The value of experimental models of colitis in predicting efficacy of biological therapies for inflammatory bowel diseases

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Valatas V, Vakas M, Kolios G. The value of experimental models of colitis in predicting efficacy of biological therapies for inflammatory bowel diseases. Am J Physiol Gastrointest Liver Physiol 305: G763–G785, 2013. First published August 29, 2013; doi:10.1152/ajpgi.00004.2013.—During the last decade, biological therapies for inflammatory bowel diseases (IBD) have increased significantly, and clinical response to the therapy of choice has been approached from the translational perspective. In the field of inflammatory diseases the majority of biologics under development have failed to reach the clinic. This review examines the ability of preclinical data from animal models of IBD to predict success or failure of biologics in human IBD. Specifically, it describes the murine models of IBD, the mechanism of disease induction, the phenotype of the disease, its relevance to human IBD, and the specific immunological features of disease pathogenesis in each model and mainly compares the results of the phase II and III trials of biologics in IBD with preclinical data obtained from studies in animal models. Finally, it examines the possible reasons for low success in translation from bench to bedside and offers some suggestions to improve translation rates.

KEY WORDS: inflammatory bowel disease; animal models; biologics

IDIOPATHIC INFLAMMATORY BOWEL DISEASE (IBD) is a chronic relapsing inflammation of the gastrointestinal tract. In most cases human IBD can be categorized, on the basis of clinical features and distinct intestinal pathology, into two separate clinical entities: Crohn’s disease (CD) and ulcerative colitis (UC). CD generally involves the ileum and colon, but it can affect any region of the intestine, often discontinuously. Pathology may show lymphoid aggregates giving rise to aphthous ulcers and noncaseating granulomas in the early phase (189). Later on, dense lymphoid aggregates extend transmurally to the submucosa and muscularis propria, potentially giving rise to large ulcers that create fistulas and abscesses usually followed by fibrosis and strictures (2).

UC almost invariably involves the rectum and may affect part of the colon or the entire colon, but not the small intestine, in an uninterrupted pattern. Inflammation is confined to the mucosa and submucosa and consists of diffuse inflammatory infiltrates of neutrophils, lymphocytes, plasma cells, and macrophages that invade the epithelium, giving rise to superficial ulcers and crypt abscesses (35). Both CD and UC are characterized by a chronic relapsing clinical course often accompanied by various extraintestinal manifestations such as joint, ocular, skin, liver, and bile duct inflammation.

Animal models of IBD have been initially described by Kirsner and Elchlepp more than 50 years ago (103). It took more than three decades from those pioneer experiments to jump from chemically induced, self-limiting models to genetically manipulated mice with various defects of immune regulation that develop chronic intestinal inflammation (106). The studies on interleukin (IL)-2, IL-10, and T cell receptor (TCR) knockout (KO) mice (112, 141, 199) and the development of the CD45RBhigh T cell transfer model have revolutionized the field of IBD research (182). Thereafter, genetic engineering, guided partly by human genome-wide association IBD studies, generated more than 50 different mouse models of acute or chronic intestinal inflammation; the most commonly used are listed in Tables 1 and 2 (106).

Experimental colitis models have widely served as tools for the preclinical evaluation of the efficacy of new drugs and especially biologics prior to clinical trial testing. Since a significant number of new biologics for IBD has already completed phase II and III clinical trials, it is now possible to reevaluate the accuracy of preclinical intervention studies based on experimental IBD models in predicting the efficacy of these drugs in human IBD. This review compares the results of the phase II and III trials of biologics in IBD with data obtained from experimental colitis studies in an effort to identify factors that could optimize preclinical evaluation strategies. An electronic search for biologics in IBD was performed in the databases of PubMed and http://www.clinicaltrials.gov and in the abstracts of the yearly meetings of Digestive Disease Week and United European Gastroenterology Week between 2003 and 2013 to identify biologics that have been through phase
Review

G764 ANIMAL MODELS IN DEVELOPMENT OF BIOLOGICS FOR IBD

Table 1. Experimental IBD models related to epithelium and innate immunity defects

<table>
<thead>
<tr>
<th>Model</th>
<th>Pathogenesis</th>
<th>Phenoype</th>
<th>Immunological Features</th>
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<tbody>
<tr>
<td><strong>Epithelial barrier defects</strong></td>
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<tr>
<td>DSS</td>
<td>Chelation of divalent cations (e.g., Ca^{2+}, Mg^{2+}) required for IEC tight junctions</td>
<td>UC-like acute or chronic self-limiting colitis</td>
<td>Innate immunity; M1 macrophages and neutrophils (216); Initially Th1, but later Th1/Th2 (42)</td>
</tr>
<tr>
<td>TNBS</td>
<td>Haptenization of colonic proteins</td>
<td>CD-like acute self-limiting colitis</td>
<td>DTH-like response; Th1 (and possibly Th2) (154)</td>
</tr>
<tr>
<td>Oxazolone</td>
<td>Haptenization of colonic proteins</td>
<td>UC-like acute self-limiting colitis</td>
<td>Th2 and NK T cells (22)</td>
</tr>
<tr>
<td>N-cadherin DN</td>
<td>Loss of IEC cadherin and cell-matrix contacts</td>
<td>CD-like chronic focal small bowel enteritis, adenomas</td>
<td>Th1; Enhanced IEC migration and death (82)</td>
</tr>
<tr>
<td>MUC1/2^{-/-}</td>
<td>Disruption of the MUC2 mucin gene</td>
<td>UC-like distal chronic colitis (254) and gastrointestinal tumors (263)</td>
<td>IEC hyperproliferation and apoptosis defects</td>
</tr>
<tr>
<td></td>
<td>Disruption of the MUC1 mucin gene</td>
<td>CD-like chronic colitis in the CD45RB^{hi} or TCR{g} KO model</td>
<td>Expansion of an innate IL-17 producing lymphoid cell population (158)</td>
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<tr>
<td><strong>Innate immunity defects</strong></td>
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<tr>
<td>CD40 mAb</td>
<td>CD40 mAb activation of myeloid cells in RAG-2^{-/-} mice (250)</td>
<td>Acute self-limiting colitis</td>
<td>IL-12/IL-23-driven inflammation</td>
</tr>
<tr>
<td>Mdr1a^{-/-}</td>
<td>Deficiency of the intestinal transporter P-glycoprotein</td>
<td>UC-like chronic colitis</td>
<td>Enhanced IEC responses to bacteria, chemokine overexpression, Th1 (166, 236)</td>
</tr>
<tr>
<td>TRUC</td>
<td>Disruption of T-bet^{-/-} in RAG-2^{-/-} mice</td>
<td>UC-like chronic transmissible colitis, malignant transformation</td>
<td>Loss of T-bet regulation of TNF-α production by DCs; Epithelial barrier disruption; IEC hyperproliferation and defective apoptosis (52, 262)</td>
</tr>
<tr>
<td>IEC/IKKγ^{-/-}</td>
<td>Conditional targeting of NEMO in IEC</td>
<td>Chronic cecum sparing colitis</td>
<td>Dysregulation of TLR signaling, loss of tolerance to commensals; Lack of defensin-3; Defects in apoptosis and epithelial integrity (153, 172)</td>
</tr>
<tr>
<td>Helicobacter hepaticus → RAG^{-/-}</td>
<td>Hh colonization of lymphopenic RAG^{-/-} mice</td>
<td>Spontaneous chronic colitis</td>
<td>IL-23-driven Th1/Th17 (88, 125)</td>
</tr>
<tr>
<td>NF-κB deficient</td>
<td>Hh in p50^{-/-} and p50^{-/-}p65^{+} mice</td>
<td>Chronic transmural colitis more severe in p50^{-/-}p65^{-/-} (53)</td>
<td>Enhanced macrophage (IL-12p40) and T cell (IFN-γ, TNF-α, IL-1β) responses</td>
</tr>
<tr>
<td><strong>Congenic models with mixed defects</strong></td>
<td></td>
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<tr>
<td>C3H/HeJBr</td>
<td>A strain of TLR-4 lacking, C3H/HeJ mice unresponsive to LPS</td>
<td>CD-like chronic colitis of the cecum and proximal colon, antibodies against microflora (33, 235)</td>
<td>IL-12-driven, Th1</td>
</tr>
<tr>
<td>SAMP1/Yit</td>
<td>Sublines of SAM mice from the AKR/J background</td>
<td>CD like, chronic segmental ileitis and typhlitis with granulomas (111, 129)</td>
<td>Early Th1 later mixed Th1/Th2; Increased epithelial permeability; PPAR-γ has been identified as a susceptibility gene</td>
</tr>
<tr>
<td>SAMP1/YitFc</td>
<td></td>
<td>Accelerated disease, perianal lesions, ulceration, and fistulas (190, 234)</td>
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</tbody>
</table>

CD, Crohn’s disease; DCs, dendritic cells; DN, dominant negative; DSS, dextran sulfate sodium; DTH, delayed-type hypersensitivity; Hh, Helicobacter hepaticus; IBD, inflammatory bowel disease; IEC, intestinal epithelial cell; IKK, inhibitor of IκB kinase; KO, knockout; LPS, lipopolysaccharide; mAb, monoclonal antibody; Mdr, multidrug resistant; MUC, mucin; NEMO, NF-κB essential modulator; NK, natural killer; PPAR-γ, peroxisome proliferator-activated receptor γ; TLR, Toll-like receptor; TNBS, trinitrobenzene sulfonic acid; TRUC, T-bet^{-/-} Rag2^{-/-} ulcerative colitis; UC, ulcerative colitis.

II/III clinical studies in IBD. A subsequent search in the databases of PubMed was performed for their biological targets combined with the keywords “murine colitis” and “experimental colitis” to identify the relevant preclinical studies.

