Crohn's disease: beyond antagonists of tumour necrosis factor

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In the past few years, antagonists of tumour necrosis factor have resulted in unforetold therapeutic benefits in Crohn's disease, but the magnitude and duration of responses are variable. New agents are therefore needed. Their development has benefited from advances in the understanding of the pathophysiology of this disease. Uncontrolled activation of the acquired immune system has an important role, and lymphocytes, cytokines, and adhesion molecules are broadly targeted for therapeutic intervention. With increasing evidence of an implication of the innate immune system and the intestinal epithelium, the therapeutic paradigm is also shifting from mere immunosuppression to the reinforcement of the intestinal barrier. We review mechanisms of actions of new drugs and the efficacy and adverse events from data from clinical trials. We discuss future directions, including new strategies with optimum endpoints.

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Introduction

Crohn's disease results from a dysregulated response of the mucosal immmune system to intraluminal antigens of bacterial origin in people who are genetically predisposed to this disease.[1,2] The traditional view of the pathogenesis of Crohn's disease is that intestinal inflammation is mediated by cells of the acquired immune system, with overly aggressive activity of effector lymphocytes and proinflammatory cytokines.[1,2] Emerging evidence suggests that disease development implicates a dysregulated dialogue between the intestinal microbiota and components of both the innate and adaptive immune systems.[3,4] The host response to the intestinal microbiota can be categorised into three basic components: the intestinal epithelium, innate immune cells of the myeloid lineages (eg, monocytes, dendritic cells, and granulocytes), and adaptive immune cells (B and T cells) (figure 1). Models with defects in each of these components have been associated with pathogenesis of inflammatory bowel disease in mice.[3,4]

Investigators have long sought to identify a micro-organism that causes inflammatory bowel disease. The present theory suggests a breakdown in the balance between putative species of protective versus harmful bacteria—a notion that has been termed dysbiosis.[5] Recent studies emphasised the potential importance of adherent invasive *Escherichia coli* in the initiation and maintenance of inflammation in Crohn's disease.[6,7] However, our understanding of the microbial flora is still incomplete. Metagenomic and computational analyses of the so-called microbiome might provide a foundation to achieve a more accurate understanding of the relevant, functional diversity of the flora in the context of inflammatory bowel disease.[4,8]

The intestinal epithelium, which is considered to be part of the innate immune system, has an active role in maintenance of mucosal homoeostasis. Epithelial cells form a tight, highly selective barrier between the body and the intraluminal environment. Failure of this barrier can result in intestinal inflammation, most likely through exposure to fecal antigens leading to inappropriate activation of the mucosal immune system.[1] In human beings, the importance of the epithelial barrier in disease predisposition is supported by the finding of abnormal intestinal permeability in first-degree relatives of patients with Crohn's disease.[4,9]

The innate immune system is the body's non-specific defence against pathogens. It is regarded as the first line of defence that reacts to the chemical properties of the antigen.[1] Evidence of the role of the innate immune system comes from the identification of *nucleotide-binding oligomerisation domain containing 2* (*NOD2*) as a susceptibility gene for Crohn's disease.[10,11] Individuals who are either homozygotes or compound heterozygotes for any one of the three germline variations of *NOD2* that are commonly identified have as much as a 40-fold increased likelihood of developing ileal Crohn's disease. The NOD2 protein is an intracellular receptor for a component of the bacterial cell wall, and is expressed in macrophages, dendritic cells, intestinal epithelial cells, and Paneth cells, providing specific support for the long-held hypothesis that Crohn's disease results from a genetically dysregulated host immune response to luminal bacteria.[4] Furthermore, natural antimicrobial peptides, such as defensins, are expressed in an NOD2-dependent manner, and patients with this disease

Search strategy and selection criteria

We did a computerised search of English and non-English language publications listed in the electronic databases of Medline (source PubMed, from 1966 to March, 2008), the Cochrane Library, and Embase (from 1980 to March, 2008). We searched for the terms: "Crohn's disease", "inflammatory bowel disease", "treatment", "biological therapy", "cytokine", "T-cell", "adhesion", "growth factors". We also hand-searched abstracts from the yearly meetings of Digestive Disease Week between 2003 and 2007, and the United European Gastroenterology Week between 2003 and 2007, and references from review articles and published trials to identify additional articles.

can have reduced defensin production in their intestine,[12] contributing to inadequate microbial clearance.

Adaptive immunity is the most proximate driver of tissue damage that arises in patients with inflammatory bowel disease, although innate immune responses seem to be a prerequisite for the excessive activation of adaptive immunity.[4] Adaptive responses toward a specific antigen are affected by a combination of resident and recruited cell populations. These populations consist of mucosal B cells producing immunoglobulins and a mixture of T cells that are dominated by a T-helper (Th) 1, Th2, or Th17 phenotype, and the coincident presence of regulatory T or B cells.[4] Th1 development is triggered by microbes that stimulate production of interleukin-12p40 and interferon γ, which then activate macrophages and the release of interleukin 1, interleukin 6, and tumor necrosis factor α (TNFα) (figure 1 and webfigure 1). Classic Crohn's disease has a Th1-type cytokine profile. Another CD4 T-cell lineage (Th17) that is distinct from Th1 and Th2 has now been linked to the pathogenesis of Crohn's disease. In a genome-wide association study involving a North American case-control cohort with this disease, typing more than 300 000 single nucleotide polymorphims, an interleukin 23R coding variant was associated with reduced risk of inflammatory bowel disease.[13] Th17-cell development is driven by transforming growth factor β (TGFβ) and interleukin 6, whereas interleukin 23 seems to expand and maintain Th17-cell populations. The interleukin-23 receptor consists of the interleukin-23R subunit and interleukin 12RB1, whereas the interleukin-23 cytokine consists of p19 and p40 subunits.[3] In addition to helper-cell activation, evidence in human beings and murine models also suggest a role for regulatory T cells producing interleukin 10 or TGFβ, or both, in maintenance of intestinal homoeostasis[4] (webfigure 1).

See Online for webfigure 1

Over the past decade, the advent of antiTNFα agent infliximab has changed the way that refractory Crohn's disease is treated. Infliximab rapidly induces and maintains response and remission,[14,15] spares steroids,[15,16] and induces and maintains fistula closure.[17,18] Nevertheless, about a third of patients do not respond at all to this drug, and an additional third has only some response. This finding can be explained by the presence

