

Experimental Models of Inflammatory Bowel Diseases



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SUMMARY

Experimental models of inflammatory bowel diseases have provided valuable insights into the complex mechanisms operative in the development and pathogenesis of these diseases. This review describes widely used models and how they help us understand the immunology of intestinal inflammation.

The understanding of the intestinal inflammation occurring in the inflammatory bowel diseases (IBD) has been immeasurably advanced by the development of the now numerous murine models of intestinal inflammation. The usefulness of this research tool in IBD studies has been enabled by our improved knowledge of mucosal immunity and thus our improved ability to interpret the complex responses of mice with various causes of colitis; in addition, it has been powered by the availability of models in which the mice have specific genetic and/or immunologic defects that can be related to the origin of the inflammation. Finally, and more recently, it has been enhanced by our newly acquired ability to define the intestinal microbiome under various conditions and thus to understand how intestinal microorganisms impact on inflammation. In this brief review of murine models of intestinal inflammation, we focus mainly on the most often used models that are, not incidentally, also the models that have yielded major insights into IBD pathogenesis. (*Cell Mol Gastroenterol Hepatol* 2015;1:154–170; <http://dx.doi.org/10.1016/j.jcmgh.2015.01.006>)

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For more than two decades, animal models of intestinal inflammation have provided a wealth of information about mucosal immunology, particularly as the latter relates to the maintenance of intestinal homeostasis or the disruption of such homeostasis and the intestinal inflammation encountered in the inflammatory bowel diseases (IBD). No single model captures the complexity of human IBD, but each model provides valuable insights into one or another major aspect of disease, and together they have led to the establishment of now a generally accepted set of principles of human IBD pathogenesis. Chief among these are that (1) different causes of induced or genetically based inflammation give rise to a finite number of common

pathways of immunopathogenesis; (2) normal resident gut microbiota can drive intestinal inflammation; (3) loss of oral tolerance and disruption of the epithelial barrier contribute to the development of intestinal inflammation; and (4) polarized T helper (T_H) cell responses as well as defects in innate immunity mediate disease. In this review, we focus on selected experimental models of intestinal inflammation that amply verify these principles and in doing so provide important contributions to our understanding of the immune dysregulation that characterizes IBD. In addition, we provide a brief summary (Table 1) of less common mouse models that are based on genetic defects that lead to increased susceptibility to gut inflammation.

DSS Colitis

Disruption of the intestinal epithelial barrier and thereby the entry of luminal bacteria or bacterial antigens into the mucosa has been clearly established as a disease mechanism in enterocolitis by the fact that intestinal inflammation can be more easily induced or may occur spontaneously in numerous murine models characterized by molecular abnormalities that cause massive barrier loss. The importance of an intact epithelium in the prevention of mucosal inflammation was initially demonstrated by Hermiston and Gordon¹ in an influential study published in the 1990s. In this study, a chimeric mouse was created in which small intestinal epithelial cell adhesion mediated by E-cadherin was undermined by the introduction of an epithelial cell transgene expressing dominant negative N-cadherin. The small intestines of these mice contained patches of villi with poorly adherent and incompletely differentiated transgenic enterocytes adjacent to patches of normal enterocytes with normal adherence. The striking finding was that inflammation occurred in the lamina propria of these mice but only in areas subjacent to the defective epithelium, strongly suggesting that entry of commensal microorganisms into an otherwise normal lamina propria can induce an inflammatory response.

Abbreviations used in this paper: bp, binding-protein; DSS, dextran sulfate sodium; Foxp3, forkhead box P3; IBD, inflammatory bowel disease; IFN- γ , interferon- γ ; IL, interleukin; LAP, latency-associated protein; NKT, natural killer T; PSA, polysaccharide A; PTEN, phosphatase and tensin homolog on chromosome 10; TGF- β , transforming growth factor- β ; T_H, T helper cell; TLR, Toll-like receptor; TNBS, 2,4,6-trinitrobenzene sulfonic acid; TNF- α , tumor necrosis factor- α ; Treg, regulatory T cell.