Experimental IBD: Positive and Negative Aspects in Modeling Human Disease

Experimental IBD mouse models can be broadly divided into congenic, chemically induced, genetically engineered, and cell transfer models (137). Genetically engineered models include the various KO and transgenic (Tg) models that develop intestinal inflammation due to targeted disruption or overexpression of known genes, and the transfer models that involve transfer of lymphocytes in lymphopenic mice. Congenic experimental IBD models are generated through crossbreeding of mice with various genetic backgrounds and therefore the genetic defects that contribute to intestinal inflammation are largely unknown (137). In the majority of the experimental IBD models, bowel inflammation develops and persists over the course of a few weeks or months and therefore it is considered “chronic.” In contrast, inflammation develops in a couple of days and lasts less than a couple...
### Table 2. *IBD* models induced by an altered balance between regulatory and effector T cells

<table>
<thead>
<tr>
<th>Model</th>
<th>Pathogenesis</th>
<th>Phenotype</th>
<th>Immunological Features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Excessive effector cell responses</strong></td>
<td></td>
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<tr>
<td>TNF&lt;sup&gt;ΔARE&lt;/sup&gt;</td>
<td>Deletion of a segment in the 3′ UTR region increases TNF-α mRNA stability and</td>
<td>CD-like chronic transmural ileitis/proximal colitis with granulomata, arthritis, alopecia</td>
<td>Th1-driven CD8&lt;sup&gt;+&lt;/sup&gt; effector T cell inflammation (109)</td>
</tr>
<tr>
<td>STAT4 tg</td>
<td>STAT4 overexpression in CD4&lt;sup&gt;+&lt;/sup&gt; T cells under control of a CMV promoter</td>
<td>CD-like chronic transmural ileitis/colitis</td>
<td>Loss of STAT4 transcriptional regulation of IL-12/IL-23 receptor signaling results in enhanced Th1 (273)</td>
</tr>
<tr>
<td>IL-7 tg</td>
<td>Enhanced IL-7 production by mucosal T cells (268)</td>
<td>UC-like chronic panniculitis</td>
<td>Sustained survival of colitogenic IL-7R-expressing memory CD4&lt;sup&gt;+&lt;/sup&gt; T cells, Th1 DCs exhibit decreased IL-10 and drive excessive Th1/Th17 (163, 173); Defective epithelial barrier (49); IEC hyperproliferation and apoptosis resistance (49); Mucosal B cell defects (162)</td>
</tr>
<tr>
<td>Goi2&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Defects of signal transduction through adenylate cyclase</td>
<td>UC-like chronic colitis, malignant transformation (196), CD features, perforation and fibrosis; Strain-dependent</td>
<td></td>
</tr>
<tr>
<td>CD45RB&lt;sup&gt;high&lt;/sup&gt;</td>
<td>Repletion of lymphopenic RAG1&lt;sup&gt;−/−&lt;/sup&gt; or SCID mice of variable genetic backgrounds with naïve CD4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Chronic transmural colitis, strain-dependent small bowel, skin, lung inflammation</td>
<td>Lack of CD45RB&lt;sup&gt;−/−&lt;/sup&gt; Tregs</td>
</tr>
<tr>
<td>IL-10&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Disruption of the IL-10 gene ± NSAID for synchronization</td>
<td>Chronic transmural colitis; Hh accelerates colitis</td>
<td>IL-12/23-dependent Th1/Th17; Th2 in late phase</td>
</tr>
<tr>
<td><em>Helicobacter hepaticus</em> → mAb IL-10R</td>
<td>Hh colonization and mAb IL-10R treatment</td>
<td>Chronic moderate colitis</td>
<td>Increased epithelial permeability (112, 284); IL-23-driven Th1/Th17 inflammation (88, 125)</td>
</tr>
<tr>
<td>IL-2&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Disruption of the IL-2 gene</td>
<td>Multiorgan inflammation, transmural chronic colitis, lymphoproliferation</td>
<td>Lack of Tregs; Deficient activation-induced (Fas-mediated) T cell apoptosis; Enhanced antigen-presentation by epithelial cells, Th1 (116)</td>
</tr>
<tr>
<td>STAT3&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Disruption of the signal transducer and activator of transcription (STAT3) gene in neutrophils and macrophages (expressing lysozyme M gene) by Cre-loxP recombination</td>
<td>Chronic transmural colitis with malignant transformation</td>
<td>Absence of the IL-10 counterregulatory effect on neutrophils and colonic macrophages; TLR-dependent enhanced IL-12p40, Th1 (239)</td>
</tr>
<tr>
<td>CD&lt;sup&gt;+&lt;/sup&gt;ITF-TGFβRII DN</td>
<td>Transgenes encoding dominant-negative TGF-β receptor II under T cell-specific (CD4&lt;sup&gt;+&lt;/sup&gt;) or colonic epithelial cell-specific (ITF) promoters</td>
<td>CD4, Chronic colitis, lung inflammation, autoantibodies and glomerular immune complex deposition ITF, Chronic colitis, susceptibility to DSS, antibodies to goblet cells</td>
<td>Enhanced Th1 and Th2 responses (73)</td>
</tr>
<tr>
<td>IL10R2&lt;sup&gt;−/−&lt;/sup&gt; × TGFβRII DN</td>
<td>Disruption of the IL-10 receptor 2 gene combined with CD4&lt;sup&gt;+&lt;/sup&gt; TGFβRII DN transgenic expression</td>
<td>UC-like chronic transmural colitis</td>
<td>Enhanced TNF-α, IL-6 production, Th1/Th17 (98)</td>
</tr>
<tr>
<td><strong>Inadequate regulatory response</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CD45RB&lt;sup&gt;high&lt;/sup&gt;</td>
<td>Chronic transmural colitis, strain-dependent small bowel, skin, lung inflammation</td>
<td>Chronic transmural colitis</td>
<td>Lack of CD45RB&lt;sup&gt;−/−&lt;/sup&gt; Tregs</td>
</tr>
<tr>
<td>IL-10&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Chronic transmural colitis; Hh accelerates colitis</td>
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<td>IL-10R2&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Multiorgan inflammation, transmural chronic colitis, lymphoproliferation</td>
<td>IL-12/23-dependent Th1/Th17; Th2 in late phase</td>
<td>Increased epithelial permeability (112, 284); IL-23-driven Th1/Th17 inflammation (88, 125)</td>
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<tr>
<td><strong>Intrathymic developmental defects with regulatory and effector T cell imbalance</strong></td>
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<tr>
<td>TCRα&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Lack of TCR-α chains</td>
<td>UC-like superficial colitis with ANCA circulating antibodies</td>
<td>B6 TCR T cells of restricted diversity, that enhance B cell responses and produce IL-4 and IFNγ (Th1/Th2); TCR-β&lt;sup&gt;−/−&lt;/sup&gt; have accessory role in Th2; Lack of Tregs (138)</td>
</tr>
<tr>
<td>Tg26</td>
<td>Transgenic CD3-epsilon chain mice, rescued by normal bone marrow</td>
<td>Chronic transmural colitis</td>
<td>Defective thymic microenvironment, intrathymic T cell and NK cell death due to excessive signal transduction, failure to negatively select aggressive T cells and to positively select Tregs (85, 264)</td>
</tr>
<tr>
<td>CD40L tg</td>
<td>Overexpression of CD40L in T and some B cells under control of the Lck promoter</td>
<td>CD-like chronic ileitis/colitis, multiorgan inflammation; Loss of lymphoid tissue architecture</td>
<td>T cell activation via CD40L interaction with CD40, on APCs; Defective thymocyte development, enhanced negative selection (30)</td>
</tr>
</tbody>
</table>

ANCA, anti-neutrophil cytoplasmic antibody; APCs, antigen-presenting cells; CMV, cytomegalovirus; DNP-KLH, dinitrophenol-keyhole limpet hemocyanin; ITF, intestinal trefoil peptide; Lck, lymphocyte-specific protein tyrosine kinase; UTR, untranslated region.
of weeks in the acute chemically induced models and therefore it is considered “acute” (137).

Experimental IBD mouse models exhibit some but not all of the phenotypic and physiological characteristics of human CD and UC. Phenotypic similarity to CD is established by the presence of some of the pathological features that characterize human CD. These include transmural inflammation of the intestinal wall with a discontinuous pattern, small bowel involvement, stricture development, granuloma formation, and perianal disease with fistulae. On the other hand, phenotypic similarity to UC refers to rectal involvement, crypt abscess formation, and superficial involvement of the bowel wall (131). Physiological similarities of the various models of experimental IBD to human CD and UC refer to the dominance of the Th1/Th17 or Th2 immune responses, respectively, during the active phases of intestinal inflammation (Tables 1 and 2). The chemically induced models are the least relevant to human IBD because of their acute, self-limiting course and the unphysiological pathogenesis that involves chemical damage of the gut epithelium and administration of haptenization agents. However, they are the most widely used because of their ease of use and flexibility (131).

Although none of the existing models fully recapitulates human disease, their study has generated some key concepts of IBD pathogenesis (230). Firstly, mucosal inflammation can arise from a variety of genetic defects that converge on a limited number of final common pathways of disease pathogenesis. This underlies the multiplicity of genetic alterations than may predispose to IBD development, a hypothesis currently confirmed by genetic studies of IBD patients (68). Secondly, intestinal inflammation is the result of loss of immune tolerance to components of the gut microbiota. This is suggested by the absence of inflammation when animals with the same genetic defects are raised germ free (52) and supports the lack of a causal relationship of known, specific enteric pathogens indicated so far by the human studies (262). Thirdly, that mucosal inflammation is the result of a polarized Th1/Th17 or Th2 T cell-mediated process, although in the majority of the models inflammation has of both innate and adaptive components. This is a major organizing concept that has helped to target our therapeutic arsenal on specific immunological pathways (Fig. 1). Furthermore, murine colitis models have helped to identify the indispensable functions of the mucosal immune system that cooperate to maintain gut homeostasis. These include the presence of an intact epithelial barrier, the development of efficient innate immune responses, the maintenance of a delicate balance between effector and regulatory T cell responses, and the establishment of physiological inflammation (232).

Undoubtedly, mouse models of experimental IBD represent indispensable tools to study IBD pathogenesis. Using genetically identical populations of inbred mice that are kept in controlled environments and harbor identical or minimally different gut microflora produces homogenous results that are easy to interpret and reproduce. Genetically engineered and cell transfer models provide the ability to dissect and individually study the contribution of different pathways in intestinal inflammation. For example, the lack of naturally occurring regulatory T cells (Tregs) in the CD45RB<sup>high</sup> transfer model (Table 2) enables to separately study the role of inducible regulatory T cells (251). Furthermore, the employment of cell-specific gene KO systems has revealed conflicting functions of some signaling molecules during intestinal inflammation. For example, inhibition of nuclear factor κB (NF-κB) activation in immune cells abrogated clinical and histological signs of trinitrobenzene sulfonic acid (TNBS)-induced colitis (156). In contrast, subsequent studies on epithelial cell-specific NF-κB essential modulator (NEMO) KO mice have provided evidence of a protective role of NF-κB activation in epithelial cells during experimental intestinal inflammation (153).