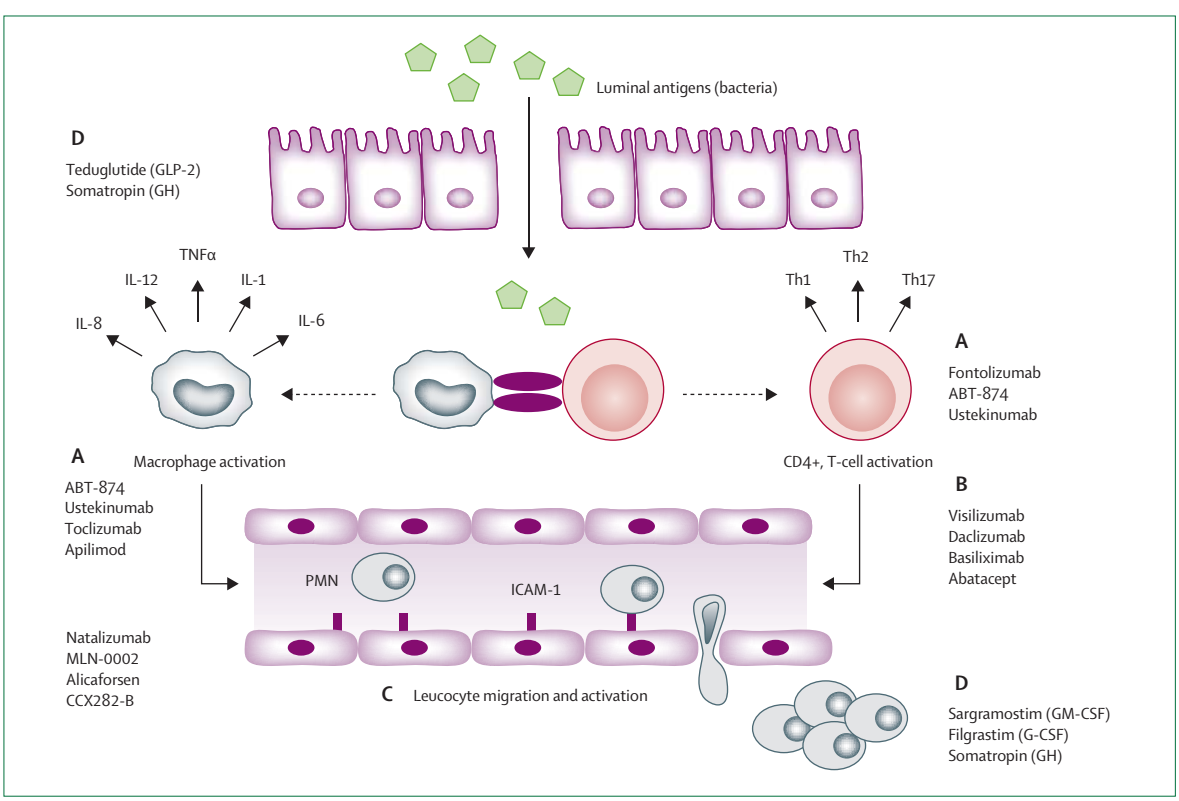

Figure 1: **Overview of therapeutic targets in Crohn's disease: cytokine therapies (A), T-cell blocking agents (B), antiadhesion molecules (C), and growth factors (D)**

The host response to the intestinal microbiota can be categorised into three basic components: the intestinal epithelium, innate immune cells of the myeloid lineages (including macrophages and granulocytes), and adaptive immune cells (including CD4+ T-helper 1 cells). The most crucial environmental factor in the pathogenesis of Crohn's disease might be the luminal flora. Defects in the intestinal innate immune barrier (consisting of several cell types including the epithelial and immune cells), leading to aberrant early innate immune response towards bacterial threats, might start an excessive adaptive immune response dominated by mucosal CD4+ lymphocytes. Classic Crohn's disease has a predominant Th1 cytokine profile that is characterised by interleukin 12 and interferon γ. TNF=tumour necrosis factor α. ICAM-1=intercellular adhesion molecule-1. GM-CSF=granulocyte-macrophage colony-stimulating factor. G-CSF=granulocyte colony-stimulating factor. GH=growth hormone. Th=T-helper. GLP-2=glucagon-like peptide 2. IL=interleukin. PMN=polymorphonuclear.

of different effector pathways in responders and non-responders.[1] Newer antiTNF drugs such as certolizumab pegol and adalimumab have similar efficacy to infliximab.[19–21] Patients who have been previously given infliximab who have lost response or become intolerant can respond to alternative biological drugs targeting TNF.[22] However, an overall decrease is noted in the absolute proportion of responses to the second agent, suggesting that some patients who previously responded might not have benefits of targeting TNF.[22] This finding emphasises the need for developing novel biological drugs for the treatment of Crohn's disease.

These advances in our understanding of the pathophysiology of inflammatory bowel disease have led to new therapeutic opportunities (figures 1 and webfigure 1).[2,23] The many therapies being investigated include cytokine and anticytokine therapies, T-cell blocking agents, antiadhesion molecules, and new immunomodulatory strategies (tables 1 and 2). In this review, we will discuss mechanisms of action of new biological drugs, their efficacy, and safety profiles, and will review previous innovative therapies and future directions for treatment of Crohn's disease (table 3).

Mechanisms of action of novel biological therapies

T-cell blockade

The T cell is pivotal in orchestration and promotion of the immune response in inflammatory bowel disease. Most therapies for inflammatory bowel disease aim to inhibit T-cell function, block the generation of T-cell pro-inflammatory cytokines, or induce apoptosis of T cells or a particular subset of these cells.[23] CD4+ T lymphocytes recognise antigens that have been processed and are presented in association with a self class II MHC molecule to initiate the immune response.[23,74] After the development of CD4+ T-cell antagonists, such as cM-T412,[24,25] other antibodies have been generated against more specific T-cell subsets: CD3+ cells (visilizumab) and CD25+ (daclizumab and basiliximab).[23] Visilizumab is a non-Fc receptor-binding antiCD3 monoclonal antibody directed against the invariant CD3ε chain of the T-cell receptor (webfigure 2).[75] Unlike the prototypic murine antiCD3 monoclonal antibodies, which induce T-cell activation by FcR binding and recruitment of antigen-presenting cells, non-FcR-binding antiCD3 monoclonal antibodies do not activate resting T cells and therefore induce less toxic effects from cytokine release in vivo.[75]

Visilizumab induces apoptosis selectively in activated T cells, and it is much more effective in this respect than are murine antiCD3 monoclonal antibodies (webfigure 2). Two antibodies against the interleukin-2 receptor (CD25)—namely, daclizumab and basiliximab, have been studied to mimic the activity of cyclosporine, which acts by disruption of the calcineurin pathway. Additionally, because interleukin 2 induces steroid resistance, blockade of this receptor should in principle abrogate this effect.[23]

Blockade of T-cell differentiation or activation

Instead of depletion of a particular T-cell subset, another approach has been to block steps in T-cell development by targeting cytokines and molecules that are involved in T-cell differentiation and activation (webfigure 1).[23] Interleukin 6 is a pleiotropic cytokine that is released in response to interleukin 1 and TNFα, with central roles in immune regulation and inflammation.[76] Accordingly, interleukin 6 offers an attractive target to interrupt inflammation in inflammatory bowel disease at several points. Increased serum concentrations of soluble interleukin 6R were detected in the active stage of inflammatory bowel disease.[77] Tocilizumab (formerly atlizumab) binds to both the membrane-bound form and the soluble form of human interleukin 6R with high affinity and specificity.[28] Fontolizumab is a humanised antibody directed against interferon γ, which is a key Th1 cytokine driving expression of MHC class II on antigen presenting cells, increasing chemokine secretion and activating macrophages, lymphocytes, and endothelial cells.[76] Because of the proinflammatory role of interleukin 23,[3] attention is now being focused on molecules specifically targeting the interleukin-23 p19 subunit in addition to developing drugs—such as apilimod mesylate (STA 5326), ABT-874, and ustekinumab (CNTO 1275)—which block both interelukin-23 and interleukin-12 activities.[32,34,78]

T cells need both antigen-specific and costimulatory signals for their full activation (figure 2).[79] ch5D12 blocks the CD40/CD40L costimulatory pathway; CD40 belongs to the TNF receptor family.[79] On the basis of a second T-cell surface molecule that is homologous to CD28—CTLA-4 (CD152), which has a 20-fold higher affinity for the CD80 and CD86 ligands than does CD28—abatacept has been developed (figure 2).[79] Another selective costimulation blocker, belatacept (LEA29Y), was designed by substitution of two amino acids in the abatacept CD80/CD86-binding domain to increase avidity to CD86 and provide the potency needed for immunosuppression in transplantation.[79] It has not yet been tested in inflammatory bowel disease.

See Online for webfigure 2

Resetting T-cells

Patients with Crohn's disease receiving allogeneic bone-marrow tansplants for unrelated disorders had extended remission of their Crohn's disease, providing evidence of the role of bone-marrow T cells (either T-helper or regulatory T cells) in this disease.[80] Although not curative, reconstitution of a normal T-cell balance by autologous haemopoietic stem-cell transplantation resulting in the elimination of all circulating T cells has been used to treat a range of autoimmune, T-cell driven diseases,[23] including multiple sclerosis and rheumatoid arthritis.[81] In theory, a transplant conditioning regimen would ablate aberrant disease-causing immune cells, whereas haemopoietic stem cells would regenerate an antigen-naive immune system.

