Animal models of ulcerative colitis and their application in drug research

Abstract: The specific pathogenesis underlying inflammatory bowel disease is complex, and it is even more difficult to decipher the pathophysiology to explain for the similarities and differences between two of its major subtypes, Crohn’s disease and ulcerative colitis (UC). Animal models are indispensable to pry into mechanistic details that will facilitate better preclinical drug/therapy design to target specific components involved in the disease pathogenesis. This review focuses on common animal models that are particularly useful for the study of UC and its therapeutic strategy. Recent reports of the latest compounds, therapeutic strategies, and approaches tested on UC animal models are also discussed.

Keywords: inflammatory bowel disease, preclinical trials, emerging therapy

Introduction to ulcerative colitis

Ulcerative colitis (UC) is an idiopathic chronic relapsing–remitting inflammatory disorder that affects the colon, characterized by diarrhea and rectal bleeding (Figure 1). The molecular etiology of UC development is complex and involves genetic, microbial, environmental, and other unknown factors (Figure 1). In this review, we discuss the underlying pathophysiology of UC and how observations from animal models that mimic UC contribute to better understanding of this disease and lead to advancement in novel treatment design.

Based on recent reports, there is a steady increase in the global incidence of UC. Currently, the prevalence in Europe and North America is 24.3 and 19.2 per 100,000 individuals, respectively, and 6.3 per 100,000 people in Asia and the Middle East. Most patients develop UC between the ages of 15 and 30 years, although individuals aged 50–70 years form another potential risk group. There are no significant differences in UC risk between sexes. The growing prevalence of this disease increases both economic and health care burdens. In the United States, an individual UC patient has an average annual expenditure of approximately $15,020 in medical costs. Thus, better and more affordable treatments and eventually a cure are greatly needed.

An individual’s genetic makeup forms one of the primary causal factors for inflammatory bowel disease (IBD) development. The first clue that there is a partial genetic component to the risk of IBD comes from the observation that a family history of IBD confers one with a higher probability of disease development. The offspring of IBD-affected mothers have a higher incidence of developing the disease as compared with the offspring of IBD-affected fathers, indicating that maternal inheritance factors predetermine UC-related genetic risk. Molecular studies have also shown evidence of...
UC-associated genetic imprinting, such that maternal inherited gene expression and functions have been directly linked to IBD development. Recent Genome-Wide Association Studies have also identified 47 UC risk alleles. In addition to direct DNA sequence variants, changes in epigenetics, including DNA methylation, histone modifications, and noncoding RNA, contribute an additional layer of genetic contribution to IBD risk.

Environment and lifestyle constitute the second major arm of UC causal factors. During infancy, early exposure to microbes protects individuals from UC development at subsequent life stages. This is in sync with the notion that improved sanitation in developed countries may lead to immaturity of an individual’s immune system during childhood and subsequently increase susceptibility to UC later in life. Seasonal changes also exacerbate UC conditions, such that symptoms occur more frequently during spring and summer seasons. Meta-analysis has shown that smokers tend to be protected from UC as compared with nonsmokers. In contrast to UC, smoking appears to worsen Crohn’s disease (CD) symptoms.

With the identification of these fundamental causal factors, the next step is to prioritize the directions for the next wave of UC research. The Crohn’s and Colitis Foundation of America composed a list of priorities for IBD research agendas, with the ultimate goal of applying bench-side discoveries to the bedside. To facilitate understanding of UC pathogenesis, animal models, particularly mouse models, have become indispensable tools to study this topic (Figure 2). Experimental colitis can be induced using chemical irritant or bacterial infection. Over the years, many transgenic (Tg) and gene knockout (KO) mouse strains have been developed, allowing the opportunity to address specific pathophysiologic questions related to UC and to test novel drug/therapeutic candidates pertinent to specific components/pathways.

Chemical and bacterial induction of colitis in animal models
A UC-like phenotype can be induced in animals easily using either chemical administration or bacterial infection. Although the majority of these reports were performed on mice, chemically induced colitis has also been tested on other...
species, ranging from lower organisms such as zebrafish and drosophila to higher organisms, including rats and porcine. The choice of animal and induction method will depend on the specific question the particular study is addressing.

Dextran sulfate sodium-induced colitis

A common method to create ulcer formation and inflammation is through administration of dextran sulfate sodium (DSS) to animals. Strictly speaking, DSS does not directly cause intestinal inflammation per se; rather, it exerts chemical injury to the intestinal epithelium, resulting in exposure of the lamina propria (LP) and submucosal compartment to luminal antigens and enteric bacteria, triggering inflammation. The effectiveness of DSS-induced colitis depends on several factors, including dosage (usually 1%–5%), duration (acute or chronic), manufacturer/batch of DSS, strain of animals (C3H/HeJ and Balb/c mice strains are more susceptible), sex of animals (male mice are more susceptible), and microbial environment of animals (eg, germ-free [GF] versus specific pathogen-free [SPF]). In addition, DSS-administered mice also show highly variable disease severity. Unless animals are treated with multiple pulses of DSS, the phenotype lacks the chronic changes observed in humans. Nevertheless, DSS-induced colitis is still commonly used, given its simplicity to administer (usually in the drinking water) and the ease of controlling the dosage (to determine severity) and duration (to study the inflammatory or recovery process). Although the earliest change in this model is characterized by a progressive disruption of colonic crypts, macrophages and cluster of differentiation 4 (CD4⁺) T cells become more prominent in areas of wound healing in the basal portion of the LP after the late recovery phase. Many chemical compounds, gene/cell therapy, and microbial interventions have been reported to be therapeutically effective in DSS-induced colitis (see review by Mizoguchi).

