Conclusion

To address the problems outlined above, a recent consensus statement from the clinical trials taskforce of The International Organization of Inflammatory Bowel Disease has outlined the current position with respect to activity indices, definition of patient subpopulations, treatment indications and efficacy endpoints in trials of patients with Crohn's disease. If this consensus (and a similar one in ulcerative colitis) is adhered to in trials of

patients with IBD, inconsistencies between trials may be reduced and comparisons facilitated [16].

Testing the mechanism of action of new drugs *in vivo* in IBD

As indicated earlier, there are many different ways of assessing how new (or old) drugs work in IBD, the methods selected varying widely with the agent and targeted pathway under investigation. We outline below some of the approaches used to date, illustrating their rationale in Figure 1, and emphasizing that the list given is inevitably comprehensive neither in range nor content (Table 1). Some of the agents recently or currently under investigation for use in IBD are shown in Table 2.

At the outset, it should be emphasized that it can be difficult, when testing an agent with a mechanism of action of limited specificity, to ascertain whether an observed reduction in, for example, mucosal production of an inflammatory mediator, reflects the primary action of that agent, or is the result of a reduction in mucosal inflammation evoked by other mechanisms. For example, a reduction in mucosal production of an eicosanoid after 4 weeks of treatment of ulcerative colitis with prednisolone or an aminosalicylate may reflect anti-inflammatory actions other than a primary effect of the drug on

Table 1 Methods for assessing the pharmacological actions of new and existing therapies in IBD.

Site and method	Variable	Example of therapy*	References
<i>Gut lumen</i>			
Faeces	Microbiology	Antibiotics, probiotics	[50]
	Cytokines		[51]
Faecal dialysis	Drug concentration	5ASA	[52]
<i>Gut mucosa</i>			
Lavage	Cytokines, immunoglobulins	Enteral nutrition	[53, 54]
	Proteins	Enteral nutrition	
Colonic perfusion	Eicosanoids	Ridogrel, 5ASA	[56, 78]
Rectal dialysis	Eicosanoids, cytokines	Zileuton, ridogrel, steroids and 5ASA	[48, 49, 79]
Filter paper	Eicosanoids, cytokines		[63, 64]
Permeability	Solute absorption	Enteral nutrition	[65]
Biopsy	Mucosa-associated flora	Enteral nutrition	[67]
	Cytokines	Infliximab	[66]
	NFkB	Infliximab	[68]
	Reactive oxygen metabolites	Anti-oxidants	[80]
Biopsy culture	Eicosanoids	5ASA	[81, 82]
	Cytokines	Anti-cytokines	
	Growth factors	Smad7 antisense	[83]
<i>Blood</i>			
	Lymphocyte count	Natalizumab	[76]
	Platelet function	Ridogrel	[49]
	Cytokines	Infliximab	[68]

*each technique can be used for a range of novel therapies, depending on their putative mechanism of action. 5ASA, 5-aminosalicylic acid, NFkB, Nuclear transcription factor kappa B.

Table 2 Potential new treatments for IBD.

Target	Agent	References
Colonic bacterial flora	Antibiotics, prebiotics and probiotics	[50, 84]
	<i>Saccharomyces boulardii</i>	[85]
	<i>Trichuris suis</i> eggs	[86]
Epithelium	Short chain fatty acids	[87]
	Growth hormone	[88]
Leucocytes		
I. Reduce numbers	Apheresis	[89]
	Anti-CD4 antibodies	[90]
	Bone marrow transplant	[91]
	Stem cell transplant	[92]
II. Reduce migration	ICAM-1 antisense	[93–95]
	Alpha 4-integrin antibody	[76, 96]
III. Modifying function		
I. stimulate granulocytes	G-CSF, G-M-CSF	[97, 98]
II. ↓ pro-inflammatory cytokines	Onercept,	[99]
	RDP1258	[100]
	Anti IL-12 antibody	[101]
III. ↑ anti-inflammatory cytokines	IL-10, IL-11	[102–104]
IV. ↓ soluble mediators	Ridogrel	[49]
	SOD	[105]
	GTN	[106]
	Fish oil	[19]
	PAF antagonists	[107]
V. Modify intracellular signalling	NFκB antisense, PPARγ agonist, MAPK inhibitor	[108–110]
Agents targeting enteric neurones	Local anaesthetics	[111, 112]
Modulation of pro-coagulant state	Heparin	[113, 114]

ICAM: cell adhesion molecule; G-CSF: granulocyte colony stimulating factor; G-M-CSF: granulocyte-macrophage colony stimulating factor; IL: interleukin; SOD: superoxide dismutase; GTN: glyceryl trinitrate; NFκB: nuclear transcription factor kappa B; PAF: platelet activating factor.

eicosanoid synthesis. One way of attempting to obviate this difficulty is to assess the production of the target mediator very early (e.g. after a few hours) after administration of the test drug, before mucosal inflammation is likely to have been down-regulated by the agent by other mechanisms [48, 49].