	Development status	Compound	Manufacturer	Target	Compound class	Clinical efficacy		Biological efficacy (CRP)	References
						Induction	Maintenance		
T-cell blockade	Phase I/II	cM-T412	Centocor, Malvern, PA, USA	CD4 on T-cell surface	Chimeric mAb	No placebo group	NA	NA	24,25
		Visilizumab	PDL Biopharma, Fremont, CA, USA	CD3 on T-cell surface	Humanised Fc IgG2 receptor -non-binding mAb	No placebo group	NA	+	26,27
Blockade of T-cell differentiation or activation	Phase III	Abatacept*	Bristol Mayers Squibb, New York, NY, USA	Blockade of CD28 costimulatory pathway	Soluble recombinant fusion protein	Not yet available	Not yet available	Not yet available	NA
	Phase I/II	Tocilizumab	Chugai Pharmaceuticals, Fremont, CA, USA	IL-6 receptor	Humanised mAb	+	NA	+	28
		Fontolizumab/ HuZAF	PDL Biopharma, Fremont, CA, USA	Interferon γ	Humanised mAb	–	NA	+	29–31
		ABT-874/ J695	Abbott, Parsipanny, PA, USA	IL-12/IL-23, p40	Humanised mAb	+	NA	NA	32
		Ustekinumab (CNTO 1275)	Centocor, Malvern, PA, USA	IL-12/IL-23, p40	Human mAb	+	NA	+	33
		Apilimod mesylate/ STA 5326	Synta Pharmaceuticals, Lexington, MA, USA	IL-12/23	Small molecule	No control group	NA	NA	34
		ch5D12	Tanox, Houston, TX, USA	CD40 on antigen-presenting cells	Chimeric mAb	No placebo group	NA	NA	35
Resetting T cells	Phase I/II	Haemapoietic stem cell transplantation†	Northwestern University, Evanston/Chicago, IL, USA	Autologous haemopoietic stem cells	Cell therapy	No placebo group	NA	NA	36
AntiTNF strategies	Phase I/II	Semapimod/CNI-1493	Cytokine PharmaSciences Inc, King of Prussia, PA, USA	JNK and p38 MAP kinases	Synthetic guanylhydrazone	No placebo group	NA	+	37,38
		Doramapimod/ BIRB 796	Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT, USA	p38 MAP kinase	Small molecule (member of the N-pyrazole-N-naphthly urea class)	–	NA	+	39
		Thalidomide	Pharmion, Camberley, UK	Antiangiogenic and anti-inflammatory (TNFα) properties	Synthetic derivative of glutamic acid	No placebo group	No placebo group	NA	40
Regulatory T-cell modulation	Phase I/II	IL-10	Shering-Plough, Kenilworth, NJ, USA	IL-10	Recombinant human cytokine	–	NA	NA	41–43
		Oprelvekin/IL-11	Wyeth, Madison, NJ, USA	IL-11	Recombinant human cytokine	–	NA	–	44–46
		Lactococcus lactis (LL-Thy12) expressing mature human IL-10	ActoGeniX, Ghent, Belgium	IL-10	Living non-pathogenic micro-organisms expressing IL-10 (TopAct system)	No placebo group	No placebo group	NA	47
Blocking cell recruitment	Phase III	Natalizumab	Elan, Dublin, Ireland	Leucocyte α4β1 and α4β7 integrins	Humanised mAb	+	+	+	48–56
		Alicaforsen/ISIS-2302	Isis Pharmaceuticals, Carlsbad, CA, USA	Endothelial ICAM-1	Phosphorothioate-modified antisense oligodesoxynucleotide	–	NA	NA	57–62
		CCX282-B	ChemoCentryx, Mountain View, CA, USA	Antichemokine receptor CCR9	Small molecule	+/–	NA	+/–	63,64
	Phase II	MLN-0002/ LDP-02‡	Millennium Pharmaceuticals, Cambridge, MA, USA	Leucocyte α4β7 integrin	Humanised mAb	+/–	NA	–	65

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AntiTNF strategies

In addition to TNF antagonists—such as infliximab, adalimumab, and certolizumab—other antiTNF approaches have been developed to treat inflammatory bowel disease. Mitogen-activated protein (MAP) kinases, belonging to the family of serine or threonine kinases, constitute major NF-kB-independent inflammatory signalling pathways from the cell surface to the nucleus. Four major groups of distinctly regulated groups of MAP kinase cascades led to altered gene expression: ERK1/2, ERK5, JNK, and p38 MAP kinase. Because only p38 MAP kinase and JNK pathways

	Development status	Compound	Manufacturer	Target	Compound class	Clinical efficacy		Biological efficacy (CRP)	References
						Induction	Maintenance		
(Continued from previous page)									
Enhancing repair	Phase II	Teduglutide/ALX-0600	NPS Pharmaceuticals, Salt Lake City, UT, USA	Intestinal GLP-2 receptors	Analogue of human peptide GLP-2	+	NA	NA	66
		Somatropin/	Eli Lilly,Indianapolis, IN, USA	Intestinal epithelium	GH peptide	+	NA	NA	67
Innate immune stimulation	Phase III	Sargramostim	Berlex (Schering AG), Berlin, Germany	Intestinal epithelium, neutrophils, monocytes	Yeast-derived recombinant human GM-CSF	+/–	NA	NA	68–71
	Phase I/II	Filgrastim	Amgen, Thousand Oaks, CA, USA	Neutrophils	E coli-derived human (G-CSF)	No placebo group	NA	NA	72
Induction of oral tolerance	Phase I/II	Aleqel	Enzo Therapeutics, Farmingdale, NY, USA	Induction of oral tolerance	Autologous colonic extracts	–	NA	–	73
		Opebacan	Xoma, Berkeley, CA, USA	Induction of oral tolerance	Autologous colon-derived antigens	Not yet available	Not yet available	Not yet available	NA

NA=not available. IL=interleukin. TNF=tumour necrosis factor. ICAM-1= intercellular adhesion molecule-1. GLP-2=glucagon-like peptide-2. mAb=monoclonal antibody. MAP=mitogen-activated protein. GH=growth hormone. GM-CSF=granulocyte-macrophage colony-stimulating factor. GCSF=granulocyte colony-stimulating factor. CRP=C-reactive protein. *Phase III trials are in progress in Crohn's disease (abatacept has been approved by the US Food and Drug Administration for rheumatoid arthritis). †Phase II trials are in progress in Crohn's disease (results presented here come from phase I trials). ‡Phase III trials are anticipated in Crohn's disease (results presented here come from phase II trials).

Table 1: **Efficacy of biological agents in clinical trials in Crohn's disease**

contribute to pro-inflammatory response, specific inhibitors, including semapimod and doramapimod (BIRB 796)[37–39] were tested in inflammatory bowel disease. Several therapies, which are presented as TNFα inhibitors, have a more general immunomodulatory effect and are fairly weak inhibitors—such as thalidomide.[40] RDP58 is a decapeptide that is orally available and reduces the activity of TNFα, interleukin 2, interleukin 12, and interferon γ.[23,82]

Regulatory T-cell modulation

Regulatory T cells function to control the inflammatory process directed by other T-helper cells. Interleukin 10 is one of the prototypic products of regulatory T cells and downregulates activation of Th-cell subsets. Interleukin 10 also inhibits macrophage inflammatory cytokine production including TNFα, interleukins 1 and 12, and T-cell associated macrophage activity. Interleukin 10 has therapeutic effects in several preclinical murine models of colitis.[23]