Oxazolone colitis

Intrarectal administration of the hapten oxazolone with ethanol into murine animals results in acute colitis. The condition is characterized by a T helper (Th)2-type immune response with a marked increase in interleukin (IL)-4 and IL-5 production, accompanied by body weight loss, diarrhea, ulcers, and loss of epithelial cells in the large intestine. Thus, it resembles UC rather than other IBD subtypes, distinguishing it from trinitrobenzene sulfonic acid (TNBS)-induced colitis (Th1-mediated immune responses), which mimics CD more closely. Natural killer T cells and their associated cytokine, mainly IL-13, are also intimately involved in oxazolone-induced colitis induction. Many studies have utilized oxazolone-induced colitis to test disease pathology and therapeutic interventions for UC. It was recently shown in oxazolone-treated mice that nicotine upregulates

Figure 2: Mouse models of inflammatory bowel disease (IBD). Many mouse models are currently available that mimic the two major subtypes of IBD (Crohn’s disease [CD] and ulcerative colitis [UC]). Specific mouse models with characteristics of CD and/or UC are summarized. Abbreviations: ΔARE, a deletion mutant in AU-rich elements; DSS, dextran sulfate sodium; Gαi2, guanine nucleotide-binding protein G(i) subunit a-2; IL, interleukin; Mdr1a, multiple drug resistance 1a; TCR, T-cell receptor; Tg, transgenic; TNBS, 2,4,6-trinitrobenzenesulfonic acid; TNF, tumor necrosis factor; TRUC, T-bet−/−XRag2−/−; ulcerative colitis; WASP, Wiskott–Aldrich syndrome protein; TGF, transforming growth factor.
the nicotine acetylcholine receptors on CD4+ T cells and increases regulatory T cell (Treg) numbers, accompanied by a decrease in the number of Th17 cells, resulting in the amelioration of the inflammatory phenotype. In contrast, nicotine treatment in TNBS-induced mice, which have CD-like Th1-associated inflammation, upregulates Th17 cell numbers associated with an exacerbation of the inflammatory response. In a separate study, mice treated with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor showed suppression of the Th1 response and attenuation of intestinal inflammation in TNBS-induced colitis, but not oxazolone-induced colitis. These examples illustrate the importance of choosing an appropriate model to answer a specific question when exploring a therapeutic potential of a drug/compound for a particular disease.

Acetic acid-induced colitis

Intrarectal administration of diluted acetic acid provides an alternative method to create chemical injury to the mucosal epithelium that induces a transient phenotype mimicking UC. The first report of this model was demonstrated by MacPherson and Pfeiffer where they instilled 10%–50% acetic acid into the rat rectum for 10 seconds, followed by flushing the lumen with saline three times. A diffuse colitis in an acetic acid dose-dependent manner was observed in these rats, with histopathological features including ulceration of the distal colon and crypt abnormalities. The ulcerated and injured mucosa, with destruction sometimes extending to the LP, begins to heal within days in mice and a few weeks later in rats. Subsequent modifications and optimization over years focused on varying the concentration of acetic acid and the contact time. As enemas of high concentration of acetic acid into the lumen often cause perforations, the latest protocol is executed using 4% acetic acid with 15–30 seconds of exposure. The advantages of acetic acid-induced colitis are its low cost and the ease of administration.

There are a large number of reports that describe compounds that can ameliorate acetic acid-induced colitis. These include compounds aiming to target reactive oxidative species such as N-acetyl cysteine, trimetazidine, vitamin E, and melatonin, suggesting that acetic acid-induced colitis may be a good model to study the efficacy of drugs that aim to interfere with reactive oxidative species pathogenesis. Of note, the epithelial injury observed within the first 24 hours of acetic acid induction is not immunologic in nature. Thus, designing drugs that target immune responses should be tested at a time point after 24 hours postinduction.

Salmonella-induced colitis

The gram-negative Salmonella typhimurium and Salmonella dublin are food-borne enteric bacterial pathogens that can cause intestinal diseases. Direct oral infection of *S. typhimurium* into mice results in systemic infection that may mask the phenotype of intestinal inflammation. However, this problem can be overcome by pretreating mice with an oral antibiotic cocktail to disrupt commensal microbial flora and allow better colonization of *S. typhimurium*, resulting in high-density growth of the bacterium within a day. The initial inflammation caused by such colonization has similar histopathological characteristics to human UC, including epithelial crypt loss, erosion, and neutrophilic infiltration. Of note, this mode of colitis induction usually results in systemic infection within 5–7 days of infection. Therefore, it is perceived that *S. typhimurium* infection is a valuable model to study the acute phase, but not later stages, of colitis.

Salmonella has been shown to function as a good vector to introduce certain gene components into the mucosa to elicit immune response for vaccines against colitis. It can efficiently invade into the intestinal epithelium and Peyer’s patches (PP). Therefore, careful and detailed characterization of its virulence factors is critical to efficiently create a safe attenuated strain for such gene therapy vaccination. Vaccinating mice with an attenuated mutant *S. typhimurium* strain that contains deletion of the znuABC operon, which encodes a zinc importer responsible for metal recruitment in the infected host, elicits effective immune responses and protects mice from subsequent Salmonella infection. In addition, elucidating how *S. typhimurium* interacts with host epithelial cells facilitates further understanding of ways to potentially prevent UC onset. For instance, blocking host inflammatory-induced proteins (eg, chitinase 3-like 1 [CHI3L1, also known as YKL-40]) in the colon using appropriate antibodies (Abs) or inhibitors can prevent colonization of *S. typhimurium* on the intestinal epithelium, thus preventing further invasion.