Another positive aspect of animal models is that they provide the ability to closely monitor disease development from symptomless early stages to full-blown syndrome. Therefore, they give the opportunity to separate early from late events in disease course, to distinguish the true pathogenetic pathways of inflammation from the epiphenomena, and to dissect pathways related to the progression of disease from those related to the resolution of the inflammation. For example, the precise temporal identification of pathologocal changes in senescence-accelerated mouse P1 (SAMP)/YitFc mice has offered the opportunity to compare the immunological phenomena that take place before and after the appearance of histologically detectable intestinal inflammatory lesions. This made it possible to separate an initial “inductive” phase characterized by a strong mucosal Th1 response from the subsequent “chronic” inflammatory phase associated with a striking increase in Th2 effector pathways (10).

However, important biological differences exist between animal models and human disease, and these differences merit careful consideration by researchers who attempt to translate findings at the bench into therapeutic strategies at the bedside. In contrast to the polygenic nature of human IBD, experimental IBD models usually involve disruption or overexpression of a single gene, therefore operating pathogenetic pathways are considered limited (Tables 1 and 2). For example, intestinal inflammation in the IL-10<sup>−/−</sup> or the signal transducer and activator of transcription (STAT)4 Tg model is generated in the absence of regulatory factors such as the IL-10 and the transcriptional regulation of IL-12/IL-23 receptor signaling, but human IBD develops in individuals expressing IL-10 or STAT-4 (112, 273). Physiological relevance is questionable in some models such as the TCRα<sup>−/−</sup> model. In this model, colitogenic TCRα<sup>−/−</sup>β<sup>+</sup> T cells, generated due to the lack of TCRα chains, are generally believed to exist only in TCRα<sup>−/−</sup> mice and not in human IBD (138). Finally, KO mice that lack IBD-associated genes previously identified by human genome association studies do not usually develop intestinal inflammation. The case of the nucleotide-binding oligomerization domain-containing protein (NOD)2 gene represents a characteristic example. Although NOD2 is the first definitive genetic risk factor identified for CD, none of the mouse strains carrying nonfunctional NOD2 develop ileitis or colitis spontaneously (90, 104). This suggests that additional immune and environmental factors are required to fully elicit intestinal inflammation, which are missing from the settings of mouse experimental IBD. Homogeneity that characterizes experimental IBD, resulting from dependence on specific operating pathways and the relative paucity of environmental and immune cofactors, enables analysis and therefore is advantageous for the study of IBD pathogenesis. However, it might be considered a disadvantage when mouse models are used for the preclinical evaluation of the efficacy of new drugs. In these settings each
individual animal model should be regarded as akin to a single patient (76).

Another important aspect that has to be taken into account when interpreting the efficacy of various therapeutic targets is the time of intervention. In the case of experimental IBD, onset of autoimmunity and inflammation occurs over tightly defined time courses that enable intervention at specific time points in disease progression. Experimental IBD studies are not designed to assess the efficacy of the therapeutic intervention per se, and this is one limitation when trying to compare results of clinical trials to data from experimental animal models. Intervention in animal studies is often used as the final proof of the existence of a novel operating pathway. In these cases, inflammatory pathways are often targeted before the development of inflammation to avoid complicating epiphenomena or changes in immunological processes that occur during disease evolution (10). However, an intervention that is able to prevent the development of inflammation may not be equally effective in resolving established disease. Such an early intervention is not possible in human patients who participate in clinical trials with clinically evident, established disease and often lack response to conventional treatment. Therefore the mode of

Fig. 1. Inflammatory and regulatory pathways involved in inflammatory bowel disease (IBD) pathogenesis that have been discovered by experimental IBD studies and have been targeted by biological therapies. Crohn’s disease (CD) is characterized by the generation of Th1- and Th17-polarized T cell responses driven by the production of interleukin (IL)-12, IL-18, IL-23, IL-6 and tumor necrosis factor (TNF)-α by dendritic cells (DC) and macrophages. Th1-polarized cells secrete IL-2, IL-17, interferon (IFN)-γ, and TNF-α. Ulcerative colitis (UC) is characterized by an atypical Th2-polarized T cell, and natural killer T cells (NKT) response mediated by IL-5 and IL-13. Polarized T cell responses initiate an inflammatory cascade that involves endothelial activation, chemokine production, and white blood cell (WBC) recruitment. Inappropriate triggering and maintenance of these pathogenic responses has been associated with innate immunity defects that include defective type I IFN production and lack of efficient control by anti-inflammatory cytokines such as IL-10 and transforming growth factor (TGF)-β produced by regulatory T cells (T_{reg}), macrophages, B cells, and stromal cells. AB, antibody; CD, cluster of differentiation; CTLA-4, cytotoxic T lymphocyte antigen-4; ICAM-1, intercellular adhesion molecule-1; MAdCAM-1, mucosal addressin cell adhesion molecule-1; pDC, plasmacytoid dendritic cell; VCAM-1, vascular cell adhesion molecule-1.
intervention employed in the animal studies, that is “preventive” or “therapeutic,” must be taken into account when evaluating the possible efficacy of new treatment strategies to be tested in humans (Tables 4 and 5).

Besides the aforementioned structural differences between experimental and human IBD studies, another limitation in the extrapolation of results from animals to humans is the different outcomes measured to evaluate efficacy. Disease activity in human clinical trials is measured by various indexes that quantify symptoms related to active disease. Most widely used are the Crohn’s disease activity index (CDAI) for patients with CD and the Mayo Clinic score for UC (19, 214). The CDAI is a numerical calculation derived from the sum of products from a list of eight items and represents a numerical estimation of a physician’s interpretation of patient symptoms. Severe disease is defined as a value of greater than 450. Most major research studies on medications in CD define response as a fall of the CDAI of greater than 70 points. Remission is defined as a fall in the CDAI to less than 150 (271). The Mayo Clinic score for UC is a combination of a numerical estimation of the severity of patient symptoms combined with an endoscopic score of colonic inflammation (214). Definitions of clinical response and disease remission in UC vary widely among clinical trials. A decrease in the Mayo score of at least 3 points is often used to define response and a Mayo score <2 with no individual subscore >1 usually defines clinical remission (197, 249). In contrast, the effects of therapeutic interventions in experimental IBD studies are estimated by using “semiquantitative” histopathology scores of inflammation in colonic tissue and differences in the rate of weight loss of the treated animals during disease evolution (212). However, a treatment able to generate statistical significance in pathology scoring in homogeneous groups of diseased animals may not be potent enough to induce clinical remission in heterogeneous groups of patients.

Development of Biological Therapies

The combination of ex vivo studies in IBD patients with in vivo studies on experimental colitis models so far supports the concept that T cell activation is an important component of intestinal inflammation in IBD. CD is characterized by the generation of Th1- and Th17-polarized T cell responses driven by the production of IL-12, IL-18, IL-23, IL-6, and transforming growth factor-β by antigen-presenting cells (APC), macrophages, and nonimmune cells of the intestinal microenvironment. Th1-polarized cells secrete IL-2, IL-17, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α, which initiate an inflammatory cascade that involves endothelial cell activation, chemokine production, leukocyte recruitment, and proinflammatory cytokine production (124). Inappropriate triggering and maintenance of these pathogenic immune responses in CD has been associated with defective functioning of intestinal innate immune defense, comprising intestinal epithelium and phagocytic cells of the lamina propria, including neutrophils and macrophages (124). Although a significant proportion of genes predisposing to CD is shared by UC, the inflammation in UC is primarily characterized by the dominance of an atypical Th2-polarized T cell and natural killer (NK) cell. T cell response mediated by IL-5 and IL-13 (64). The lack of efficient control by anti-inflammatory cytokines such as IL-10 and transforming growth factor (TGF)-β, regulatory T cell elements, and defective T cell apoptosis results in chronic intestinal inflammation (94, 233). Therefore, biological therapies in IBD aim to reduce of pathogenic T cell activation via blockage of proinflammatory cytokines, administration of anti-inflammatory cytokines, induction of T cell apoptosis, inhibition of T cell proliferation, prevention of inflammatory cell recruitment, and enhancement of innate immune responses (Fig. 1). Existing types of biological therapies for IBD and respective targets are summarized in Table 3.

Blockage of proinflammatory cytokines produced by effector T cells. TNF inhibitors were the first biologics approved for the treatment of IBD. The first successful open-label trial of anti-TNF-α therapy in CD (259) was justified mainly by its successful use in rheumatoid arthritis (RA) clinical trials and refractory CD cases (41). That initial open-label study followed by a randomized-controlled study on the effectiveness of infliximab for moderate to severe refractory CD (243) have provided the initial jumpstart for many subsequent trials and the development of many agents that target TNF-α (Table 3). Some are currently used in the clinic such as adalimumab and certolizumab pegol, and others are being tested in various phases of the clinical trial process (39). Etanercept is a dimeric fusion protein consisting of the extracellular portion of the p75 TNF receptor linked to the Fc domains of a type 1 human immunoglobulin (IgG1) (140). Despite efficient TNF-α neutralization and an established role in the treatment of RA (142), etanercept has proven ineffective in CD (210). This intriguing difference from infliximab has been investigated by subsequent studies showing that infliximab but not etanercept induced apoptosis of activated peripheral blood and lamina propria lymphocytes, possibly via reverse signaling through transmembrane TNF-α (135, 253).

The anti-TNF-α approach for IBD treatment has not been adequately tested in experimental colitis models before its first use in humans. Interestingly, studies in CD45RB<sup>B</sup> high transfer experimental colitis prior to the first randomized controlled trial (RCT) in humans have suggested only partial protection by anti-TNF-α treatment with no long-term immunomodulatory effects compared with IFN-γ neutralization approaches (183). Subsequent animal studies have retrospectively confirmed the efficacy of anti-TNF-α strategies in the prevention and treatment of experimental colitis in the majority of colitis models (Table 4). However, anti-TNF-α treatment has not been successful in all models. In the case of acute dextran sulfate sodium (DSS) colitis TNF-α neutralization had no effect or even aggravated inflammation and delayed recovery (108, 164). In IL-10<sup>−/−</sup> mice, anti-TNF-α failed to prevent or treat disease (115, 188) with the exception of the study of Myers et al. (150), in which antisense oligonucleotide blockage of TNF-α was used. Treatment outcomes in animal models may reflect the differences of some of them such as the DSS colitis from human IBD. On the other hand they may indicate the presence of alternative inflammatory pathways, which could be relevant to those patients that do not respond to anti-TNF-α or lose response over time.