Blocking cell recruitment

Most of the strategies detailed above aim to interrupt cytokine activity through the inhibition of pathways promoting cytokine production, or by directly blocking cytokine action. A different approach aims to block leucocyte migration to sites of inflammation by interfering with cell-adhesion molecules.[23] Most leucocytes, including lymphocytes, monocytes, eosinophils, and basophils, express α4 integrin adhesion molecules.[83,84] The integrins are a large family of heterodimeric, transmembrane glycoproteins that are capable of mediating both cell–cell and cell–matrix interactions.[85] Subunits of α4 integrin are most frequently found adjoined with β1 and β7 subunits. Endothelial ligands for α4 integrins include members of the immunoglobulin superfamily of adhesion molecules, vascular cell adhesion molecule-1 (VCAM-1), and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) (figure 3).[83,84] Inhibitors of selective adhesion molecules interfering with the migration of leucocytes from the bloodstream to the sites of inflammation—a process known as diapedesis—have been developed. Natalizumab and MLN-0002 bind specifically to α4 integrins, whereas the anti-intercellular adhesion molecule-1 (ICAM-1) antisense oligonucleotide ISIS-2302 (alicaforsen) blocks the endothelial cell adhesion molecules (figure 3). ICAM-1 binds β2-integrin leucocyte function-associated antigen-1 (LFA-1). CCX282-B is a drug that targets the chemokine receptor 9 (CCR9), which is a highly specific receptor expressed by T cells migrating selectively to the digestive tract. CCR9-positive cells are recruited into the epithelium of the small intestine because they respond specifically to the CCR9 ligand CCL25 (also called TECK [thymus-expressed chemokine]) expressed by small-intestine cells, and to a lesser extent by colonic cells.[85] Another means to prevent cells from migrating to sites of inflammation uses extracorporeal devices (for instance Adacolumn, Otsuka, Tokyo, Japan) to filter out or lyse leucocytes subsets from whole blood.[86]

Enhancing repair

Rather than interfering with inflammation, teduglutide, a dipeptidyl peptidase IV resistant glucagon-like peptide-2 (GLP-2) analogue,[66] and somatropin, a growth hormone, can overcome the detrimental effects of the inflammatory process by driving restitution of the epithelium.[67] GLP-2 is a 33 aminoacid peptide, which is

secreted from enteroendocrine L cells in the distal ileum and colon in a biphasic pattern, in response to nutrient ingestion including glucose, fatty acids, and dietary fibre; it stimulates intestinal growth through poorly understood paracrine or neural pathways, or both.[87] Action of growth hormone is mostly mediated by growth-hormone-dependent hepatic production of insulin-like growth factor-1, which has been defined as

	Compound	Half-life	Mode of administration	Adverse effects	Immunogenicity (% antibodies against compound in the active group)	References
T-cell blockade	cM-T412	NA	Intravenous	Fever, chills, headhache; sigmoid perforation (one case)	NA	24,25
	Visilizumab	NA	Intravenous	Transient increase of transaminases, headache, pyrexia, cytokine release syndrome	NA	26,27
Blockade of T-cell differentiation or activation	Abatacept*	13 days	Intravenous	Headache, nasopharyngitis, dizziness, infusion reactions*	1·4%*	NA
	Tocilizumab	4 days	Intravenous	Common cold, nausea, pharyngolaryngeal pain, headache, retching, vomiting, insomnia; gastrointestinal bleeding (two cases), paralytic ileus (one case)	None	28
	Fontolizumab/HuZAF	18 days	Intravenous	Asthenia, chills, fever	3–8%	29–31
	ABT-874/J695	NA	Subcutaneous	Injection-site reactions	5%	32
	Ustekinumab (CNTO 1275)	NA	Intravenous, subcutaneous	One case of disseminated histoplasmosis	NA	33
	Apilimod mesylate/STA 5326	NA	Oral	Nausea, dizziness, headache, fatigue	NA	34
	ch5D12	8–10 days	Intravenous	Pyrexia, arthralgia, myalgia, headache	11%	35
Resetting T-cells	Haemapoietic stem-cell transplantation	Not applicable	Not applicable	Fever, neutropenia	Not applicable	36
AntiTNF strategies	Semapimod/CNI-1493	NA	Intravenous	Transient rise of liver enzymes, injection-site reactions	NA	37, 38
	Doramapimod/BIRB 796	NA	Oral	Transient increase of liver enzymes	NA	39
	Thalidomide	5–8 h	Oral	Sedation, neuropathy	NA	40
Regulatory T-cell modulation	IL-10	1·5–3 h	Subcutaneous	Diarrhoea, arthralgia, headache, fever, dizziness, back pain, anaemia, thrombocytopenia	None	41–43
	Oprelvekin/IL-11	2–3 h	Subcutaneous	Injection-site reactions, fever, arthralgia, raised platelet count, headache, oedema, hypereosinophilia (one case)	None	44–46
	Lactococcus lactis (LL-Thy12) expressing mature human IL-10	Not applicable	Oral	Only minor adverse events	Not applicable	47
Blocking cell recruitment	Natalizumab	132–161 h	Intravenous	Headache, nausea, abdominal pain, influenza, hypersensitivity-like reactions, nasopahryngitis; progressive multifocal leucoencephalopathy (one per 1000 treated patients), basal cell carcinoma (three cases)	8–9·5%	48–56
	Alicaforsen/ISIS-2302	1·5 h	Intravenous (or subcutaneous in one study)	Fever, chills, myalgia, arthralgia, headache, injection-site reactions	1%	57–62
	CCX282-B	NA	Oral	Headache	NA	63,64
	MLN-0002/LDP-02	9–12 days	Intravenous	Similar to placebo†	24%†	65
Enhancing repair	Teduglutide/ALX-0600	7 min (for intravenous formulation)	Subcutaneous	Abdominal pain and injection-site reactions; possible intestinal malignancy in animal studies	NA	66
	Somatropin	3 h	Subcutaneous	Oedema, headache; renal adenocarcinoma (one case), benign schwannoma impinging on the spine (one case)	NA	67
Innate immune stimulation	Sargramostim	NA	Subcutaneous	Injection-site reactions and bone pain	1·3%	68–71
	Filgrastim	3·5 h	Subcutaneous	Bone pain, viral-like syndrome (one case)	NA	72
Induction of oral tolerance	Aleqel	Not applicable	Not applicable	No treatment-related adverse events	Not applicable	73
	Opebacan	Not applicable	Not applicable	Not yet available	Not applicable	NA

NA=not available. TNF=tumour necrosis factor. IL=interleukin. *Data from trials in rheumatoid arthritis. †Data from trials in ulcerative colitis.

Table 2: **Adverse effects of biological agents (with their method of administration, half-life, and immunogenicity) in clinical trials in Crohn's disease**

an important intestinal growth factor.[88] Studies in both human beings and animals have shown that recombinant human growth hormone can enhance protein synthesis, promote tissue recovery, and stimulate intestinal epithelial growth.[89,90] However, the mechanism underlying its protective effect on the intestinal mucosa barrier is not understood.

Innate immune stimulation

Growth factors have emerged as a new therapeutic class of molecules. Clinical trials investigated efficacy and safety profile of two immune stimulatory molecules: sargramostim, a granulocyte-macrophage colony-stimulating factor (GM-CSF) targeting neutrophils, monocytes, and epithelial cells; and filgrastim, a granulocyte colony-stimulating factor (G-CSF) that is widely used for treatment of neutropenic oncological patients receiving chemotherapy for malignancies, acting earlier in haemopoietic lineage and targeting specifically neutrophils.[72] Growth hormone can also have stimulatory effects on neutrophil function.[91]

Induction of oral tolerance

Oral tolerance, a long-recognised method of inducing immune tolerance or systemic hyporesponsiveness induced by feeding protein, has been used to prevent or treat many T-cell-mediated autoimmune disorders.[92] Crohn's disease is characterised by breakdown of tolerance to the intestinal bacterial flora,[93] and feeding colonic extracts prevented colitis in an animal model of inflammatory bowel disease.[94] Therefore, induction of oral tolerance by feeding autologous colonic extracts can offer a new targeted therapy for patients with inflammatory bowel disease.[73]

Efficacy of novel biological drugs in clinical trials

T-cell blockade

Table 1 shows the efficacy of new biological drugs in clinical trials. First attempts to block CD4+ T-cell function with cM-T412 have failed in two open-labelled pilot studies enrolling 24 patients.[24,45] Visilizumab has been assessed in both luminal and fistulising Crohn's disease in two phase I trials[26,27] and in ulcerative colitis.[95] Eight patients