Adherent–invasive *E. coli*

The commensal adherent–invasive *Escherichia coli* (AIEC) strain was originally isolated from the ileum of CD patients and was shown to exacerbate intestinal inflammation in an opportunistic manner. However, AIEC can adhere to both small and large intestinal epithelial cells (IECs) with equal affinity. Induction of colonic inflammation in animal models using AIEC infection requires mild epithelial damage, such as low-dose DSS treatment, during the entire course of the infection. The phenotype of the colonic inflammation...
mimics UC, including body weight loss, presence of blood in stool, and colonic neutrophilic infiltrations. A recent study showed that AIEC encodes a pathogenic form of chitinase, chiA, that is distinguishable from other non-pathogenic E. coli and is utilized to adhere to host epithelial cells by binding with colonic inducible protein CHI3L1. Administration of chitin microparticles (1–10 µm in size) into mice ameliorates colonic intestinal inflammation, presumably by blocking the interaction of bacterial-derived factors (such as AIEC chiA) with host CHI3L1. Similarly, using anti-CHI3L1 Abs also resulted in an ameliorative effect.

Effective invasion into, and colonization of, AIEC in the mucosal epithelium is usually hindered by mucosal biofilm formation of probiotic bacteria, such as Lactobacillus casei. Certain antibiotics result in the disruption of the intestinal microflora, including the probiotic biofilm, creating an ideal environment for the opportunistic AIEC to adhere to and invade IECs and macrophages. It has been shown that AIEC does not adhere efficiently in non-antibiotic-treated mice, but colonizes well in the antibiotic-treated animals. This result suggests that restoration of a beneficial microbiota, either through probiotic intake or other methods such as fecal microbial transplantation, can theoretically prevent further exacerbation of intestinal inflammation by commensal pathogenic bacteria.

Transgenic and gene knockout animal models of ulcerative colitis

IL-7 Tg mice

IL-7 is a pleiotropic cytokine and a candidate risk gene associated with UC. IECs express IL-7, which serves as a regulatory factor for the development and homeostasis of lymphocytes that express IL-7 receptor (IL-7R). In UC patients, IL-7 protein expression is significantly upregulated and exerts its optimal effects in maintaining long-lived memory CD4+ T cells in colonic mucosa. IL-7 appears to mediate the persistence of chronic colitis through the IL-7Rα chain expressed specifically on CD4+ T cells, but not on other cell types. Thus, blocking IL-7R functions has been shown to be effective in suppressing adaptive T-cell-mediated intestinal inflammation in mice. Administration of specific anti-IL-7R Ab into murine colitis models (eg, Helicobacter bilis-infected Mdr1 KO mice) also controls macrophage and dendritic cell (DC) expansion.

IL-7 Tg mice expressing the murine IL-7 complementary DNA spontaneously develop acute colitis at 1–3 weeks of age, characterized by a mixed cellular infiltration that includes neutrophils and lymphocytes. At 8–12 weeks of age, the Tg mice display rectal prolapse and remittent intestinal bleeding, with rectal erosion, goblet cell loss, and occasional crypt abscesses. Upregulation of IL-7R on mucosal lymphocytes is also associated with disease progression. Thus, an IL-7 Tg mouse model is useful to understand T-cell-mediated pathogenesis of colitis for therapeutic interventions targeting T-cell functions.

TCRα KO mice

In 1993, Mombaerts et al showed that T-cell receptor α chain (TCRα) KO mice spontaneously developed chronic colitis, which was mediated by a Th2-type immune response closely resembling human UC with an inflammatory pattern restricted primarily to the colonic mucosa. At 4–6 months of age, approximately 60% of TCRα KO mice produced soft stools, associated with loss of goblet cells, and a mixed cellular infiltration mainly consisting of lymphocytes and neutrophils in the affected LP. Spontaneous colitis develops in TCRα KO animals when raised in a helicobacter-free/SPF facility, but not in GF or conventional (CV) environments. When SPF-born TCRα KO mice were subsequently transferred into a CV environment, the mice developed attenuated mild colitis. This supports the notion that early life exposure to environmental microbes may be protective against colitis risk later in life.

Several therapeutic interventions have been tested in TCRα KO mice with efficacy. Daily oral administration of 3 mg/kg dexamethasone, a member of the glucocorticoid class of steroid drugs, into TCRα KO mice was effective in preventing goblet cell loss and leukocyte infiltration. Immunotherapy treatment using anti-IL-4 Abs has also been shown to suppress both clinical and histological signs of colitis by controlling Th2-type cytokine productions. Administration of purified immunoglobulin G, with a mixture of monoclonal auto-Abs reactive against colonic epithelial cells, can attenuate colitis in B-cell-deficient TCRα KO mice. Carbon monoxide, a prominent component in cigarette smoking, exerts anti-inflammatory effects in TCRα KO mice through suppression of IL-1β, tumor necrosis factor-α (TNFα) and IL-4, as well as through induction of IL-10 production, providing molecular insights into how smoking has protections against UC. In addition, oral administration of chitin microparticles into TCRα KO colitic mice also showed suppression of IL-4 and TNFα production and increased interferon-γ (IFNγ) production in the mesenteric lymph nodes (MLNs).

It was found that chitin treatment in TCRα KO mice normalizes intestinal bacterial composition, as compared with...
control groups that exhibit expansion of a certain genre of commensals microbes.

Wiskott–Aldrich syndrome protein KO mice

Patients with Wiskott–Aldrich syndrome have not only immunodeficiency but also often autoimmune manifestations, with 5%–10% of patients developing colonic inflammation.\cite{44,45} They either lack or express a defective form of Wiskott–Aldrich syndrome protein (WASP), an intracellular molecule specific to hematopoietic cells. Given the major biological role of WASP in actin polymerization, it is crucial for multiple cellular functions such as cell motility, activation, and signaling.\cite{46} Like their human counterpart, WASP KO mice on the 129 SvEv background also develop spontaneous colitis from 4 months of age. Full penetrance was observed at 6 months of age. The pancolitic pattern of inflammation along with elevations in Th2 cytokines in the colonic LP associated with this model mirror features of UC.\cite{47} Initially, it was hypothesized that aberrantly activated effector T cells as well as deficient and dysfunctional Tregs associated with WASP deficiency bore the sole responsibility for colitis development, but more recent studies have revealed a role of WASP KO innate immune cells in disrupting mucosal regulation.\cite{47,48} The advantages of using this model for studying UC pathogenesis are the Th2-skewed cytokine profile mimicking human disease, aberrant natural Treg and innate immune cell function, and a human correlate where a subset of patients with the same genetic defect also suffer from colitis.