Gut lumen

Analysis of gut luminal contents by any of the range of methods shown in Table 1 gives information both about luminal factors, such as flora, likely to be important in the pathogenesis of IBD, and release into the lumen of inflammatory mediators and products.

Faeces Microbiological culture of faeces has proved of limited value to date in the evaluation of the mechanism of action of antibiotics and probiotics. Indeed, analysis of mucosa-associated flora (see below) is likely to be more useful in defining the ways in which these therapeutic approaches may influence the pathogenesis of IBD [50].

Faeces from patients with IBD can also be analysed for cytokines such as TNFα [51], but this method has not, to our knowledge, been applied to assessing the effects of anticytokine therapies as yet.

In vivo faecal dialysis, in which the contents of dialysis bags swallowed and subsequently excreted per rectum by the patient are analysed, has given useful information about the pharmacokinetics of the aminosalicylates [52]. It has not been used as yet to study the mechanism of action of novel therapies.

Collection and analysis of faeces (and of dialysis bags passed with them) is unpleasant for patient and technician alike, and the stability of chemicals released from mucosa in stored faeces (or dialysis fluid) is uncertain. Approaches utilizing faeces therefore seem unlikely to achieve widespread application for the study of mechanisms of action of new therapies.

Whole gut lavage Whole gut lavage, in which rectal effluent is collected after oral or naso-gastric administration of large volumes of nonabsorbable fluid, has been used particularly to study the mechanism of action of enteral nutrition. Elemental diets, for example, have been shown to reduce intestinal release of immunoglobulins, IL-1 β and IL-8 [53], as well as of proteins [54].

Again, this technique is time-consuming and unpleasant for patients, so that it is unlikely to be used widely.

Colonic perfusion Perfusion of the entire colon or of isolated colorectal segments [55] has been used to measure the effects of thromboxane synthase inhibitors on release of eicosanoids in ulcerative colitis [56]. Although the same technique could be used to assess mucosal release of cytokines, it is tedious to undertake. Both balloon inflation and perfusion themselves may alter colonic function and quantification of results may be inaccurate. The more recently developed techniques of rectal dialysis and application of filter paper to the rectal mucosa (see below) are likely to make colonic perfusion obsolete for pharmacological studies.

Rectal dialysis This method was originally devised for investigation of rectal mucosal transport of water and electrolytes [57] and later adapted for measurement of release from the rectal mucosa of prostaglandins and other soluble mediators [58]. A cylindrical bag made of cellu-

lose dialysis membrane is filled with fluid of known composition and inserted into the rectum where it apposes the rectal mucosa for a defined period. Cytokines and mediators released from the mucosa move across the dialysis membrane into the fluid in the bag; their concentration is measured after removal of the bag by analysis of its contents. This *in vivo* technique has been used to study the effects of drugs on release of eicosanoids [48, 49, 59], and to clarify drug pharmacokinetics by assay of their luminal concentrations [60].

Rectal dialysis is simple and safe. Insertion of the dialysis bag does not appear to alter mucosal function unless undertaken very frequently over a short period [61]. If equilibrium dialysis is to be performed, the procedure can be lengthy, lasting up to 4 h; however, patients can be ambulant during this time [62]. Other disadvantages include the possibility of degradation of short-lived mediators in the dialysate during the procedure, its inapplicability to compounds of molecular weight too large to allow their penetration of the dialysis membrane, difficulties in interpretation caused by faecal contamination of the bag, and the possibility that rectal mucosal function is not representative of the remainder of the intestine (particularly in Crohn's disease).

Filter paper technique In an effort to circumvent some of the problems associated with rectal dialysis, a simple filter paper technique has recently been devised. Through a standard rigid sigmoidoscope a small piece of filter paper is apposed to the rectal mucosa until visibly soaked with peri-mucosal fluid. Sufficient soaking occurs in 30–60 s, after which the filter paper is removed and washed in buffer, which is subsequently analysed for cytokines [63] and/or eicosanoids [64]. This technique is quick, cheap and easy to perform. No bowel cleansing preparation is required. Again, mediators and cytokines to be assayed with this technique require to be stable and nonadherent to the filter paper after washing [64].