	Manufacturer	Mechanisms of action	Compound class	Other indications
Phase II				
THC:CBD (high CBD)	GW Pharmaceuticals, Salisbury, UK	Cannabinoid receptor agonist	Small molecule (botanical extract)	Rheumatoid arthritis
Mesenchymal adipocyte stem cells/ Cx401	Cellerix, Madrid, Spain	Autologous stem-cells transplantation	Cell therapy	
Mesenchymal stem cells	Osiris Therapeutics, Columbia/Baltimore, MD, USA	Ex-vivo cultured adult human mesenchymal stem cells	Cell therapy	
Phase I				
C326	Avidia, Mountain View, CA, USA	IL-6 antagonist	Avimer (non-immunoglobulin protein)	
MDX-1100	Medarex, Princeton, NJ, USA	Chemokine IP-10 (also known as CXCL10) antagonist	Fully human mAb	
AntiVAP-1 antibody	BioTie Therapies Corp, Turku, Finland	Human antiVAP-1 (also known as SSAO) antagonist	Fully human mAb	Psoriasis
Guanilib (SP-304)	Callisto Pharmaceuticals, New York, NY, USA	Guanylyl cyclase receptor agonist, with both anti-inflammatory and antiproliferative effects	Small molecule	Colon and haematological malignancies
r-IL-18 BP	Merck Serono SA, Geneva, Switzerland	IL-18 antagonist	Recombinant IL-18 binding protein	Rheumatoid arthritis and atherosclerosis
Preclinical studies				
VT-214	Viron therapeutics, London, ON, Canada	Chemokine antagonist	Protein originating from viral sources	Lupus
VT-346	Viron therapeutics, London, ON, Canada	Cytokine antagonist	Protein originating from viral sources	Rheumatoid arthritis
HuMax IL-15 (AMG-714)	Genmab, Amgen, Immunex, Thousand Oaks, California, USA	IL-15 antagonist	Fully human mAb	Rheumatoid arthritis and psoriasis
Thymosin beta4	RegeneRx, Bethesda, MD, USA	Actin binding protein (wound repair)	Peptide (43 aminoacids)	Sepsis
IPL-42	Inflazyme Pharmaceuticals LtdRichmond, BC, Canada	PDE4 inhibitor	Small molecule	Asthma
Revlimid (CC-5013)	Celgene Corp, Summit, NJ, USA	TNFα inhibitor, angiogenesis inhibitor, IL-10 agonist, with both anti-inflammatory and antiproliferative effects	Small molecule (thalidomide derivative)	Oncology
AntiCD103 antibody	LigoCyte Pharmaceuticals, Bozeman, MT, USA	CD103 (also termed HML-1 or αEβ7 integrin, higly expressed on intraepithelial and lamina propria T cells) antagonist	Murine mAb	Graft *vs* host disease, psoriasis, chronic pulmonary obstructive disease
Antiβ7 antibody	Genentech, South San Francisco, CA, USA	β7 integrin antagonist	mAb	

TNF=tumour necrosis factor. mAb=monoclonal antibodies. IL=interleukin. VAP-1=antivacular adhesion protein-1. SSAO=semicarbazide-sensitive amine oxidase. THC=tetrahydrocannibinol. CBD=cannabinoid. PDE4=phosphodiesterase 4.

Table 3: **Pipeline compounds in inflammatory bowel disease**

with luminal Crohn's disease refractory to infliximab therapy received 10 μg/kg intravenous visilizumab once every day for 2 days and were followed up for at least 59 days. Three complete clinical remissions were recorded throughout the study and one other at day 59. Six of the eight patients had 100-point clinical response at day 59. Concentrations of C-reactive protein decreased quickly after start of treatment and remained low in two patients who achieved clinical remission.[26] These encouraging results led to the start of a phase I/II dose-finding[95] and placebo-controlled phase III study in patients with severe steroid refractory ulcerative colitis; however, an interim analysis of 90 randomised patients in this placebo-controlled trial did not show efficacy and study enrolment was stopped.[96]

Visilizumab was not effective for fistula closure in a small series of 28 patients,[27] and development of this drug for inflammatory bowel disease has been stopped. No data are available for daclizumab and basiliximab in Crohn's disease. In ulcerative colitis, four studies assessed daclizumab (humanised antibody)[97,98] and basiliximab (chimeric antibody),[99,100] with varying results. Because prelimary data suggested that clinical and endoscopic responses did not differ between active and placebo groups at week 8,[98] development of daclizumab has been stopped. Two uncontrolled trials enrolling 30 patients showed that basiliximab can be effective in steroid-resistant ulcerative colitis.[99,100] A phase II trial is in progress in ulcerative colitis.

Blockade of T-cell differentiation or activation

Two phase III trials have established the efficacy of abatacept for the treatment of rheumatoid arthitis.[101,102] Abatacept is approved in the USA for patients with rheumatoid arthritis who failed any other type of disease-modifying drug, and in Europe for those who failed other disease-modifying drugs including TNF antagonists.[103] Phase III studies of abatacept are in progress in Crohn's disease and ulcerative colitis. No phase I or II studies have been done in inflammatory bowel disease.

Tocilizumab has shown promising results in a small phase I/II study (n=36) that met its primary endpoint. At 12 weeks, response rates (with response defined as a reduction of Crohn's Disease Activity Index [CDAI] ≥70) were higher in patients given an 8 mg/kg infusion of tocilizumab every 2 weeks than in those given placebo (80% *vs* 31%; p=0·019), and were accompanied by a decrease in C-reactive protein concentrations. However, only two of ten patients went into remission, compared with none of 13 in the placebo group (p=0·092), and no significant improvement in mucosal healing was noted compared with placebo.[28] A European phase II trial (CHARISMA)[104] compared several tocilizumab doses with and without concomitant methotrexate in 359 patients with active rheumatoid arthritis despite receiving methotrexate treatment. The American College of Rheumatology 20% (ACR20) responses differed significantly with tocilizumab compared with placebo plus continued methotrexate (p<0·05 without and p<0·0001 with methotrexate).[104] Phase III trials are underway in rheumatoid arthritis.[103]

Fontolizumab has been assessed in three phase I/II dose-ranging studies (0·1, 1, 4, or 10 mg/kg) enrolling a total of 374 patients with moderate to severe Crohn's disease.[29–31] The results of only two studies have been reported.[29,30] Fontolizumab at doses of up to 4·0 mg/kg improved endoscopic lesions and decreased concentrations of C-reactive protein,[29,30] but no study met its primary endpoint, which was defined as induction of clinical response at 1 month;[29–31] thus the development of fontolizumab for Crohn's disease has been stopped.

Interleukins 12 and 23 are targeted by two humanised interleukin 12/23 antibodies, ABT-874 and ustekinumab, and a small molecule apilimod mesylate (STA-3526). ABT-874 has shown promising results in a phase II dose-ranging study including 79 patients with Crohn's disease.[32] 7 weeks of uninterrupted treatment with 3 mg/kg ABT-874/J695 resulted in higher response rates than did placebo administration (75% *vs* 25%; p=0·03). Ustekinumab has been shown to be efficacious in psoriasis.[78] A phase IIa study, assessing this drug in Crohn's disease has been completed.[33] Patients received either one intravenous infusion (4·5 mg/kg) or four consecutive subcutaneous injections (90 mg) every week of ustekinumab. Preliminary results combining intravenous and subcutaneous groups (n=104) suggested that 49% of patients receiving ustekinumab were in clinical response at week 8 with 100 points or more CDAI reduction compared with 30% in the placebo group (p=0·05).[33] In a phase I/IIa dose-escalating trial (n=73), apilimod mesylate was well tolerated (primary objective). At 1 month, remission rates with daily doses of 28 mg and above ranged from 15% to 36%. A decrease in endoscopic score was recorded in a subgroup of 35 patients.[34] An additional phase II dose-escalating trial of apilimod mesylate was undertaken, but the results have not been made public (registered with ClinicalTrials.gov, number NCT00138840). The development of this drug for Crohn's disease has been stopped.

Only one trial has assessed the effects of an antibody directed against CD40 in inflammatory bowel disease. In this phase I study, the efficacy of one infusion of ch5D12 in 18 patients with Crohn's disease was not convincing, with low remission rates (22%) and no dose-response effect.[35]

Resetting T cells

Autologous haemapoietic stem-cell transplantation has shown favourable results in a phase I study (n=12), with substantial and rapid decrease in disease activity, accompanied by gradual improvement in endocopic lesions and quality of life.[36] Phase II trials are underway in Crohn's disease.