Several treatment strategies have been investigated using WASP KO animals. WASP-expressing retrovirus was transduced into WASP-deficient hematopoietic stem cells before transfer into lethally irradiated recipient mice, resulting in the attenuation of colitis along with normalized populations of mature B and T cells compared with chimeric mice with control retrovirus-transduced WASP-deficient bone marrow cells that developed disease.\cite{49} In addition, given that UC patients were found to produce lower levels of intestinal alkaline phosphatase, WASP KO mice were treated with oral intestinal alkaline phosphatase and were found to effectively attenuate colitis with less cellular infiltration and reduced production of IL-4 and IFNγ.\cite{50,51} Furthermore, direct neutralization of IL-4, but not IFNγ, with weekly injection of anti-IL-4 antibody for 8 weeks ameliorated disease.\cite{52} Lastly, administration of a newly formulated IL-10-immunoglobulin fusion protein completely abrogated colitis development in chimeric mice with WASP-deficient innate immune cells.\cite{48}

**Mdr1a KO mice**

*Mdr1a* KO mice lack the multiple drug resistance 1a (*mdr1a*) gene, encoding for the cell surface P-glycoprotein (P-gp) transporter that pumps small amphiphilic/hydrophobic molecules across the cell membrane. Approximately 25% of these mice develop colitis between 8 and 36 weeks of age when raised in an SPF facility, but not in a GF facility.\cite{52} Histological findings in this model include mucosal thickening and loss of goblet cells that is also accompanied by crypt abscesses and ulceration in the colon.\cite{53} *Mdr1a* KO mice are devoid of the proper ability to dispose of bacterial breakdown products in epithelial cells. The accumulation of these bacterial products increases excess/abnormal antigen presentation to neighboring T cells, leading to a marked T-cell activation state that drives the colitis. Recently, T-cell involvement in the development of colitis in the *Mdr1a* KO model has been increasingly characterized. The lack of *Mdr1a* (P-gp) restricts the development of inducible Treg cells, thus producing fewer functional forkhead box P3 (Foxp3)-positive Treg cells and therefore less IL-10 production to control and regulate intestinal inflammation.\cite{54} Hematopoietic-specific *Mdr1a* deficiency results in a more severe colitis than mice that have *Mdr1a* deficiency only in IECs, suggesting a critical role of immune cell-derived P-gp in colitis development.\cite{55}

Consistent with the finding that *Mdr1a* KO mice in a GF facility do not develop colitis, prophylactic treatment using broad-spectrum oral antibiotics greatly reduces the incidence of colitis development.\cite{56} *Mdr1a* KO mice fed with a diet containing a polyphenol compound called curcumin (commonly found in spices used in Asian food) demonstrated upregulation of xenobiotic metabolism as well as downregulation of proinflammatory pathways and associated attenuation of histological signs of colitis.\cite{57} Meta-analysis has identified several polymorphisms in the *Mdr1a* locus in human UC, but not CD, patients that affect its gene expression and regulation.\cite{58} Loss of *Mdr1a* expression was implicated in UC development, but not in CD patients.\cite{59} Administration of the probiotics *Lactobacilli* upregulated P-gp expression under both normal and inflammatory conditions, and reduces myeloperoxidase activity and histological signs of injury in DSS-treated mice.\cite{60} These results suggest that *Mdr1a* KO mice can be utilized in the design of drugs targeting intestinal epithelial barrier dysfunction and in elucidating the mechanisms underlying the benefits of probiotic treatment.

**IL-2 KO mice**

IL-2 is an effective regulatory cytokine produced by CD4+ T cells and amplifies stimulatory responses by promoting
T lymphocyte expansion. IL-2 KO mice are viable and develop normally before 4 weeks of age, thereafter displaying 50% mortality. The remaining mice develop colon-restricted inflammation with 100% penetrance by 6 weeks of age when maintained in a CV environment, but not in GF conditions. The clinical and histological characteristic of this inflammation bears striking resemblance to human UC, including crypt abscesses, ulceraions, and loss of goblet cells. Of note, GF-reared IL-2 KO mice showed signs of other non-UC phenotypes such as disturbances in bone marrow hematopoietic cells, lymphocytic hyperplasia, hemolytic anemia, and generalized autoimmunity, but not colitis. SPF-maintained mice begin to show signs of colonic inflammation only at 17–20 weeks of age, and heightened T-cell and B-cell activation were found to mediate the colitis. These T cells are presumably activated through altered antigen presentation by DCs. In IL-2 KO mice, colonic DCs increase four- to five-fold under inflammatory conditions and localize within the T- and B-cell aggregates, expressing high levels of major histocompatibility complex II, CD80, CD86, CD40, CD205, and C-C chemokine receptor type 5 molecules. These changes in DC phenotype may be induced by certain colitogenic bacteria or antigens. It was shown that Bacteroides vulgatus mpk monoclonized IL-2 KO mice do not manifest colitis but instead exert protective effect, unlike E. coli mpk monoclonized IL-2 KO mice that develop disease. Bacteroides vulgatus mpk-infected IL-2 KO mice increase IL-6 expression and semimaturation of LP DCs. It was reported that intestinal bacterial flora and endogenous antigens, but not environmental antigens, are the main contributors affecting SPF-associated colitis phenotype in this murine model.