IFN-γ is the end proinflammatory product of activated T cells, NK cells, and macrophages (201). Increased numbers of IFN-γ producing cells and increased levels of IFN-γ are found in human CD inflamed mucosa (27, 66). IFN-γ blockage or deficiency seems to be protective in many different models such as the SAMP1/YitFc, IL-10<sup>−/−</sup>, TNF<sup>ΔΔARE</sup>, and the
<table>
<thead>
<tr>
<th>Biological Agent</th>
<th>Mechanism of Action</th>
<th>Targeted Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abatacept</td>
<td>Soluble recombinant fusion protein containing cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4)</td>
<td>CD/UC</td>
</tr>
<tr>
<td>Adalimumab</td>
<td>Human monoclonal antibody against TNF-α</td>
<td>CD/UC</td>
</tr>
<tr>
<td>AJM-300</td>
<td>Orally active antagonist to α4β7 integrin</td>
<td>CD</td>
</tr>
<tr>
<td>Alicaforse</td>
<td>Antisense oligonucleotide against intercellular adhesion molecule-1 (ICAM-1) messenger RNA</td>
<td>CD/UC</td>
</tr>
<tr>
<td>AMG 827</td>
<td>Human monoclonal antibody against IL-17R</td>
<td>CD</td>
</tr>
<tr>
<td>AMG 181</td>
<td>Human monoclonal antibody against α4β7 integrin</td>
<td>UC</td>
</tr>
<tr>
<td>Amrakinuzumab</td>
<td>Humanized monoclonal antibody against IL-13</td>
<td>UC</td>
</tr>
<tr>
<td>Basiliximab</td>
<td>Chimeric mouse-human monoclonal antibody against IL-2Rα</td>
<td>UC</td>
</tr>
<tr>
<td>BMS-945429</td>
<td>Humanized monoclonal antibody against IL-6</td>
<td>CD</td>
</tr>
<tr>
<td>Briakinumab</td>
<td>Human monoclonal antibody against the common p40 subunit of IL-12/23</td>
<td>CD</td>
</tr>
<tr>
<td>C326</td>
<td>Avimer protein that inhibits IL-6</td>
<td>CD</td>
</tr>
<tr>
<td>CCX-025</td>
<td>Antagonist of chemokine receptor CCR9</td>
<td>CD</td>
</tr>
<tr>
<td>CCX282-B (Traficet-EN)</td>
<td>Antagonist of chemokine receptor CCR9</td>
<td>CD</td>
</tr>
<tr>
<td>Certolizumab pegol</td>
<td>PEGylated Fab fragment of a humanized TNF inhibitor monoclonal antibody</td>
<td>CD</td>
</tr>
<tr>
<td>Daclizumam</td>
<td>Humanized monoclonal antibody against IL-2Rα</td>
<td>UC</td>
</tr>
<tr>
<td>Debiaerce</td>
<td>TNF-α inhibitor, TNF-α kinnin</td>
<td>CD</td>
</tr>
<tr>
<td>Dersalazine</td>
<td>Combination through an azo bond, of a platelet activating factor (PAF) antagonist (UR-12715) with 5-aminosalicylic acid (5-ASA)</td>
<td>UC</td>
</tr>
<tr>
<td>Etrolizumab (rHuMab b7)</td>
<td>Humanized monoclonal antibody against the β7 integrin</td>
<td>UC</td>
</tr>
<tr>
<td>Firaigrelast (SB-683699)</td>
<td>Oral inhibitor of α4 integrin</td>
<td>CD</td>
</tr>
<tr>
<td>Fontolizumab</td>
<td>Humanized monoclonal antibody against IFN-γ</td>
<td>CD</td>
</tr>
<tr>
<td>GLPG0974</td>
<td>Orally available inhibitor of free fatty acid receptor 2 (FFA2) that reduces migration of neutrophils</td>
<td>CD</td>
</tr>
<tr>
<td>Golimumum</td>
<td>Human monoclonal antibody against TNF-α</td>
<td>UC</td>
</tr>
<tr>
<td>GSK1070806</td>
<td>Humanized monoclonal antibody against IL-18</td>
<td>CD</td>
</tr>
<tr>
<td>GSK1070806</td>
<td>Humanized monoclonal antibody against IL-18</td>
<td>CD</td>
</tr>
<tr>
<td>Infliximab</td>
<td>Chimeric mouse-human monoclonal antibody against TNF-α</td>
<td>CD/UC</td>
</tr>
<tr>
<td>Interferon beta</td>
<td>Human type I interferon</td>
<td>CD/UC</td>
</tr>
<tr>
<td>MDX-1100</td>
<td>Human monoclonal antibody against IP-10 (CXCL10)</td>
<td>UC</td>
</tr>
<tr>
<td>MEDI2070 (AMG139)</td>
<td>Human monoclonal antibody against the p19 subunit of the IL-23</td>
<td>CD</td>
</tr>
<tr>
<td>Muromonab (OKT3)</td>
<td>Murine monoclonal antibody against CD3 on T cells</td>
<td>UC</td>
</tr>
<tr>
<td>Natalizumab</td>
<td>Humanized monoclonal antibody against the cell adhesion molecule α4-integrin</td>
<td>CD</td>
</tr>
<tr>
<td>NI-0401</td>
<td>Human monoclonal antibody against CD3 on T cells</td>
<td>CD</td>
</tr>
<tr>
<td>NN5555 (IPH 2301)</td>
<td>Human monoclonal antibody against NKG2D, an activating receptor on NK cells and CD8 T cells</td>
<td>CD</td>
</tr>
<tr>
<td>OvaSave</td>
<td>OVA-specific Type 1 regulatory T-cell therapy</td>
<td>CD</td>
</tr>
<tr>
<td>Ozoralizumab</td>
<td>Humanized monoclonal antibody against TNF-α</td>
<td>CD</td>
</tr>
<tr>
<td>PDA-001</td>
<td>Human placenta-derived stem cell therapy</td>
<td>CD</td>
</tr>
<tr>
<td>PF 04236921</td>
<td>Human monoclonal antibody against IL-6</td>
<td>CD</td>
</tr>
<tr>
<td>PF-547659</td>
<td>Monoclonal antibody against mucosal addressin cell adhesion molecule-1 (MAdCAM)</td>
<td>CD/UC</td>
</tr>
<tr>
<td>QAX576</td>
<td>Monoclonal antibody against IL-13</td>
<td>CD</td>
</tr>
<tr>
<td>Remestemcel-L</td>
<td>Human mesenchymal stem cells</td>
<td>CD</td>
</tr>
<tr>
<td>rhIL-11</td>
<td>Recombinant human IL-11</td>
<td>CD</td>
</tr>
<tr>
<td>Rituximab</td>
<td>Chimeric mouse-human monoclonal antibody against CD20 on B cells</td>
<td>UC</td>
</tr>
<tr>
<td>Sargramostim</td>
<td>Recombinant human granulocyte-macrophage colony-stimulating factor</td>
<td>CD</td>
</tr>
<tr>
<td>SCH900222 (MK-3222)</td>
<td>Humanized monoclonal antibody against the p19 subunit of IL-23</td>
<td>CD</td>
</tr>
<tr>
<td>Secukinumab (AIN457)</td>
<td>Human monoclonal antibody against IL-17A</td>
<td>CD</td>
</tr>
<tr>
<td>Tenovil</td>
<td>Recombinant human IL-10</td>
<td>CD</td>
</tr>
<tr>
<td>Tocilizumab</td>
<td>Humanized monoclonal antibody against IL-6R</td>
<td>CD</td>
</tr>
<tr>
<td>Tofacitinib (CP-690550)</td>
<td>Inhibitor of the enzyme Janus kinase 3 (JAK3)</td>
<td>CD/UC</td>
</tr>
<tr>
<td>Tralokinumab</td>
<td>Human monoclonal antibody against IL-13</td>
<td>UC</td>
</tr>
<tr>
<td>TRK-170</td>
<td>Orally active α4β7/α2β1 integrin antagonist</td>
<td>CD</td>
</tr>
<tr>
<td>Ustekinumab</td>
<td>Human monoclonal antibody against the common p40 subunit of IL-12/23</td>
<td>CD</td>
</tr>
<tr>
<td>Vategizumab</td>
<td>Humanized monoclonal antibody against α4 integrin</td>
<td>UC</td>
</tr>
<tr>
<td>Vedolizumab (MLN0002)</td>
<td>Humanized monoclonal antibody that targets the α4β7 integrin</td>
<td>CD/UC</td>
</tr>
<tr>
<td>Vidofludimus</td>
<td>Oral immunomodulatory drug that inhibits dihydro- orotate dehydrogenase, lymphocyte proliferation, IL-17A and IL-17F production</td>
<td>CD/UC</td>
</tr>
<tr>
<td>Visilizumab</td>
<td>Humanized monoclonal antibody against CD3 on T cells</td>
<td>CD/UC</td>
</tr>
</tbody>
</table>

CD45RB<sup>hi</sup> transfer models (Table 4) (10, 17, 40, 109, 183). Interestingly, IFN-γ has been identified as an important mediator of intestinal inflammation even in the absence of T cells since neutralization of the cytokine partly protects from disease in the anti-CD40 colitis model (250). However, the efficacy of IFN-γ blocking to ameliorate established colitis has not been addressed except in the case of IL-10⁻/⁻ colitis, where it was found unsuccessful (Table 4) (17, 40, 115). Fontolizumab is a monoclonal antibody against IFN-γ currently being tested in patients with IBD. The fontolizumab phase II studies reported
that the primary efficacy end point that was defined as clinical response at day 29 was not reached but a significant proportion of patients that received multiple doses had clinical response at later time points compared with placebo (86, 187). The partial success of the anti-IFN-γ/H9253 approach has been predicted by its limited therapeutic efficiency in the treatment of established experimental colitis (17, 40, 115).

IL-13 has been shown to be pathogenic in experimental UC by affecting epithelial tight junctions, apoptosis, and colonic epithelial cell restitution (80). Evidence for its pathogenic role is produced mainly from studies in oxazolone colitis, in which IL-13-producing NK T cells and CD4 helper T cells have been identified as mediators of disease. IL-13 neutralization, IL-13 deficiency in CD4 T cells, and NK T cell depletion all protected mice from colitis (81, 87). In contrast, Wilson and colleagues (270) have shown that enhancement of IL-13 activity via knocking out of the IL-13R/H92512 decoy receptor protected IL-10/H11002/H11002/H11003 mice against the induction of colitis by non-steroid anti-inflammatory drugs (NSAIDs) or Trichuris muris infection and that IL-13 neutralization restored colitis suscep-

Table 4. Effects of targeting proinflammatory cytokines for the treatment of experimental IBD

<table>
<thead>
<tr>
<th>Target</th>
<th>Approach</th>
<th>Effective</th>
<th>Ineffective</th>
</tr>
</thead>
</table>
| TNF-α   | Ab prevention  | Chronic DSS (146) | Acute DSS (164) IL-10⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽⁽=Value of 10.220.33.5 on October 14, 2017 http://ajpgi.physiology.org/ Downloaded from http://ajpgi.physiology.org/ by 10.220.33.5 on October 14, 2017

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*IL-17 neutralization or deficiency aggravates colitis.
tibility. In that study IL-13 suppressed Th1-Th17 inflammatory responses, and decreased the levels of IFN-γ and IL-17. These data indicate that enhancement of IL-13 activity would be beneficial in patients with CD (270). Monoclonal antibodies against IL-13 include anrakinumab and tralokinumab that are currently in phase IIA trials to assess pharmacokinetics and safety in patients with UC but results of efficacy have not yet been reported, as assessed in http://www.clinicaltrials.gov. In CD there is no evidence from animal studies to suggest a pathogenic role for IL-13 and there are no data on the possible results of IL-13 blockade. Despite the lack of preclinical data, QAX576, an IL-13 antagonist, is also currently being used in phase II trials for the treatment of perianal CD, and results of its efficiency are pending (1).