AntiTNF strategies

A phase I study suggested a clinical, biological, and endoscopical benefit of semapimod,[38] but a phase II study of 152 patients did not meet its primary endpoint, which was defined as a decrease in CDAI score of 70 points or more at day 29.[37] Doramapimod (BIRB 796), a p38 an inhibitor of mitogen-activated protein kinase, did not show clinical or endoscopic efficacy in a large (n=284) phase II study.[39] Only a transient, but significant, decrease in concentrations of C-reactive protein was recorded compared with placebo. All together, p38 mitogen-activated protein inhibitors are not effective in Crohn's disease.

RDP58 is not further expanded in inflammatory bowel disease because of failed phase II trials (data unpublished). In a retrospective study, thalidomide seems an effective short-term to medium-term treatment in some patients with refractory luminal and fistulising Crohn's disease.[40]

Regulatory T-cell modulation

Recombinant human interleukin 11[44–46] and interleukin 10[41–43] were not effective for Crohn's disease in phase II studies enrolling a total of 285 and 489 patients, respectively; thus their development has been stopped. The use of genetically modified bacteria for mucosal delivery of interleukin 10 might renew this notion, with encouraging results in ten patients included in a phase I placebo-uncontrolled trial.[47]

Blocking cell recruitment

After encouraging results from phase II trials,[48–50] phase III studies have shown that natalizumab was effective for both induction and maintenance of clinical remission in Crohn's disease.[51,52] A large (n=905) induction trial did not meet its primary endpoint: the proportion of patients with a response (defined as a reduction in the CDAI score of at least 70 points from week 0) at the prespecified time of week 10 . At week 10, 56% of patients (408/724) in the natalizumab group had a response (≥70 point decrease in baseline CDAI score) compared with 49% in the placebo group (88/181; p=0·051). A subgroup analysis showed efficacy in patients with C-reactive protein greater than the normal range. In another phase III induction trial in patients with active Crohn's disease—who were randomly assigned to receive natalizumab 300 mg or placebo intravenously at weeks 0, 4, and 8 and had C-reactive protein above the upper limit of normal confirmed efficacy—more patients in the natalizumab group were in response at both weeks 8 and 12 than were those in the placebo group (48% [124/259] *vs* 32% [81/250]; p<0·001).[52] Remission at both weeks 8 and 12 was also achieved in a higher proportion of patients in the natalizumab group than in the placebo group (26% [68/259] *vs* 16% [40/250]; p=0·002).[52] In a phase III maintenance study, 354 patients who had initially responded to natalizumab were randomly assigned again to receive maintenance therapy with placebo or natalizumab 300 mg every 4 weeks. Both primary endpoints (70-point response and clinical remission) were significantly met through 36 weeks. Natalizumab maintenance therapy continued to provide statistically better sustained remission rates at week 60 than did placebo.[51] Steroid-free remission rates were also significantly higher in the active group than in the placebo group through 60 weeks.

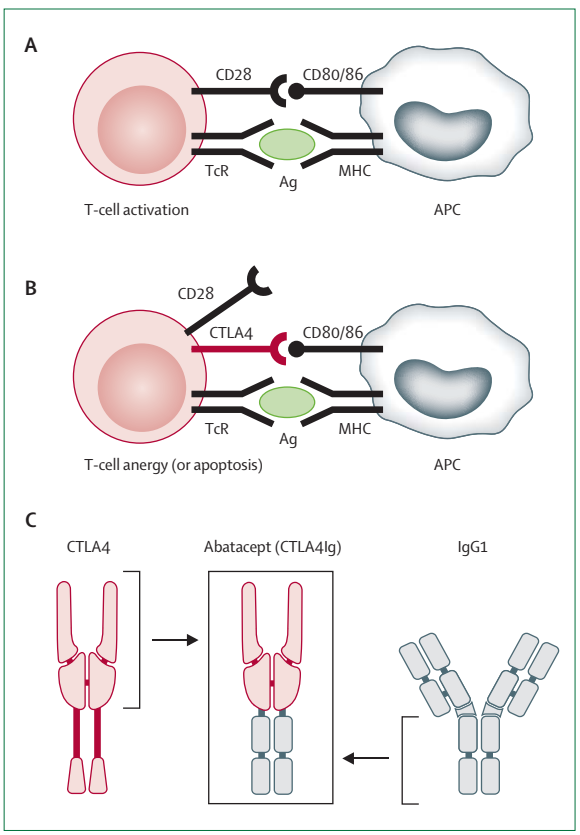

Figure 2: **Structure and mechanism of action of abatacept**
(A) Naive T cells need antigen (Ag)-specific interactions (T-cell receptor [TcR] with the MHC) and costimulatory signals for their full activation. CD28 interaction with its ligands, CD80, and CD86 is essential for initiation of antigen-specific T-cell responses. CD28 is expressed constitutively on the surface of most human T cells. CD86 is constitutively expressed on antigen-presenting cells (APCs), including B cells, macrophages, and dendritic cells, whereas CD80 expression is induced by extended T-cell stimulation. (B) Cytotoxic T lymphocyte-associated antigen 4 (CTLA4) is a higher-avidity receptor for CD80 and CD86 than is CD28. Native CTLA4 and abatacept (CTLA4Ig) interrupt CD28 costimulation of T cell, resulting in an attenaution of interleukin 2 and interleukin-2 receptor (T-cell anergy) and arrest of T cells at the G1 phase of the cell cycle (T-cell apoptosis). (C) Abatacept is a soluble recombinant fusion protein.

Alicaforsen was first assessed in five studies yielding mixed results.[56–61] Two large randomised, placebo-controlled studies enrolling a total of 331 patients confirmed that this molecule is ineffective in Crohn's disease.[62] The encouraging results from a phase II induction trial (n=71) with CCX282-B[63] prompted initiation of a 12-week phase III trial (the PROTECT-1

trial).[64] Preliminary results from this study in 162 patients showed that 68% and 42% of all patients (pooled results of the placebo and CCX282-B groups) were in clinical response and remission, respectively. These results are difficult to interpret because data for the placebo group have not yet been presented.

Leucocyte apheresis remains an invasive procedure, despite some promising results for efficacy and its use in Japan in refractory Crohn's disease.[105] Additionally, a phase III sham-controlled trial in ulcerative colitis did not show efficacy.[106]

Preliminary results from a 6-week, randomised, placebo-controlled, phase II study (n=185) suggested that MLN-0002 might be effective for Crohn's disease.[65] At 57 days, the primary endpoint—a decrease of greater than 70 points on the CDAI—was not achieved. However, the investigators recorded a significant difference between the 2 mg/kg (36·9%) and placebo (20·7%) groups in achieving remission, the secondary endpoint, and a trend towards dose response.[65] Phase III studies are anticipated.

Enhancing repair

In a phase II dose-ranging study (n=100), investigators reported a fairly small decrease in CDAI and no statistical difference in remission rates at week 8 (primary endpoint) with teduglutide 0·2 mg/kg per day compared with placebo.[66] A phase II trial (n=37) in which somatropin (growth hormone) was prescribed in association with a high-protein diet reached its primary endpoint—a decrease in CDAI score at 4 months.[67]

Innate immune stimulation

Although a pilot study (n=15) of sargramostim showed encouraging results,[68] a phase II trial (n=124) did not meet its primary endpoint—a 70-point response at day 57.[69] However, 70-point response rates at other times, as well as 100-point response and remission rates on day 57 and improvements in the health-related quality of life, were significantly higher than they were with placebo.[69] Sargramostim significantly increased the rate of patients achieving corticosteroids-free remission compared with placebo in a phase II study (NOVEL 2; n=127),[70] but sargramostim was not better than was placebo in a large phase III induction study (NOVEL 4; n=286).[71] Thus, development of sargramostim for Crohn's disease has been stopped.

In a small open-label trial (n=20) assessing filgrastim, 11 patients (55%) showed a decrease of at least 70 points in CDAI scores from baseline (primary endpoint) at week 11, and five (25%) achieved a sustained remission.[72] Phase II trials are in progress.