Specific targets have been identified to control the severity of colitis in IL-2 KO mice. It was noted that 2,4,6-trinitrophenol-ovalbumin-immunized IL-2 KO mice displayed much more severe intestinal inflammation as compared with untreated mice. In contrast, mice administrated monoclonal Abs against the αβ integrin together with 2,4,6-trinitrophenol-ovalbumin immunization demonstrated attenuated disease associated with a reduction in CD4+ cells and IFNγ production in the LP. In addition, treating 8-week-old IL-2 KO mice with green tea polyphenol extract in the drinking water reduced IFNγ and TNFα production after 1 week of treatment and displayed further improvement in the general histological scores of the spontaneous colitis after 6 weeks.

Gαα2 KO mice

Guanine nucleotide-binding protein G(i) subunit α2 (Gαα2) KO mice exhibit distinct lethal diffuse colitis phenotype within 5–7 weeks of age, associated with clinical and histopathological features resembling UC. These include colonic thickening, lymphocyte and neutrophilic infiltrations, crypt and goblet loss, and crypt abscesses. Cell analysis also showed a marked increase in memory CD44+CD45RBlow and CD62Llow CD4+ T cells in LP. Transfer of Gαα2 KO splenic CD3+ T cells, but not MLN CD3+ T cells, into immunodeficient mice causes severe colitis. B cells within the hematopoietic compartment appear to be an important regulatory factor in controlling the colitis phenotype in Gαα2 KO mice, as demonstrated by a reduction in LPS-induced proliferation and IL-10 production. Indeed, cell transfer of B cells isolated from wild-type MLNs can protect Gαα2 KO mice from colitis.

Testing of therapeutic agents in Gαα2 KO mice has shown positive results in ameliorating colitis. Intrapерitoneal injection of the acellular Bordetella pertussis vaccine into Gαα2 KO mice demonstrated an increase in regulatory IL-10 production in the intestine, accompanied by a significant reduction in colitis. Excessive proliferation of CD4+ T cells was controlled upon treatment, along with an increase in apoptosis of activated Th1-type CD4+ T cells. Ex vivo cultures of colons obtained from Gαα2 KO mice respond to the anti-inflammatory agent methyl-prednisolone in a similar manner as colons from mice that had been orally treated with the same drug, as determined by inflammatory-associated gene expression. Hence, colonic culture systems, rather than in vivo testing, can be utilized to validate future IBD therapies.

Comparative evaluation of various animal ulcerative colitis models in drug development

With the current spectrum of animal models available to study UC, together with the explosive information of the underlying molecular pathogenesis of the disease, these resources can be carefully leveraged to determine the best approaches to design therapeutic drug targets to combat the disease (Figure 3).

The most obvious dogma is to start testing using lower organism models, including Caenorhabditis elegans, drosophila, and zebrafish, that provide a convenient and fast approach to doing large-scale screenings of both drugs and genetic targets (see review by Lin and Hackam). These lower organisms are ideal subjects to study microbial response, as well as understanding the genetics of signaling pathway or intestinal physiology. Upon identifying drug candidates or genetic targets from pilot tests in lower
organisms, further precise investigation can be performed using mouse models. It is important to first identify the most appropriate model that best represents the component in which the drug is exerting its effect: T-cell-mediated UC (IL-7 transgene, TCRαKO, WASP−/− mice, Mdr1a−/− mice), B-cell biology (Gαi2−/− mice), intestinal barrier dysfunction (Mdr1a−/− mice), and signaling pathway dysregulation (Gαi2−/− mice). Of note, the condition of animal husbandry and the cleanliness of the mouse facility (e.g., GF, SPF, or CV) will have robust effects on the results, including the penetrance and severity of colitis in the mice.

If the drug candidate does not pertain to any specific pathway/process, chemically induced colitis mice may be a good approach. The advantage of using these methods, as compared with spontaneous models, is the ease of controlling the chemical dosage, which affects the extent and severity of colitis. This is particularly useful when multiple doses of the drug are to be administered. In addition, the duration of the induction is also highly controllable, hence allowing one to dissect between onset, acute, and recovery phases of the colitis.

When the desired outcome of the new candidate drug/therapy is observed in the rodent colitis models, further investigation can be extended to larger animal models, such as porcine or sheep. The morphology and physiology of pig intestine share a high degree of similarity to those of humans and thus may better reflect responses in patients. Finally, positive findings of a novel therapeutic agent in preclinical testing in animal models can then be extended into a Phase I clinical trial for human UC patients.

Figure 3 Ulcerative colitis (UC) models that address the specific framework of disease pathology. Invertebrate (e.g., Caenorhabditis elegans, drosophila) and vertebrate (e.g., zebrafish, mouse) models have their respective advantages in the study of UC pathogenesis. Different models can be utilized and carefully chosen to more appropriately address particular questions, mainly altered enteric microbial membership, intestinal epithelial dysregulation, and aberrant immune responses.

Abbreviations: AIEC, adherent–invasive Escherichia coli; DSS, dextran sulfate sodium; Gαi2, guanine nucleotide-binding protein G(i) subunit α-2; IL, interleukin; Mdr1a, multiple drug resistance 1a; TCR, T-cell receptor; Tg, transgenic; WASP, Wiskott–Aldrich syndrome protein.

Role of animal models in the development of alternative and emerging ulcerative colitis treatments

Current drug treatment for UC patients includes 5 aminosalicylic acid (5-ASA), corticosteroids, thiopurines, and anti-TNFα Abs. Except for 5-ASA, the mechanisms of action of which is not entirely clear, these therapies aim to exert immunosuppression to control the extent of inflammation. However, long-term efficacy is achieved only in approximately one-third of the patients with moderate to severe
disease and is accompanied and restricted by adverse effects, including risks of infections, lymphoma, and nonmelanoma skin cancer. Animal models have provided a platform for insights into emerging therapies that hopefully will be more efficacious and/or safe (Figure 4).