Recent experimental data indicate that there is a Th1 and a Th17 component to both human and experimental IBD, but it is still unclear which one might be more important. The involvement of the Th17 immune response in the development of experimental IBD is supported by studies showing that experimental colonic inflammation is more dependent on IL-23 than IL-12 (88, 114). More importantly, a single nucleotide polymorphism in the IL-23R gene has been associated with decreased susceptibility to both CD and UC in humans (45). However, subsequent studies have indicated a regulatory role for IL-17 that could be more critical than its pathogenic role in experimental intestinal inflammation (Table 4) (159). Transfer of T cell populations from IL-17-deficient mice to immunodeficient mice led to earlier onset of colitis and higher levels of IFN-γ production than transfer of cell populations from wild-type mice (159). Moreover, neutralization or deficiency of IL-17 aggravates DSS colitis in mice (161, 283). These studies indicate a significant role of IL-17 in maintaining intestinal homeostasis, which has been attributed to its regulatory effects on innate epithelial immune responses and lymphocyte T-bet expression (231, 283).

The IL-17 family of cytokines includes IL-17A (commonly referred to as IL-17), IL-17B, IL-17C, IL-17E, and IL-17F. Among them IL-17A and IL-17F are 50% homologous and share many biological properties (168). Both IL-17A and IL-17F have been targeted for the treatment of human IBD. Secukinumab, a monoclonal antibody against IL-17A, has only been tested in a proof-of-concept study. The trial involved 59 patients with CD, and results showed that secukinumab actually worsened disease outcome and increased infection risk compared with placebo (89). A recent phase II trial of AMG 827, another human anti-IL-17 receptor antibody, also resulted in a disproportionate number of cases of worsening CD in subjects with active CD and no evidence of meaningful efficacy (242). The results from these trials were correctly predicted by the aforementioned studies on animal models of transfer and DSS colitis (159, 161, 283). On the contrary, vidofludimus, an oral inhibitor of both IL-17A and IL-17F release, through inhibition of STAT3 and NF-κB-signaling pathways has shown promise (59). The single-arm, open-label ENTRANCE study reported remission rates up to 53.9% in 26 patients suffering from UC or CD following treatment with vidofludimus (83). It is currently unknown whether the promising effects of the drug are mediated through inhibition of T-lymphocyte proliferation, or they are relevant to inhibition of IL-17A and IL-17F signal transduction pathways, or even both.

Blockage of the downstream signaling of cytokine receptors represents an alternative approach for reducing inflammatory response in IBD. The Janus kinases (JAK) JAK1, JAK2, and JAK3 are tyrosine kinases that interact with cytokine receptors and play a crucial role in growth, survival, development, and differentiation of immune cells. Specifically, JAK3 mediates signal-transduction activity involving the common gamma chain of the surface receptors of IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 (70). Deficiency of JAK1 leads to lack of response to type I and type II IFNs, common γ chain cytokines, and gp130 subunit-utilizing cytokines (193). JAK2-deficient cells fail to respond to hormone-like cytokines, such as erythropoietin, thrombopoietin, and granulocyte-macrophage colony-stimulating factor (GM-CSF) (169). Tofacitinib (formerly known as CP-690550) is an orally administered small-molecule antagonist of JAK1 and JAK3 and, to a lesser extent, of JAK2. Tofacitinib inhibited IL-2-dependent differentiation of Th2 and Th17 helper T cells in vitro and improved disease by inhibiting the production of inflammatory mediators and suppressing STAT1-dependent genes in an animal model of collagen-induced arthritis.

Tofacitinib has been assessed in phase II trials for both moderate to severe CD and moderate to severe UC without any preclinical evaluation in experimental IBD animal models (209). In the CD trial, which involved 139 patients, the primary end point of clinical response at 4 wk was not achieved despite significant reductions in serum C-reactive protein and fecal calprotectin, both markers of active inflammation, in patients receiving the highest dose of tofacitinib (208). In the UC trial, 194 patients were randomized to receive one of four doses of tofacitinib or placebo, twice daily for 8 wk, and were assessed for clinical response at week 8. There were significant dose-dependent responses in patients receiving the study drug, the week 8 clinical response rates being 61–78% in the groups receiving the two highest doses compared with 42% of patients receiving placebo. Furthermore, high remission rates of 41–48%, compared with 10% in the placebo group, were also observed. On the basis of these results, tofacitinib is currently being evaluated in phase III clinical trials (1).

In summary, some of the data from experimental colitis models have predicted the failure of IL-17 neutralization and the partial success of IFN-γ neutralization reported by CD clinical trials. IL-13 has been suggested as a possible therapeutic target in UC by preclinical studies but results from human trials are not yet available. JAK inhibition has been tried successfully in animal models of arthritis but not in experimental IBD and results from phase II clinical trials in UC are promising.

**Blockage of proinflammatory cytokines produced by APCs.** There are accumulating data from experimental colitis and human genetic studies that the initiation and maintenance of Th1- and Th17-polarized T cell responses during chronic intestinal inflammation depends on the production of IL-12 (p35/p40) and IL-23 (p19/p40) from resident APCs (4). However, there are only a few animal studies demonstrating effectiveness of anti-p40 in the treatment of established colitis (40, 154). The majority of animal studies implicate IL-12 and IL-23 in colonic inflammation based on the successful prevention of colitis development by either neutralizing antibodies or p19, p40, and STAT-4 genetic deficiency (see Table 4). Interestingly, many of the studies have used colitis models in lym-
phophenic mice, where intestinal inflammation develops via mechanisms of innate immunity in the complete absence of T and B cells, which suggests important additional roles of IL-23 and IL-12 outside the context of the Th17 and Th1 differentiation pathways (88, 105, 250).

Briakinumab and ustekinumab are antibodies against p40, whereas SCH900222 and AMG139 are antibodies against the p19 subunit that selectively target IL-23. All four are currently being studied in phase II/III trials for CD, but results are only available for ustekinumab. Satisfactory initial clinical response rates of 53–75% have been reported for ustekinumab and attributed to reduction of IL-12-driven IFN-γ secretion as well as the induction of Fas-mediated apoptosis of Th1 cells (65). Large randomized trials report response rates up to 40% for patients that had failed anti-TNF treatment with a significant proportion of responders to maintain remission (206, 207). Thus preclinical experimental data were able to correctly predict favorable treatment responses in human IBD despite the fact that most of the data were produced by prevention studies, and the long-term efficacy of targeting IL-12 and IL-23 in established intestinal inflammation has only been evaluated in two studies (Table 4).

IL-6 is a central cytokine in IBD that contributes to enhanced survival and resistance to apoptosis of lamina propria T cells (144). IL-6 also contributes to Th17 differentiation of T cells, and increased levels of IL-6 and soluble IL-6R are associated with increased disease severity in IBD (144). A polymorphism within the IL-6 gene has also been linked with early-onset CD (200). Most of the animal IBD studies have evaluated targeting of the IL-6 receptor in the basis of its importance in activated T cell survival. Neutralizing antibodies against IL-6R have been found to prevent disease development and to ameliorate established experimental colitis in the adoptive transfer, TNBS, and IL-10−/− colitis models through the induction of T cell apoptosis (Table 4) (8, 145, 279). Atreya et al. (8) have shown that this effect was mediated via blockage of the STAT-3 signaling pathway responsible for the upregulation of expression of the antiapoptotic genes such as bcl-2 and bcl-xL. Inhibition of soluble IL-6 receptor signaling by gp130-Fc can both prevent and treat SAMP1/Yit colitis, whereas hyper-IL6, an IL-6-soluble IL-6 receptor fusion protein, exacerbates disease (136).

IL-6 and its receptor have been selected as therapeutic targets in CD patients. PF 04236921 is a human monoclonal antibody against IL-6 that is currently being tested in phase II clinical trials in CD, and tocilizumab is a monoclonal antibody against IL-6R that has been approved for the treatment of refractory RA (1, 3). Data are only available for tocilizumab from a single placebo-controlled phase I study in CD (92). The study showed adequate response rates of 80%, but only 20% of the patients went into remission despite the lack of circulating antibodies against tocilizumab (92). Reduction in acute-phase response markers and enhanced apoptosis of lamina propria mononuclear cells was detected in the treated patients (92). Thus previous work in experimental IBD models correctly predicted initial clinical responses and the effect of tocilizumab in T cell apoptosis (Table 4). The effectiveness of tocilizumab in achieving remission cannot be evaluated by using the available clinical data because of the small number of patients included and the relatively few dose regimens administrated in the aforementioned study (92).

The cytokine IL-18 was initially identified as a potent IFN-γ-inducing factor and a stimulator of TNF-α, IL-1β, IL-8, IL-6 production (152, 184). Increased IL-18 mRNA transcripts have been found in both intestinal epithelial cells (IECs) and lamina propria mononuclear cells from CD patients (178). IL-18 administration or Tg overexpression induces colitis and increases susceptibility to DSS colitis (29, 91). IL-18 neutralization or inhibition of expression via IL-18 antisense mRNA prevents and treats experimental colitis (Table 4) (97, 220, 224, 244, 272). In contrast, IL-18−/− and IL-18R−/− mice developed more severe DSS colitis associated with high lethality and more histopathological abnormalities compared with control mice (237). Recent studies have also suggested that tonic activation of NACHT, LRR, and PYD domains containing protein (NLRP3) and IL-18 production at the level of IECs is critical for the integrity of the epithelial barrier. Mice deficient for Nlrp3 or apoptosis-associated speck-like protein containing a CARD (ASC) and caspase-1 were highly susceptible to chemically induced experimental colitis (46, 287). Furthermore, reduced IEC levels of IL-18 have been recently associated with alterations of the colonic microflora characterized by expansion of the colitogenic bacterial phyla Bacteroidetes and TM7 that result in exacerbation of DSS murine colitis (51). Current evidence from animal studies suggests that activation of NLRP3 and IL-18 at the level of IECs promotes intestinal homeostasis, whereas activation of NLRP3 and IL-18 in the lamina propria promotes inflammation (288). At the moment, the role of inflammasomes in human IBD pathogenesis remains undefined and the effect of neutralizing IL-18 in human IBD is highly unpredictable. Despite the inability of existing experimental evidence to predict success, GSK1070806, which targets IL-18, is currently in phase I clinical trial in healthy volunteers and results are pending (1).