Gut mucosa

Study of luminal contents inevitably provides a less direct measure of mucosal events than investigation of mucosa itself, either through assessment of permeability or, more usefully, biopsies taken at endoscopy.

Permeability Intestinal mucosal permeability can readily be measured by assay of urine collected after oral administration of poorly absorbable solutes such as ^{51}Cr -EDTA or sugars (e.g. lactulose, sucrose, mannitol). Such techniques give an overview of epithelial function and have been used to assess mucosal integrity in patients given elemental diets as therapy for Crohn's disease [65].

Mucosal permeability, however, is unlikely to be a direct target for novel therapies, changes in this variable probably reflecting alterations in the extent or intensity of mucosal inflammation.

Mucosal biopsies Endoscopically obtained biopsies have been processed in a variety of ways in order to shed light on mechanisms of action of new drugs.

Immunohistochemistry can be used to assess the effect of new drugs on the inflammatory cell infiltrate in the mucosa of patients with IBD. With or without *in situ* hybridization to assay expression of mRNA, this method can also be used to study production of mucosal cytokines and adhesion molecules. Such an approach has, for example, been used in patients with Crohn's disease to evaluate the actions of infliximab [66] and enteral nutrition [67]. The inhibitory action of infliximab on the concentration of the nuclear transcription factor NF κ B p65 sub unit in colorectal mucosal biopsies has also been reported [68].

Mucosal production of reactive oxygen metabolites in response to drugs administered *in vitro* has been assessed using chemiluminescence [69], and the technique could be applied to study the pharmacological effects of novel antioxidant therapies.

Mucosa-associated bacterial flora can be studied using microbiological culture, polymerase chain reaction and *in situ* hybridization [70] but such techniques have not yet been used in assessing the efficacy of probiotics or antibiotics in altering intestinal flora.

Mucosal biopsies can also be cultured for periods of up to 24 h to assess the effects of novel therapies given *in vivo* on release into the incubate of cytokines and mediators such as eicosanoids and platelet-activating factor [49, 71]. Furthermore, mononuclear cells can be isolated from mucosal specimens obtained at surgery and their release of cytokines and reactive oxygen metabolites, for example, studied before and after administration to patients of novel therapies [72, 73].

Rectal mucosal biopsies are readily available from patients with ulcerative colitis undergoing sigmoidoscopy before and during treatment with novel agents. However, such an approach is much less feasible in Crohn's disease, in which diseased bowel is usually more proximal and less accessible to the endoscopist. Production of mediators can be artefactually modified by the trauma associated with biopsy [74, 75]. Furthermore, biopsy is associated with a small risk to patients of bleeding and perforation, particularly in inflamed bowel. For these reasons, assessments of mechanisms of action of new therapies involving mucosal biopsy have usually been confined to study of the effects of the test agent *in vitro* rather than before and after their administration *in vivo*.

Blood

Flow cytometry can be used to identify lymphocyte subpopulations. In Crohn's disease, they have been employed to confirm the predicted effect of natalizumab, an $\alpha 4$ integrin antibody, on circulating B- and T-lymphocytes numbers [76]. Analogous methods were applied to platelets in studies of the possible mechanism of action of ridogrel [49].

Concentrations of pro-inflammatory cytokines such as TNF α are increased in active IBD [77]; the secretion capacity for TNF α in a whole-blood cytokine assay was reduced 1 day after infliximab infusion in patients with Crohn's disease [68]. Blood concentrations of cytokines and mediators are likely to give a less direct indication of mucosal pathology than tests involving direct assessment of mucosal specimens. Nevertheless, the easy availability of venous blood samples makes assay of cytokines and mediators whose production is targeted by novel therapies an appealing approach.

Conclusions

Although advances in immunology, biotechnology and pharmaceutical science have initiated the development of a wide range of specifically targeted novel therapies for IBD, there is still no entirely effective safe or cheap drug for the suppression for IBD, let alone its cure. Efforts to develop new drugs for IBD will need to utilize techniques of the types outlined in this paper to confirm successful hits of purported pathophysiological targets. Clinical pharmacologists and other investigators must be aware of the limitations of each of these techniques, and of the many pitfalls associated with undertaking clinical trials in patients with IBD.

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