Antibodies targeting immune cell trafficking to the gut
A potential target to treat chronic intestinal inflammation is to intervene with the process of immune cell recruitment and infiltration into the intestine. Leukocytes rapidly circulate through the microvasculature unless there is a specific signal for cells to exit the circulation and penetrate target organs. This process requires signaling between integrins on the endothelial surface (called adhesion molecules) and their receptors on the leukocyte surface membrane. Pertinent to the gut, mucosal addressin cell adhesion molecule-1 (MAdCAM-1) is expressed only in the intestinal tract and its associated lymphoid tissue and is recognized by its receptor α4β7 on the leukocyte surface, whereas vascular cell adhesion molecule-1 is expressed on endothelial cells in other organs besides the intestinal mucosa and functions as a ligand for both receptors α4β7 and α4β1. Studies in animal models from more than a decade ago demonstrated the efficacy of inhibiting these signals in the treatment of chronic colitis. Podolsky et al74 and Hesterberg et al75 demonstrated attenuation of acute colitis in cotton-top tamarins by administration of anti-α4 monoclonal antibody and anti-α4β7 monoclonal antibody, respectively. At the same time, Picarella et al76 demonstrated the efficacy of Abs to β7 and to MAdCAM-1 in ameliorating colitis induced by transfer of CD4+CD45RBhi cells into lymphopenic mice. These preclinical studies demonstrated the proof of concept that interfering with leukocyte trafficking to the intestine could be effective colitis treatment.

Given that expression of mucosal adhesion molecules MAdCAM-1, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 have been described in mucosal tissues from patients with either CD or UC, Abs to these molecules and their corresponding receptors were deemed favorable targets as novel therapies for IBD (Figure 5).77 Natalizumab, a monoclonal antibody to α4 integrin, was...
approved in 2008 by the US Food and Drug Administration (FDA) for severe, refractory CD after efficacy was documented.\(^\text{78,79}\) However, its use is highly limited due to the risk of progressive multifocal leukoencephalopathy, a potentially fatal demyelinating disease, given its interference with leukocyte homing to the central nervous system through blockade of \(\alpha_4\beta_7\) signaling. Therefore, targeting \(\alpha_4\beta_7\) more specifically with vedolizumab (aka MLN02 and MLN0002) should theoretically avoid this risk. Data from Phase III clinical trials demonstrate benefit in inducing and maintaining response in patients with moderately to severely active CD or UC, with no case of progressive multifocal leukoencephalopathy reported thus far, despite more than 2,000 patients having received at least one dose of vedolizumab in a clinical trial setting.\(^\text{80}\) At this moment, vedolizumab is awaiting FDA approval for both CD and UC.

Similar to the idea of blocking \(\alpha_4\beta_7\), an antibody against \(\beta_7\) and other specific inhibitors of trafficking to the gut are also actively being investigated, given encouraging results from animal studies. Based on the effect of \(\beta_7\) antibody on the murine colitis model of CD45RB\(^{hi}\) transfer and the positive results from a Phase I study showing safety and a hint of efficacy, etrolizumab (aka rhuMAB\(\beta_7\)) is undergoing Phase II testing for UC.\(^\text{76,81}\) Similarly, MAdCAM-1 monoclonal antibody PF-00547,659 demonstrated some efficacy for UC.\(^\text{82}\) Even though alicaforsen (ISIS 2302), an inhibitor of intercellular adhesion molecule-1 (ICAM-1), was not obviously efficacious in CD, an enema formulation of an ICAM-1 inhibitor was found to be comparable with mesalamine (a commonly used medication) in efficacy for left-sided UC.\(^\text{83–85}\) Overall, these results establish inhibitors of gut-homing molecules to be effective in treating IBD, as was seen in animal models in the 1990s.

**Inhibition of cytokine signaling**

Instead of inhibiting particular cytokines, one successful method of treatment has been through blocking the signaling pathway shared by multiple cytokines. Receptors within the IL-2R family (IL-2, IL-7, IL-9, IL-15, and IL-21) comprise two subunits, a shared common gamma subunit, and a second subunit that is specific to the particular cytokine.\(^\text{86}\) The common gamma subunit signals through activation of...
of the protein tyrosine kinase Janus kinase 3 (Jak3), which is expressed solely in immune cells. Jak molecules then transduce the cytokine signal through different isoforms of signal transducer and activator of transcription (STAT), leading to downstream gene activation. As STAT3 activation is important in IL-6-mediated T-cell proliferation and in T-cell-mediated colitis, given STAT3 KO CD4+CD45RBhi cells are unable to induce colitis when transferred into a lymphopenic host, and given STAT3 variants have been associated with increased risks of UC, a selective inhibitor of Jak3 represents a promising novel therapeutic option for colitis without other unwanted effects. Preclinical data are limited to one study demonstrating efficacy of intraperitoneal injection of Janex-1, an inhibitor of Jak3, in attenuating TNBS-induced colitis, but given known benefits in transplant rejection, rheumatoid arthritis, and psoriasis, it was tried on UC. Phase II data revealed efficacy of tofacitinib, an oral Jak3 inhibitor, in inducing response and remission in moderately to severely active UC in a dose-dependent manner. Phase III clinical trials are undergoing, with hopes of FDA approval in the near future. If confirmed to be effective, tofacitinib would become the only nonsteroidal oral treatment to induce remission for moderately to severely active UC.