Therefore, data from experimental colitis models have at least successfully predicted the partial success of anti-p40 and anti-IL-6R treatment for human CD. In the case of IL-18, its various functions in multiple levels of mucosal immunity have generated controversial experimental data that cannot safely predict the consequences of IL-18 neutralization in human IBD.

Inhibition of pathogenic T cell responses via administration of anti-inflammatory cytokines. Increasing the amount of anti-inflammatory cytokines in an effort to restore immune tolerance represents an alternative approach to treat IBD. Potential therapeutic targets include IL-10, IL-11, and IFN-β. The importance of IL-10 in the maintenance of intestinal tolerance is highlighted by the fact that IL-10 deficiency results in spontaneous colitis development in mice and is supported by human genetic studies that link variants of the IL-10 gene to increased susceptibility to UC (63). IL-10 administration is protective against the development of IBD in multiple animal models such as the IL-10-deficient, the Helicobacter hepaticus-infected recombination activating gene (RAG2)−/−, the CD45RBhigh transfer and TNBS colitis models (17, 44, 183, 266). An initial trial of recombinant human IL-10 treatment in steroid-resistant CD has showed a promising 50% remission rate (257). Unfortunately, large clinical trials that followed failed to show adequate efficacy of the IL-10 treatment in
inducing remission or preventing recurrence in patients with CD (32, 57, 213). The suboptimal efficacy of the IL-10 treatment strategy in IBD can be traced back to the relevant studies in animal models. Despite its efficacy in preventive regiments (17, 44, 183, 266), IL-10 was only partly able to suppress established colitis even if the inflammation was due to the single genetic abnormality of IL-10 deficiency (17). This implies that early exposure to IL-10 may be required for the efficient generation of immunoregulatory cells able to suppress intestinal inflammation. Alternatively, the dosage and application mode of IL-10 might have been suboptimal, with systemic administration of IL-10 resulting in very little of it reaching the site of inflammation. A few pharmacokinetic parameters need also to be taken into account. Firstly, that recombinant IL-10 had a short half-life of 1.5–2.5 h. Secondly, that absorption and systemic exposure to IL-10 were dose dependent but not dose proportional over the range of doses studied, meaning that the increase in systemic exposure was not analogous to the increase of the administered dose (57). Lastly, a bell-shaped dose-response curve was observed with less efficacy at higher doses, similarly to other studies with cytokine therapy in CD and in RA (57). Interestingly, high concentrations of IL-10 appear to induce the expression of T-helper cell proinflammatory cytokines (246). Oral administration of genetically modified IL-10-producing Lactococcus lactis has been suggested to improve IL-10 bioavailability. The method has been initially tested in chronic DSS colitis and IL-10−/− mice, which demonstrated a 50% of improvement of intestinal pathology (228). This approach has been found safe but lacked clinical efficacy in phase II clinical trials (23). Similarly to IL-10, administration of recombinant human IL-11 has also been tested. IL-11 has been shown to protect Toll-like receptor (TLR)2−/− mice with defective epithelial barrier function from lethal colitis induced by Citrobacter rodentum and ameliorates colitis in the rat human leukocyte antigen (HLA)-B27 model (71, 176). Although safe with response rates of 33% in initial trials in CD, response was considered inferior compared with prednisolone (84).

The use of type I IFNs for the treatment of IBD has been proposed on the basis of their therapeutic efficacy in multiple sclerosis (MS). IFN-β reduces attack frequency in patients with MS and has become the established treatment of choice for the relapsing remitting form of MS (25, 96). Several mechanisms have been proposed to explain the anti-inflammatory effects of type I IFNs. Specifically, they have been found to increase expression of anti-inflammatory factors (including IL-10, IL-27, TGF-β, IL-1R antagonist, soluble TNF receptors, suppressor of cytokine signaling) and to decrease proinflammatory mediators (including IL-1, IL-6, IL-8, IL-12, IL-18, TNF-α, IFN-γ, osteopontin, prostaglandin E, and cyclooxygenase-2) (96). However, studies on experimental IBD have generated controversial data concerning their role in intestinal inflammation. IFN-α/β production induced by TLR9 and TLR7 signaling reduced severity of DSS colitis partly by suppressing macrophage proinflammatory activity (99, 202). IFNAR1−/− mice have been found more susceptible to DSS colitis. However, the colonic administration of IFN-β via Tg Lactobacillus acidophilus increased levels of proinflammatory cytokines and exacerbated DSS colitis (133). Recent evidence suggests that IFN-β might worsen clinical status in diseases with a prominent Th17 immune response (9). Furthermore, there have been reports of spontaneous development of IBD in patients receiving systemic IFN-β therapy for MS (194).

Initial trials of IFN-β administration to patients with steroid-refractory UC have reported remission rates of 88%, and the response was sustained for 13 mo (148). However, subsequent open-label and double-blind, placebo-controlled studies in patients with UC produced significant controversy, with only some of the studies indicating a therapeutic effect (126, 147, 149, 157, 175). A meta-analysis of those studies does not support the efficacy of type I IFNs for induction of remission in patients with UC (217). A more recent trial with IFN-β in patients with CD also reported no difference between the IFN-β and placebo (174).

In conclusion, there have been no consistent experimental data indicating that established colitis can be effectively treated by increasing the amount of anti-inflammatory cytokines such as IL-10 and IL-11. Data from human trials have confirmed lack of effectiveness of this approach compared with targeting effector T cell proinflammatory responses. A possible reason could be that effector T cell responses might no longer be amenable to regulation by the time of diagnosis. Moreover, issues on timing, dose and bioavailability have also been raised. In the case of type I IFNs human trials reproduce the controversial results of the experimental colitis models and confirm that lack of response to IFN-β may be expected in chronic intestinal inflammation associated with high levels of IL-17 and IL-6 (126).

**Targeting T cell activation, proliferation, and apoptosis.** Activated T cells that infiltrate the intestinal lamina propria of patients with CD and UC are resistant to apoptosis. Removal of potentially harmful cells by induction of apoptosis is considered a powerful therapeutic tool in IBD. Some agents currently used for the treatment of IBD act at least partly via induction of apoptosis in monocytes or lymphocytes (122). FcR-nonbinding antibodies against the CD3 chain of the TCR may prime T cells for activation-induced death upon antigen rechallenge (28). This is mediated at least partly through activation of caspase 3- and 8-dependent pathways (286). Preclinical studies have demonstrated the efficacy of anti-CD3 antibody treatment in animal models of autoimmunity such as in the nonobese diabetic (NOD) mouse (14) and in experimental autoimmune encephalomyelitis (107). However, with the exception of a single study in DSS colitis, there have been no data on the efficacy of anti-CD3 in experimental colitis models (134).

**Visilizumab** is a monoclonal antibody against the CD3 that enhances T lymphocyte apoptosis. Initial studies in severe steroid-refractory UC have shown that visilizumab effectively induces remission and report clinical response rates up to 84% (180). However, a phase I/II dose-escalation trial of visilizumab and a subsequent placebo-controlled trial in UC reported limited efficacy and high frequency of cardiac and vascular adverse events (13, 204). Furthermore, another trial in CD reported frequent liver injury attributed to visilizumab-induced cytokine release syndrome (12). NI-0401, a fully human anti-CD3 monoclonal antibody, has been tested in a phase I study in CD but reported no significant improvement in CDAI despite some improvement in endoscopic activity scores (256). Oral administration of muromonab (OKT3), a murine antibody against CD3 used for treatment of allograft rejection...
of solid organ transplants, is currently tested in phase I/II studies in UC as assessed in http://www.clinicaltrials.gov.

B cells have been proposed to play a pathogenic role in human UC by the production of autoantibodies against epithelial cells that may participate in epithelial cell damage (48). Rituximab targets the CD20 component of the TCR on B cells with the aim of inducing apoptosis. Rituximab has been extensively investigated in RA, a disease with well-described autoimmune B cells (223). Animal models of IBD that have addressed the role of B cells in UC yielded contradictory results. In the adoptive transfer model in SAMP1/YitFc mice, cotransfer of mesenteric B cells with CD4 T cells aggravated intestinal inflammation in severe combined immunodeficiency (SCID) recipients through mechanisms that might involve inhibition of Treg cell function (165). In contrast, studies in the Gut2−/− and TCRα−/− colitis models demonstrate a protective role for B cells via the production of IL-10 and the regulation of pathogenic TCRα−/− CD4 T cells, respectively (38, 139). Additionally, CD19−/− mice exhibited more severe DSS colitis, attributed to the absence of a regulatory B cell population that represents 1–2% of splenic B cells, and produce IL-10 (280). The above findings suggest that the role of B cells in experimental colitis pathogenesis may depend on the animal model used. A phase II randomized controlled trial of rituximab in 24 patients with steroid-refractory moderate UC showed only short-term clinical responses with no difference from placebo in inducing remission (118). Furthermore, there have been case reports of UC development in patients with no prior history of bowel disease that were treated with rituximab (7). Interestingly, UC exacerbation has also been reported with rituximab and was associated with depletion of IL-10-producing B cells (72).

Cytotoxic T-lymphocyte antigen 4 (CTLA4) is an immunoregulatory molecule that is involved in the negative regulation of the proliferation of activated T cells by competing with CD28 binding to CD80/86 on the antigen-presenting cells (31). Concomitantly, CTLA4 ligation plays an important role for the Treg-mediated immunosuppression (186). CTLA4 blockage leads to deterioration of T cell transfer colitis via abrogation of the regulatory properties of Foxp3-expressing Tregs (120, 121). However, anti-CTLA4 treatment during priming in TNBS colitis has been reported to induce IL-10 producing Tregs that can suppress TNBS colitis, indicating that outcomes may differ depending on the time of administration (34).