Induction of oral tolerance

Efficacy of an extract of autologous colonic proteins that has been tested in a phase II trial (n=31) remains to be proven.[73] Phase II trials are in progress.

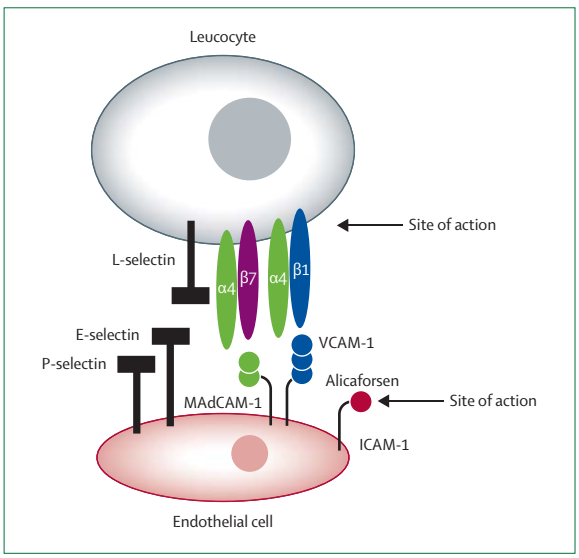

Figure 3: **Mechanisms of action of antiadhesion molecules**
Most leucocyctes express α4 integrin adhesion molecules—a family of cell surface glycoproteins—which is crucial in leucocyte migration and can contribute to maintain a chronic inflammatory state through direct leucocyte activation. The α4 integrin subunits are most frequently found adjoined with β1 and β7 subunits, which bind to vascular cell adhesion molecule-1 (VCAM-1) and mucosal vascular addressin cell-adhesion molecule-1 (MAdCAM-1) that are expressed on endothelial cells. The humanised (contains >90% human sequences) monoclonal antibody natalizumab recognises both α4β1 and α4β7 integrins. MLN02 binds specifically to α4β7 integrin, which is primarily associated with recruitment of leucocytes to the gut. Alicaforsen (ISIS-2302) is a phosphorothioate-modified antisense oligodesoxynucleotide that is designed to sequence-specifically reduce intercellular adhesion molecule-1 (ICAM-1) messenger mRNA level.

Adverse effects of novel biological drugs

Table 2 shows the adverse effects of biological drugs that are in clinical trials. Most adverse events were mild or moderate in severity with T-cell blocking agents. No lymphoproliferative or life-threatening adverse events were reported in patients given visilizumab, but two serious adverse events were noted, although no details were given.[26] The long-term safety profile of visilizumab remains to be established in a large cohort of patients. A cytokine release syndrome (fatigue, nausea, chills, and headache) that is detected in up to 80% of patients receiving visilizumab might need systematic medication with 1000 mg of acetaminophen 1–2 h after each drug administration.

Except for influenza-like symptoms, molecules for blockade of T-cell differentiation of activation were generally well tolerated.[28–31,34,35] Two cases of gastrointestinal bleeding and one paralytic ileus occurred in patients given tocilizumab, which were regarded as possibly-related to this drug by the investigators. Despite one case of dissiminated histoplasmosis that was noted in a patient given ustekinumab, short-term treatment with interleukin 12 or 23 antagonists was generally well tolerated.

No serious adverse events were noted with autologous haemopoietic stem-cell transplantation. Other than fever that was either neutropenic or disease related, the

procedure for resetting T cells was well tolerated without a documented infection.[36]

An increase in transaminase concentrations, which was seen more frequently in patients given MAP kinase inhibitors than in those given placebo, restricts the use of antiTNF strategies in inflammatory bowel disease.[37,39] The use of thalidomide is limited by its toxic effects; in a recent study, 12 of 25 patients discontinued treatment because of adverse effects (three sedation, two abdominal pain, one leucopoenia, six neuropathy).[40]

As with molecules blocking T-cell differentiation or activation, recombinant interleukin 10 and interleukin 11 for regulatory T-cell modulation were generally well tolerated except for influenza-like symptoms. Topical delivery of immunomodulatory proteins, such as interleukin 10, avoids systemic side-effects.[47] However, long-term safety of living bacterium that is genetically modified is unknown. Anaemia and thrombocytopenia occurred more frequently in the patients given recombinant interleukin 10 than in the placebo population.[42]

Concerns have arisen about the safety profile of natalizumab as a result of three cases of progressive multifocal leucoencephalopathy (PML), which occurred in patients given the drug; two cases were fatal.[53–55] Two cases occurred in patients with multiple sclerosis receiving concomitant β-1a interferon treatment, and one of the two fatal cases occurred in a patient with Crohn's disease receiving natalizumab as part of an open-label extension trial. This patient had been given several therapies, including long-term daily azathioprine, and had had severe intermittent lymphopenia for about 6 years. Results of extensive safety analyses of more than 3000 patients who had participated in clinical trials of natalizumab have identified no additional confirmed case of PML, suggesting a risk of development of PML of roughly one in 1000 patients given this drug.[56] So far, more than 20 000 patients have been given natalizumab worldwide (4500 in clinical studies and 21 900 after marketing) (Elan, Dublin, Ireland; data on file). No other cases of PML have been reported. MLN-0002 binds specifically to α4β7 integrin, which is primarily involved in the recruitment of leucocytes to the gut. This specificity for the vasculature of the gut might reduce the risk of PML. PML is a rare but mostly fatal opportunistic brain infection caused by reactivation of a latent JC virus infection. Recently, JC viral loads were shown to be significantly higher in patients with immunosuppressed Crohn's disease than in healthy volunteers.[107] The ENACT trials reported one each of varicella pneumonia (after exposure to a child who had varicella–zoster) and cytomegalovirus hepatitis (judged by the investigator not to be serious), both in patients receiving natalizumab. Both cases resolved with appropriate treatment with no sequelae.[51] No opportunistic infections were reported during the ENCORE trial.[52]

Growth-promoting properties of agents enhancing intestinal repair in animal studies, including teduglutide,[108] argue against their use in patients with inflammatory bowel disease who are at increased of developing colon cancer, even if resolution of the inflammatory process might counterbalance this potential increased risk. One renal adenocarcinoma and one benign schwannoma impinging on the spine were identified during a trial of growth hormones, but no colorectal tumours were noted.[67]

One of the most common side-effects of sargramostim and filgrastim is bone pain, usually in the lower back or pelvis and lasting only a few days. Another common side effect is a flu-like syndrome.

Autologous colon-extracted proteins and mesenchymal adipocyte stem cells were well tolerated by all patients.[73]

Short-term perspective

None of the T-cell blocking agents developed so far have reached phase III studies in Crohn's disease, despite a well established Th1-type cytokine profile. In molecules that are capable of blocking T-cell differentiation or activation, a lot is expected from abatacept, since infusion every month of abatacept significantly reduced disease activity in patients with rheumatoid arthritis and an inadequate response to methotrexate.[101] Abatacept has been approved by the US Food and Drug Administration (FDA) for rheumatoid arthritis.[103] Similarly to rheumatoid arthritis, abatacept might be beneficial to patients with Crohn's disease and inadequate response to TNF antagonists. Although the notion of innate immune stimulation to potentially restore defects in the intestinal innate immune barrier (composed of several cell types including the epithelial and immune cells) seemed promising in Crohn's disease, a phase III study yielded disappointing results for sargramostim, and development has stopped.[71] Finally, long-term risk-benefit ratio of strategies resetting T cells or inducing oral tolerance remains to be defined.[36,73]

Overall, apart from TNF antagonists, only anti-adhesion molecules have shown efficacy in Crohn's disease (table 1). In January, 2008, because the risk-benefit ratio of natalizumab looked favourable in this disease, the FDA approved natalizumab for moderate-to-severe Crohn's disease in patients who did not respond to or could not tolerate other available therapies, including TNF antagonists. Similar to its approval in multiple sclerosis, the FDA approved natalizumab for Crohn's disease with the requirement of mandatory participation in a risk management and registry programme. Its use is contraindicated in immunocompromised patients and in those concomitantly receiving immunosuppressants or modulators. The approval of natalizumab might accelerate the start of a phase III study for MLN-0002 in Crohn's disease.[64]

Future directions

Table 3 shows the compounds that are being developed for inflammatory bowel disease.