Appendectomy

Appendectomy, especially during childhood, is inversely associated with the risk of UC development and has been increasingly supported by both clinical and experimental studies. One of the earliest observations was reported in 1987 by Gilat et al in a study in search of childhood causal factors of IBD. Subsequently, a large Swedish cohort analyzing 212,963 patients (with matched controls) who had undergone appendectomy before the age of 50 years was followed for development of UC. It was found that inflammatory-associated appendectomy (eg, appendicitis or lymphadenitis), but not nonspecific abdominal pain, correlated with the lower risk of subsequent UC. Therefore, inflammation of the appendix preceding appendectomy, rather than appendectomy itself per se, appears to confer the protection against UC. Importantly, this correlation is specific to patients who have undergone appendectomy before the age of 20 years.

One of the earliest reports on the effect of appendectomy on experimental colitis in animal models was reported by Mizoguchi et al using the TCRα KO mice. When appendectomy was performed in young (3–5 weeks) TCRα KO mice, only 3.3% developed spontaneous intestinal inflammation after 6–7 months, with reduced MLN cell population, whereas up to 80% of sham-operated TCRα KO mice developed colitis during the same period. The structure of the appendix lymphoid follicle is highly similar to intestinal PP, and cellular proliferation rate in the appendix lymphoid follicle is twice that in the PP. Thus, it appears that appendix-associated/derived lymphocytes may hold key roles in UC development.

In a separate report, 7-day treatment with 2.5% DSS of mice that underwent appendectomy showed delayed onset and course of colitis development as compared with sham-operated mice. This was also accompanied with lower colonic damage scores and a reduction in ulcerated mucosal surface area. Interestingly, splenectomy did not show any benefits in DSS-induced colitis. Thus, although spleen enlargement is a frequent phenotype during DSS treatment, the fact that splenectomy did not confer protection suggests that this enlargement is a consequence, not a cause, of DSS-induced colitis.

Finally, the protective effect of appendectomy was also nicely demonstrated in a T-cell transfer colitis experiment. Fluorescence-labeled colitis-inducing CD62L+ CD4+ cells that were transferred into immunodeficient severe combined immunodeficiency (SCID) mice were found to have a 3.5-fold preferential homing toward the appendix than the colon and to express high levels of α4β7 adhesion molecule costimulatory molecule CD154. Therefore, with the help of various UC animal models, detailed characterization of the components within the appendix may further shed light on the pathophysiology underlying UC development to facilitate future therapeutic intervention attempts.

Helminth therapy

Increasing reports on both colitis mouse models as well as human UC clinical trials demonstrate that helminth infection provides beneficial effects on UC. Studies on helminth infection in UC mouse models have provided mechanistic insights into how the nematode induces tolerogenic DCs that can block colitis development and regulate T-cell responses. In addition to an immunoregulatory effect on adaptive immunity, evidence has supported that helminth infection can promote IL-22-mediated mucosal barrier regulation and gut microbiota to improve intestinal inflammation. The identification of these specific helminth factors can provide alternatively effective and safe treatment. It was demonstrated that injection of the hookworm Ancylostoma caninum-derived excretory/secretory factors can induce IL-4+ IL-10+ CD4+ T-cell response and ameliorate DSS-induced colitis in mice. To specifically determine the precise
factors that have such protective effect, Cantacessi et al.\textsuperscript{98} have characterized the transcriptome of \textit{Trichuris suis} using next-generation sequencing. Recently, Du et al.\textsuperscript{99} reported that subcutaneous injection into colitic mice with a recombinant of a helminth excretory–secretory protein called \textit{Trichinella spiralis} 53 kDa protein reduces disease activity index and macroscopic and microscopic inflammation score. Testing in UC animal models will hopefully further identify therapeutically effective products among helminth-derived factors to broaden clinical treatment options.

Amidst the success of helminth therapy in mouse models and human clinical trials, several reports have provided different effects and perspectives. Recently, Bager et al.\textsuperscript{100} demonstrated in population-based cohort studies that infection of the helminth pinworm that causes enterobiasis does not lower the risk of UC. They suggested that, in contrast to pinworms, hookworms or schistosomes thrive in tropical countries, and thus the higher rates of chronic inflammatory disease, including UC, in nontropical regions may be an effect of the absence of tropical helminth. In addition, Wang et al.\textsuperscript{101} have also reported an exacerbation of disease by the tapeworm \textit{Hymenolepis diminuta} infected into oxazolone-induced colitic mice through IL-5-mediated immune responses. These data suggest that perhaps not all helminth are protective, and careful characterization of helminth-based therapy in mouse models is critical to identify the efficacy and safety aspects before proceeding to human clinical trials.\textsuperscript{102}

Stem cell-based therapy

Pluripotent cell-based therapy for CD is in Phase I trials, which may provide informative insights in the treatments for UC patients as well.\textsuperscript{103} Colitic \textit{IL-10} KO mice that were injected with nondifferentiated embryonic stem cells tagged with yellow fluorescence protein showed homing in the colon, small intestine, liver, and thymus tissues, but not in the spleen or bone marrow, associated with improved colitis inflammatory scores upon transplantation.\textsuperscript{104} In addition, transplantation of IECs from in vivo predifferentiated embryonic stem cells into mice was also found effective in reducing inflammation and in restoration of immune balance. A similar test using \textit{IL-10} KO mice with active colitis was performed through intracolonic infusion of colonic stem cells, which showed an ameliorative effect on colitis.\textsuperscript{105} The authors therefore suggested that colonic stem cells provide a safer option for colitis treatment, as compared with those of systemic stem cell administration.