Abatacept is a soluble recombinant fusion protein containing cytotoxic T-lymphocyte-associated antigen 4 that is approved for use in RA and is currently being trialed for IBD (69). In TNBS and oxazolone murine colitis models, prophylactic treatment with abatacept demonstrated efficacy as measured by prevention of weight loss, reduction in proinflammatory cytokine production, and reduction in mucosal damage. However, in the same murine model, abatacept failed to ameliorate inflammation in established colitis, suggesting that targeting T cell activation alone may not be efficacious in established disease (281, 282). Despite the lack of efficacy suggested by some experimental studies, abatacept has moved into human IBD trials in patients with CD and UC. The results indicated that, although abatacept was well tolerated with no significant difference in adverse effects compared with placebo, the rate of remission with abatacept was not significantly different from placebo (205). Treatment with abatacept did not change the number of Foxp3+ Tregs in colonic tissue, but studies on a possible effect on Treg function were not performed (205).

IL-2 functions as a T cell growth factor and regulator of the expansion and apoptosis of activated T cells (192). In parallel, signaling through the IL-2Rα (CD25) receptor seems to be critically required for maintaining the competitive fitness of CD25+Foxp3+ regulatory T cells that restrain peripheral T cell responses (60). Most studies on experimental colitis have focused on the importance of the later in the maintenance of immune tolerance in the gut. Both IL-2Rα−/− and IL-2−/− mice represent a paradigm for perturbed lymphocyte homeostasis. They develop massive enlargement of peripheral lymphoid organs associated with T and B cell expansion and autoimmune diseases including bowel inflammation (269). Restoration of the CD25+Foxp3+ regulatory T cell population in the periphery is able to prevent the chronic accumulation of lymphoid cells in the peripheral compartments and the development of autoimmunity (5). Comparable results have been observed in mice following neutralization of IL-2 and in the CD45RBhigh adoptive transfer model, where transfer of naive CD4 T cells in the absence of CD25+Foxp3+ regulatory T cells results in chronic colitis (182, 218). Up to now there are no studies that show a positive effect of IL-2 neutralization or CD25 blocking in experimental animal models of colitis, a situation that could predict therapeutic failure in human IBD.

Despite its importance for the maintenance of regulatory T cells IL-2 has been investigated as a target for therapeutic intervention in UC. Trials were justified by the effectiveness of the calcineurin inhibitor cyclosporin in the treatment of steroid-resistant UC, which acts at least partly through inhibition of IL-2 production by activated T cells (119). Investigated biologics are basiliximab and daclizumab, both monoclonal antibodies against IL-2Rα. Initial open-label trials of basiliximab and daclizumab in steroid-resistant UC produced promising results with clinical remission rates up to 65–70% (36, 37, 260). In contrast, a larger randomized controlled trial reported that steroid-refractory UC patients treated with daclizumab were not more likely to be in remission after 8 wk of treatment compared with patients given placebo despite the low placebo response and the good safety profile of the drug (261). The lack of efficacy was attributed to the lower affinity of daclizumab for the CD25 receptor, possibly resulting in less cytokine activity compared with basiliximab and the low penetrance of the antibody to the lamina propria (261). Lack of efficacy was not attributed to depletion of CD25-expressing regulatory T cells since no exacerbation of colitis was observed with higher drug dosing.

In conclusion, efforts to treat IBD by blocking T cell or B cell activation have failed so far. In the case of B cells animal models have not yet provided consistent data on their role in colitis pathogenesis. In contrast, data from experimental colitis models have consistently shown that general blockade of T cells may be detrimental if it includes T-regulatory cell blockade. Furthermore, in established disease, additional factors besides dysregulation of T cell activation, such as disruption of intestinal epithelial barrier function and inflammatory mediators leading to recruitment of leukocytes, likely create a hurdle that cannot be overcome by blocking T cell activation alone (205).

Enhancement of the innate immune system. Defects of innate immunity have generated a variety of experimental colitis...
models (Table 1). Specifically, defects related to the production of mucin and defensins, the integrity of the epithelial barrier, the regulation of TLR signaling, and the control of macrophage and NK T cell responses can produce IBD-like phenotypes in mice (Table 1). This has generated the hypothesis that impaired clearance of mucosa-associated microbacteria may result in uncontrolled inflammatory responses that trigger IBD and especially CD (128). Defective neutrophil recruitment, reported more than 30 years ago, and recently discovered alterations in macrophage functions indicate the presence of impaired bacterial clearance in CD patients (215, 225). Genomewide association studies support this hypothesis as polymorphisms of genes associated with innate immunity such as the NOD2, autophagy-related protein (ATG)16L1, and immune-related GTPase family M protein (IRGM) have been strongly associated with increased susceptibility to CD (11).

In this context, enhancement of innate immunity through administration of recombinant GM-CSF could theoretically protect from colitis development. GM-CSF deficiency or neutralization increased susceptibility piroxicam-induced ileitis and DSS colitis (77, 275). There are only two studies in experimental DSS colitis that showed a beneficial effect of GM-CSF administration. The proposed mechanisms of action included the expansion of plasmacytoid dendritic cells that enhanced type I IFN production (203) and the increase in epithelial cell proliferation and migration that accelerated mucosal repair (18).

Based primarily on the therapeutic activity of colony stimulating factors in congenital phagocyte disorders, clinical trials of GM-CSF were undertaken in CD (195). Sargramostim is recombinant human GM-CSF that has already been tested mainly in adult and pediatric CD patients. Despite a good safety profile and promising results from the open-label and phase II studies, a phase III multicenter RCT trial in 286 patients with active CD has failed to demonstrate superiority over placebo (55, 110, 252). In conclusion, the therapeutic potential of GM-CSF was not adequately explored in experimental colitis models. Data from human trials demonstrate a limited efficacy for this approach in human CD.

**Inhibition of leukocyte migration to mucosal sites.** The process of lymphocyte homing and extravasation into the mucosal tissues is governed by the expression of integrins and chemokine receptors (CCR) on leukocytes and adhesion molecules on endothelial cells (78). Integrins and their interaction with endothelial adhesion molecules represent potent and organ-specific targets for the therapeutic modulation of T cell-mediated inflammatory diseases of the gastrointestinal tract. Predominant targets include the integrins α4β1, α4β7, and α5β2, which interact with vascular cell adhesion molecule (VCAM-1), mucosal addressin cell adhesion molecule-1 (MAdCAM-1), and intercellular adhesion molecule-1 (ICAM-1), respectively, on endothelial cells (227).

Both naïve and effector T lymphocytes use α4β7 integrin to extravasate from the blood to gut mucosal tissues by interacting with MAdCAM-1. MAdCAM-1 is constitutively expressed on high endothelial venules of Peyer’s patches and mesenteric lymph nodes and on postcapillary venules of gut lamina propria. Targeting the α4β7-MAdCAM-1 interaction has been proved efficacious in ameliorating intestinal inflammation in most experimental colitis models (Table 5). However, some controversy has been generated by few studies. Sydora et al. (236) found no difference in the onset and severity of colitis in IL-2-deficient mice crossed to Itgα7−/− mice. Furthermore, in the adoptive transfer model, delayed onset of colitis was noted over 9 wk in recipients of T cells from β7-deficient mice, although these mice progressed to severe inflammation at 25 wk. In SAMP1/YitFc mice, antibody blockade of β7 integrin or MAdCAM-1 alone failed to attenuate the ileitis, but these same antibodies were effective when combined with L-selectin blockade. Blockade of two pathways (L-selectin and MAdCAM-1 or α4 integrins) was required to improve ileitis (24, 191).

The success of targeting the α4β7/MAdCAM-1 interaction in the treatment of murine colitis consistently reported by numerous experimental studies (Table 5) predicts the clinical success of related biologics. Natalizumab, a humanized monoclonal antibody against α4 integrin, is the only biological approved for use in CD besides anti-TNF agents (61). RCTs before approval report clinical response rates of 48% and remission rates of 26% in moderate to severe CD and recent

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**Table 5. Effects of targeting lymphocyte trafficking for the treatment of experimental IBD**

<table>
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<tr>
<th>Target</th>
<th>Approach</th>
<th>Effective</th>
<th>Ineffective</th>
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<tbody>
<tr>
<td>MAdCAM-1/α4β7</td>
<td>Ab prevention</td>
<td>DSS (100)</td>
<td>SAMP1/YitFc (191); DSS (226)</td>
</tr>
<tr>
<td></td>
<td>Ab treatment</td>
<td>Chronic DSS (54; 245)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>CD45RB&lt;sup&gt;h&lt;/sup&gt; (177)</td>
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<td></td>
<td>KO</td>
<td>SAMP1/YitFc (74)</td>
<td>IL-2&lt;sup&gt;−/−&lt;/sup&gt; (236)</td>
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<td></td>
<td></td>
<td>CD45RB&lt;sup&gt;h&lt;/sup&gt; (171)</td>
<td>CD45RB&lt;sup&gt;h&lt;/sup&gt; (236)</td>
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<tr>
<td>ICAM-1/α4β7</td>
<td>Ab prevention</td>
<td>DSS* (16)</td>
<td>SAMP-1/Yit adoptive transfer* (24)</td>
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<tr>
<td></td>
<td>Ab treatment</td>
<td>TNBS (211; 285)</td>
<td>DSS (226)</td>
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<tr>
<td>VCAM-1</td>
<td>Ab prevention</td>
<td>DSS (15)</td>
<td>SAMP-1/Yit adoptive transfer* (24)</td>
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<td></td>
<td>Ab treatment</td>
<td>TNBS (211)</td>
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<td>Cotton-top tamarin colitis (181)</td>
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<td>DSS (226)</td>
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*ICAM-1 antisense oligonucleotide approach; †combined with P-selectin deficiency; ‡when blocked alone.
Review

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meta-analysis of all controlled trials concluded that the therapy is superior to placebo for induction of remission (62, 241). However, the increased risk of progressive multifocal leukoencephalopathy (PML), estimated to occur in one of 1,000 treated patients, restricts its use to second-line therapy in patients that have failed or lost response to anti-TNF-α agents (21).

The rest of the biological agents that target integrins with available clinical data include vedolizumab, PF-547659, etrolizumab, and AMJ300. Vedolizumab, which targets α4β7, is currently in phase III trials for CD and UC following favorable results from previous trials. Results from phase II trials reported clinical response rates of 52% in CD and 50% in UC, whereas experimental data indicated a lower risk of PML (56, 58, 170). PF-547659, which targets MadCAM-1, and etrolizumab, which targets β7, are both in phase I clinical studies with promising results on safety and efficacy (198, 265). AMJ300, a small molecule inhibitor of the α4-integrin subunit, is also being tested in phase I clinical trials in patients with CD, but initial results showed no significant difference in clinical response compared with placebo (238).