In the near future, present technologies, such as humanised or chimeric monoclonal antibodies, will still be used to target new pathways. For example, an anti-vacular adhesion protein-1 (VAP-1) monoclonal antibody is being investigated. VAP-1 is a cell-associated, inflammation-inducible, endothelial cell-adhesion molecule that mediates the interaction between leucocytes and activated endothelial cells in inflamed vessels.[109] Because Crohn's disease is associated with a dysregulation of many variables of the immune response, its successful management might need a combination of biological agents targeting several pathways sequentially or concomitantly.[50]

A promising class of antibodies called avimers (from avidity multimer) that are highly expresssed in bacteria is being developed.[110] These non-immunoglobulin proteins, which can bind to several sites on a ligand, have been generated by sequential selection of individual binding domains, each of which recognises a different epitope. Therefore, these antibodies can have high ligand affinity (in the picomolar and even subpicomolar range) and specificity, and could bind simultaneously to many targets. Potent anti-inflammatory activity and low immunogenicity of interleukin-6 inhibitory avimer protein have been shown in mice, but need to be confirmed in human beings.[110] Low immunogenicity can be related to their high thermodynamic stability, small size, high disulphide content of A-domains, and use of human A-domain sequences. Additionally, avimers present a way to circumvent intellectual property restrictions and produce simple and inexpensive molecules in bacteria.[111]

Interest in oral, small molecules, is greater than ever. Oral bioavailability, access to intracellular targets, and cost and ease of production are advantages that small-molecule drugs generally have over large-molecule drugs such as biological agents, natural products, and peptides. Several small molecules, such as SP-304—a guanylyl cyclase receptor agonist with both anti-inflammatory and antiproliferative effects—are being developed in inflammatory bowel disease.

Research also focuses on compounds with novel mechanisms of action. For instance, the inhibitory effects of cannabinoids on intestinal inflammation, nausea, diarrhoea, and abdominal pain[112] have led to development of molecules with a high ratio of cannabidiol (a non-psychoactive natural cannabinoid) to tetrahydrocannibinol (the main constituent of the plant *Cannabis sativa*, with psychotropic effects). A phase II trial is in progress. Because mesenchymal stem cells were reported to have both regenerative and immunomodulatory properties, efficacy of adipose mesenchymal stem-cell transplantation was assessed in fistulising Crohn's disease.[113] The accumulating evidence for a central role of luminal flora in pathogenesis of this disease has led to the supposition that manipulation of intestinal flora might have therapeutic benefits. However, no evidence supports the notion that probiotics are effective in Crohn's disease.[114,115] Compounds that are able to restore defects in the intestinal innate immune barrier can also be an attractive approach. Stimulation of pattern-recognition molecules, such as NOD2 and toll-like receptors, can contribute to maintain intestinal homoeostasis.[116]

As a result of decades of intensive research, treatment for inflammatory bowel disease is undergoing a transition from the era of TNF antagonists to an era of novel biological agents, including those that are able to stimulate the innate immune system. In parallel, clinicians are working on new strategies aimed at modification of the natural history of Crohn's disease, including an early aggressive therapeutic approach.[117] Therefore, the mere measurement of disease activity with the CDAI and of quality of life with the inflammatory bowel disease questionnaire could become insufficient. Mucosal healing is already regarded as an important therapeutic goal in inflammatory bowel disease.[118] Development of new indices assessing intestinal damage (which are similar to the Sharp score in rheumatoid arthritis) and loss of physical function also termed disability (similar to the health assessment questionnaire disability index) is also needed.[103]

Finally, safety concerns have clearly become important.[119,120] As with any therapeutic decision, the potential reward should outweigh the potential risk. A better patient selection is now needed to identify individuals with factors of disabling course[121] and to make best possible use of the potential benefit of novel therapeutic approaches. The development of biomarkers with predictive and prognostic capabilities is eagerly expected.[120]

Conflict of interest statement

LP-B has received consulting fees from Abbott Laboratoires and UCB Pharma; lecture fees from speaking at continuing medical education events from Centocor; and grant support from AstraZeneca and UCB Pharma. PD has received consultancy fees from or has been on paid advisory boards for Biofortis, Danisco France SAS, Danone France, Ferring, Giuliani SpA, Roquette, UCB Pharma, and Txcell; received lecture fees from speaking at continuing medical education events from Procter and Gamble, Ferring, Schering Plough, Shire Pharmaceuticals, UCB Pharma; and received grant support AstraZeneca, Danisco France SAS, Danone France, Ferring, Giuliani SpA, Lesaffre, Ocera Therapeutics, Roquette, Sanofi-Synthelabo, UCB Pharma, and Yoplait. WJS has received consultancy fees from or has been on paid advisory boards for Abbott Laboratories, ActoGeniX NV, AGI Therapeutics, Alba Therapeutics, Alizyme, Alza, AstraZeneca, Avidia, Berlex Pharmaceuticals, BioBalance, Boehringer-Ingelheim, Bristol Meyers Squibb, Celegene, Centocor, Cerimon Pharmaceutical, Chemocentryx, CombinatoRx, CoMentis, Corautus Genetics, Cosmo Technologies, Effective Pharmaceuticals, Eisai Medical Research, Elan Pharmaceuticals, Enzo Therapeutics, Eurand, FlexPharm, Genencor International, Genentech, GlaxoSmithKline, H3 Pharma (name changed to Debiopharm SA in 2005), Hoffman LaRoche, Hutchison Medipharma, Inflabloc Pharmaceuticals (previously named Pharmadigm), Inotek Pharmaceutical, ISIS Pharmaceuticals, Jacobus Pharmaceutical Company, Johnson & Johnson Pharmaceutical Research & Development, LigoCyte Pharmaceuticals, McNeil Consumer and Specialty Pharmaceuticals, Medarex, Millenium Pharmaceuticals, Nisshin Kyorin Pharmaceutical Co, Novartis, NPS Pharmaceuticals,

Ocera Therapeutics, Ono Pharma USA, Otsuka American Pharmaceuticals, PDL Biopharma, Pfizer, Procter and Gamble, Prometheus Laboratories, Renovis, Salix Pharmaceuticals, Schering Plough, Serono, Shire Pharmaceuticals, Synta Pharmaceuticals, Targacept, Teva Pharmaceuticals, Therakos, UCB Pharma, and ViaCell; lecture fees from speaking at continuing medical education events from Abbott Laboratories, Axcan Pharmaceuticals, AstraZeneca, Centocor, Elan Pharmaceuticals Falk Pharma, Otsuka American Pharmaceuticals, PDL Biopharma, Procter and Gamble, Prometheus Laboratories, Salix Pharmaceuticals, Schering Plough, Shire Pharmaceuticals, and UCB Pharma; and grant support from Abbott Laboratories, Bristol Meyers Squibb, Centocor, Chemocentryx, Elan Pharmaceuticals, Otsuka American Pharmaceuticals, PDL Biopharma, Procter and Gamble, Shire Pharmaceuticals, and UCB Pharma. J-FC has received consultancy fees from or has been on paid advisory boards for Abbott Laboratories, ActoGeniX NV, AstraZeneca, Berlex, Boehringer-Ingelheim, Bristol Meyers Squibb, Centocor, Cosmo Technologies, Danone France, Elan Pharmaceuticals, Genentech, GlaxoSmithKline, Millenium Pharmaceuticals, Ocera Therapeutics, Otsuka American Pharmaceuticals, PDL Biopharma Schering Plough, Shire Pharmaceuticals, Synta Pharmaceutical, Teva Pharmaceuticals, Therakos, and UCB Pharma; lecture fees from speaking at continuing medical education events supported with unrestricted educational grants from Abbott Laboratories, AstraZeneca, Centocor, Elan Pharmaceuticals, Falk Pharma, Otsuka American Pharmaceuticals, PDL Biopharma, Schering Plough, Shire Pharmaceuticals, UCB Pharma, and Ferring; and grant support from AstraZeneca and Ferring.

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