Bone-marrow derived cells (BMDCs) have also been demonstrated to control the extent of inflammation when transplanted into colitic mice. Lethally irradiated DSS-induced colitic mice transplanted with BMDCs from green fluorescence protein (GFP) Tg mice showed the presence of GFP in vimentin+ colonic interstitial cells, but not Ki-67+ proliferating cells, cytokeratin+ epithelial cells, or CD31+ endothelial cells.\textsuperscript{106} The transplanted GFP BMDCs frequently transdifferentiated into subepithelial myofibroblasts and fibroblasts that reside in the colonic subepithelium even after recovery. To fully correct the immunodysregulation in colitis, it was demonstrated that total body irradiation followed by transplantation of BMDCs is more effective in ameliorating colitis in mice.\textsuperscript{107}

Systemic infusion of mesenchymal stem cells (MSCs) in DSS-induced colitis mice also ameliorated colitis, characterized by downregulation of TNF\textsubscript{$\alpha$} and IL-1\textbeta.\textsuperscript{108} Mechanistically, MSC systemic infusion induced transient T-cell apoptosis via the Fas/Fas ligand-dependent apoptotic pathway.\textsuperscript{109} The apoptotic T cells then trigger macrophages to produce high levels of transforming growth factor-\beta, resulting in the upregulation of CD4+CD25+Foxp3+ Treg production and, eventually, enhanced immune tolerance. Colitic rats showed the presence of the transplanted MSCs only in the LP.\textsuperscript{110} In addition, topical implantation, rather than systemic transplantation, of MSCs into the chemically injured intestinal area is sufficient to promote the healing process.\textsuperscript{111} This success utilizing stem cell therapy in animal models is promising as an emerging therapy for human disease and has been studied in clinical trials.\textsuperscript{103} Further investigation in animal models will aid in determining the long-term fate of the engrafted stem cells, to ensure long-term safety of this mode of UC therapy.

Fecal microbiota transplant

Aiming to balance the dysregulated intestinal microbiome has been attempted through probiotics treatment, with some degree of success in clinical and animal studies.\textsuperscript{112} However, the outcome is nevertheless variable and modest, and so probiotics are considered supplementary and not substitutes for conventional therapy. Therefore, the potential of fecal microbiota transplantation (FMT) to regulate the homeostasis of the abnormal intestinal microbiota now poses an attractive alternative method for UC treatment.

One of the pioneering investigations of FMT as a UC treatment was reported by Bennet and Brinkman,\textsuperscript{113} where Bennet himself was suffering from UC and self-experimented with FMT, reporting improvements after 3 months with resolution of symptoms after 6 months. Although it is perceived that the normal flora present in FMT donors can
restore the normal balance in dysregulated microbial flora in IBD patients, the proper mechanism behind the FMT effect is currently still obscure. Mice that were deficient in nucleotide-binding oligomerization domain 2 and given FMT showed a reduced disease risk and long-term changes in microbiota compositions.114 Conversely, wild-type mice that were transplanted with the dysregulated fecal microbiota derived from nucleotide-binding oligomerization domain 2 KO mice showed increased disease risk, suggesting the presence of particular microbial subsets that may be protective or colitogenic. 

Recent survey results have revealed that the majority of UC patients show keen interest in considering FMT in their treatment regimen.115 With the emerging technology and maturation of humanized mouse models, mouse models of UC will serve as a valuable tool to elucidate the mechanism behind FMT beneficial effects, as well as to define proper safety issues and long-term effects.

Herbal and plant extracts

The therapeutic effects of herbal or plant extracts have been exploited for centuries, and identifying and characterizing these components can provide alternative treatment options for UC patients. These include both Western-derived herbs as well as traditional Chinese medicine. Many of the reported randomized clinical trials compared the efficacy of these alternative agents with that of standard treatments (ie, 5-ASA, sulfasalazine, and steroids) or placebo. A literature review reported that the number of subjects who have undergone clinical trials using a herbal treatment for UC from 1947 to 2013 ranges from 14 to 224 subjects, with treatment duration lasting for 4–12 weeks.116 No major adverse reactions, other than minor side effects, including nausea, constipation, and flatulence, were reported to be associated with these different herbal treatments.116 The major herb/plant extracts that have been shown to have potential benefit for UC include aloe vera, Boswellia serrata, butyrate, licorice, slippery elm, tormential extracts, wheat grass, curcumin, bromelain, and psyllium (see review by Ke et al117). One major aim of the treatment is to induce remission of UC, whereby aloe vera, wheat grass, and HMPL-004 (Andrographis paniculata extract) appear to have some efficacy when compared with placebo controls.118-120 Another major goal of herb/plant extract therapy is to maintain remission in UC, in which curcumin, myrrh, chamomile extracts, and coffee charcoal treatment in patients results in a relapsed rate that is comparable with 5-ASA-treated patients in randomized clinical trial reports.121,122 However, caution needs to be taken in interpreting these observations, given many of these studies were done on patients with only mild disease, treatment effects were not rigorously tested with objective endpoints in all studies, and long-term safety and efficacy are not known.

Given some hint of efficacy, it is important to understand the underlying mechanisms behind the potential therapeutic effects of these herbs and plant extracts by using UC animal models. For instance, A. paniculata extract (HMPL-004) has been demonstrated to reduce splenic CD4+ T cells as well as inhibit CD4+ T-cell proliferation and differentiation into Th1/Th17 effector cells in a murine CD45RBβ cell transfer model.123 In Mdr1a KO colitic mice, reduction of colitis histological signs by curcumin treatment is associated with upregulation of xenobiotic metabolism and also down-regulation of proinflammatory pathways.55 These studies provide further in vivo mechanistic understanding of how each individual herb/plant extract may work, facilitating the choice of the most appropriate herbal treatment for defined groups of patients.

Conclusion

Animal models have become indispensable tools to study mechanisms and treatments of diseases. Insights learned from such models allow us to design novel therapeutic strategies and to define, in a preclinical setting, the safety and efficacy of such novel treatments before human clinical trials. The fact that there are currently numerous models available also indicates that no single model is perfect, and it is therefore essential to define the specific question in mind, in order to identify the ideal model for study.

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