Other molecules that aim to inhibit leukocyte migration to mucosal sites include alicaforsen (ISIS-2302), an antisense oligonucleotide against ICAM-1 messenger RNA. Expression of ICAM-1 and VCAM-1 is sequentially induced on endothelial and stromal cells during active inflammation to facilitate leukocyte recruitment in inflammatory sites (47, 221). Strategies targeting ICAM-1 and VCAM-1 to prevent and treat experimental intestinal inflammation have been assessed mainly for DSS and TNBS colitis models with variable success (Table 5). A single study in a more CD relevant model, the SAMP-1/Yit adoptive transfer ileitis model, reported that blocking both ICAM-1 and VCAM-1 but neither alone is able to treat established ileitis (24).

Despite promising results from phase I studies large RCT failed to demonstrate significant efficacy of alicaforsen compared with placebo in CD (276–278). Furthermore, in an effort to deal with possible bioavailability issues, a phase II study of an enema formulation of the compound was conducted in UC but also failed to show significant differences from placebo after 6 wk of treatment. Alicaforan enema did, however, produce a prolonged reduction in disease activity relative to baseline from weeks 18 to 30 compared with placebo (258). The limited success of this approach has been well predicted by the controversial data reported from studies in experimental colitis models.

Finally, another approach tried is targeting of the chemokine receptors involved in leukocyte migration to inflamed intestinal mucosal sites. The CCL25/CXCR9 chemokine/receptor pair is a key regulator of leukocyte migration to the small intestine. CCL25 is strongly expressed by the small intestinal epithelium (167) and regulates trafficking of gut-specific memory/effector T cells via CXCR9 (229). Targeting CXCR9 has not been adequately evaluated in experimental models of intestinal inflammation. A study in DSS colitis demonstrated that CXCR9 deficiency aggravates colitis. The observed effect was accompanied by an altered balance of dendritic cell subsets in the mesenteric lymph nodes of the colitic animals, characterized by a relative increase in the numbers of plasmacytoid dendritic cells (274). However, a study of CCX282-B, a small molecule that inhibits CXCR9/CCL25-dependent chemotaxis, has been shown to ameliorate the severity of ileitis in the TNF3ARE mouse model (267). Both CCX282-B and CCX-025 target CXCR9, but clinical data are available only for CCX282-B. A double-blind, placebo-controlled trial of CCX282-B reports significant response rates of 61% and remission rates of 47% for patients with CD compared with a placebo response of 47 and 31%, respectively. A significant decrease of the need for steroid rescue therapy was also observed (50, 101, 267). Because of these encouraging results CCX282-B is currently being evaluated into phase III clinical trials (1).

In summary, the strategy of inhibition of leukocyte migration has been adequately tested in experimental colitis models for some of the relevant targets. In cases where adequate data were available, the results from the experimental studies efficiently predicted outcomes in human clinical trials. Specifically for the α4β7 and ICAM-1, the experimental studies indicated possible efficacy for the former and limited efficacy for the later. However, in the case of CXCR9 there is not a sufficient amount of experimental data generated to make predictions for the efficacy of this approach in human trials.

Discussion

Animal models of human diseases are often employed as predictive models for the discovery and quantification of the impact of a treatment in humans (255). However, a previous review (75) of the most highly cited interventional animal studies published between 1980 and 2000 reported that only 10% of these interventions were subsequently approved for use in patients, with a median time to replication to be 7 years. Biologics have increased the specificity and efficacy of therapeutic targeting so they are expected to increase this percentage. This may be true in the field of RA, in which roughly 30% of new treatment strategies have been translated in efficient drugs in the clinic (79). This has not been the case for IBD. Despite the variety of animal models that mimic various aspects of human IBD, summarized in Tables 1 and 2, the likelihood of translation of interventional animal studies into clinical use remains rather limited. From up to 60 new therapeutic targets (Table 3) evaluated in more than 600 phase I–III clinical trials, only three anti-TNF-α agents and natalizumab have been able to reach the clinic so far (1). At first glance these figures suggest a failure of preclinical studies to predict efficacy in the clinical trials.

However, it has to be taken into account that many therapies have been trialed despite controversial data from animal studies or even in the virtual absence of animal data. The comparison of data from animal studies and human clinical trials suggests that if the data from animal models are considered in sufficient depth, failure or success can be efficiently predicted. In the majority of cases of biological therapies presented in this review, approaches that are consistently efficacious in animal models usually succeed in human trials. In contrast, therapeutic strategies with inconsistent or negative results in animal studies usually fail in human trials. Naturally there is a certain lack of external validity of experimental colitis models, which can be attributed to the differences between murine experimental colitis and human IBD in terms of genetics, environmental triggers, and intestinal microflora. This limitation is highlighted by cases of successfully translated treatment strategies such as the anti-TNF-α and the anti-α4 integrin agents, where there have been isolated animal studies reporting lack of
efficacy (Tables 4 and 5). However, the majority of preclinical data in the case of anti-TNF-α and the anti-α4 integrin agents have correctly predicted success in human trials (Tables 4 and 5). This indicates that when multiple different models are used efficacy or lack of it in humans can be more accurately predicted.

The validity of experimental colitis as a tool to study efficacy of new therapeutic approaches is further highlighted by the cases of anti-IL-17, anti-CTLA-4, and anti-IL-2ra where worse outcome or no effect in the animal studies correctly predicted failure in human trials (Tables 4 and 5). In the same context, the failure of promising interventions to translate in the clinic may also relate to overoptimistic expectations about efficacy based on inadequate animal data (255). In the case of type I IFN and antibodies against CD20, ICAM-1, IL-13, and IL-18 animal studies have produced controversial data so far and were followed by clinical trials showing lack of efficacy for the compounds related to the first three treatment targets, whereas in the case of anti-IL-13 and anti-IL-18 results from human trials are pending (Tables 4 and 5). Preclinical data on GM-CSF and agents that block CD3 were also inadequate and were followed by clinical trials that reported lack of efficacy in IBD.

Another important point that has to be taken into account when evaluating the results of preclinical studies is the time of administration of the drug in relation to the onset of inflammation. There have been many promising therapeutics with beneficial effects when administered prior to the onset of inflammation in the preclinical level, which, however, failed to treat established experimental or human disease in the same or subsequent studies. Characteristic paradigms are the exogenous administration of recombinant IL-10 and the IFN-γ-blocking antibody fontolizumab. Although both efficiently suppressed the induction of inflammation in various animal models, they failed to attenuate preexisting intestinal inflammation (Table 4). Not surprisingly, both approaches proved inefficient when tested in human trials. This does not actually devaluate the predictive value of preclinical testing but rather highlights that the design of some clinical trials failed to acknowledge the limitations of efficacy reported by animal studies (255).

In addition, failures in some clinical trials have been attributed to inadequate dosing of the agent in test. For example, failure to achieve significant remission rates with tocilizumab, despite adequate response rates of 80%, could be attributed to lack of provision of sufficient treatment agent (92). Multiple drug doses and administration schedules can be tested in experimental IBD studies, and effectiveness of the agent in question can be established when some level of statistical difference from control groups is achieved. In contrast, doses and administration schedules in clinical trials are defined from phase I safety trials or prior clinical testing in other diseases, and effectiveness is established only if predefined outcome measures of clinical remission and clinical response are met. Patient stratification can also significantly affect outcome of human IBD trials. Different responses to the same agent in test are often observed and indicate the presence of different patient subgroups. For example, ustekinumab trials have reported higher response rates in patients that have failed anti-TNF-α treatment (207). However, there are no reliable biomarkers that can help identify these subgroups of patients in advance.

Furthermore, clinical trials in IBD often involve patients that are resistant to conventional treatment or even anti-TNF-α agents (207). This clinical situation is hardly modeled by existing animal models, which theoretically mimic responses of treatment-naive patients. This limitation may explain some of the failures or inadequate responses observed in cases with favorable preclinical data. We believe that the scientific community should focus on ways to improve animal modeling of human trials in IBD to improve its predictive value. In such an effort this and other issues, such as attention to sex of the animals (many animal models, e.g., DSS and IL-10−/−, are exclusively performed with females), poor diversity of the intestinal microflora, and nutritional status of the animals, have to be taken into consideration to mimic the variability of the human situation as much as possible. Another point that has to be taken into account when accessing the validity of preclinical evaluation data in predicting the efficacy of clinical studies is publication bias, in other words the practice of publishing only positive-outcome data while interventional studies reporting negative or nonsignificant effects remain unpublished. Indeed, it is hard to find published preclinical studies reporting neutral or negative outcomes in top-tiered journals. This creates an erroneous perception of consistency in preclinical data that drives progression of possibly inefficient treatment approaches in clinical trials without adequate preclinical assessment. The true impact of this phenomenon is hard to estimate. Publication of preclinical evaluation studies in a freely accessible database similarly to human trials could be a step to address the issue. Peer reviewing in this process may help to reduce methodological flaws related to randomization, blinding, sample size calculation, and statistical analysis that threaten the internal validity of preclinical evaluation studies (255).

Translation rates have been considerably higher for RA compared with IBD (79). This difference cannot be attributed to better performance of RA animal models in predicting efficacy of new treatment agents. Similarly to IBD, the validity of experimental studies in RA has also been challenged by the results from human studies. The studies on IL-1 receptor antagonism represent such a case where excellent preclinical efficacy contradicts the limited effectiveness observed in human studies (20). The observed difference in translation rates rather involves intrinsic differences between the two autoimmune diseases. Firstly, a greater multiplicity of operating pathogenetic pathways has been suggested for IBD, reflected by the more than 50 relevant animal models as opposed to less than 10 for RA (20, 106). Secondly, the complexity of interactions with the diverse gut microflora in IBD pathogenesis is only recently beginning to be explored (185). Thirdly, the diversity of genetic predisposing factors has been greater for IBD (102) with 99 nonoverlapping genetic risk loci identified so far vs. around 38 genetic risk loci for RA (179).

In conclusion, the rate of translation of efficacious treatment strategies in IBD from animal studies into clinical practice is relatively low despite the development of numerous animal models of IBD. This phenomenon should not be attributed to the described limitations of experimental colitis models in accurately reproducing human disease but rather to the failure of acknowledging these limitations in the process of human trial planning and design. Therefore, to improve translation rates in parallel to a continuous effort to improve the internal
and external validity of preclinical studies we need to be more thorough in preclinical testing and result interpretation before initiation of clinical trials.

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V.V. and G.K. conceived and designed the research; V.V., M.V., and G.K. analyzed data; V.V., M.V., and G.K. drafted manuscript; V.V. and G.K. edited and revised manuscript; V.V., M.V., and G.K. approved final version of manuscript.

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Review


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