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Table 1. Experimental Models of Inflammatory Bowel Disease Due to Specific Genetic Defects

| Model | Underlying Defect | Major Consequences | Significance |
|-----------------------------------|--|--|--|
| Muc2 Colitis ^{147,148} | Deficiency of Muc2, the main gastrointestinal mucin. | Spontaneous colitis (severe in the distal colon) that is enhanced by DSS administration and develops into colorectal cancer. Inflammation is associated with increases in intestinal lymphocytes, TNF- α , and IL-1 β . | Loss of epithelial barrier function resulting from Muc2 deficiency causes inflammation. |
| Related models ^{149,150} | Lack of C3GnT (β 1,3-N-acetylglucosaminyltransferase), an enzyme involved in the synthesis of core 3-derived O-glycans that are components of colonic mucins. ¹⁴⁹ | Increased susceptibility to DSS colitis and DSS/AOM-induced tumorigenesis; T _H 1 and T _H 7 proinflammatory cytokine production is elevated. | Enhanced intestinal permeability due to deficiency in core 3-derived O-glycans and reduced Muc2 levels increases susceptibility to colitis and colorectal cancer. |
| | Targeted deletion of core 1-derived O-glycans, also components of mucins. ¹⁵⁰ | Spontaneous colitis (severe in the distal colon and rectum) driven by TNF- α -producing macrophages and granulocytes rather than lymphocytes. Inflammation is independent of TLR signaling. | Core 1-derived O-glycans deficiency leading to a reduced inner mucus layer and decreased Muc2 enhances intestinal permeability to both proteins and bacteria and causes inflammation, that is associated with epithelial cell expression of the Tn antigen, observed in some UC patients. |
| MDR1a colitis ¹⁵¹ | Lack of P-gp, the product of the <i>MDR1a</i> gene alters epithelial barrier function. | Spontaneous colitis driven by T _H 1 cytokines. | A barrier defect arising from P-gp deficiency causes increased intestinal permeability and translocation of bacteria into the lamina propria, and the development of colitis. ^{152,153} |
| TRUC model ¹²² | Disruption of the transcriptional regulator T-bet in the innate immune system of <i>RAG2</i> ^{-/-} mice. | Spontaneous colitis driven by intestinal flora and increased production of TNF- α and IL-23. Cohousing of TRUC mice with wild-type mice demonstrate transmission of colitis to a normal host and thus reveal the existence of a "colitogenic" microflora (such as <i>Proteus mirabilis</i> and <i>Klebsiella pneumoniae</i> or <i>Helicobacter typhlonius</i>). ¹⁵⁴ | First and only demonstration of the existence of a colitogenic intestinal microflora. However, treatment of TRUC mice with anti-TNF- α therapy prevents inflammation and bacterial populations from becoming "colitogenic," indicating that the development of the latter requires an abnormal mucosal immune system. |
| NEMO Colitis ¹⁵⁵ | Intestinal epithelial cell-specific disruption of NF- κ B function via targeted deletion of NEMO, an essential regulatory subunit of NF- κ B also known as IKK- γ . | Spontaneous and severe chronic intestinal inflammation. | Lack of NF- κ B signaling results in heightened TNF- α sensitivity and apoptosis of colonic epithelial cells followed by inflammation caused by translocation of bacteria into the mucosa. Highlights role of TNF- α in maintenance of epithelial cell barrier function. |
| Related model ¹⁵⁶ | Specific deletion of the catalytic subunit of IKK- β in intestinal epithelial cells. | Severe intestinal inflammation after infection with the gut-dwelling parasite <i>Trichuris muris</i> and reduced expression of TSLP. | IKK- β in intestinal epithelial cells promotes T _H 2-cell dependent immunity and limits chronic inflammation. |

Table 1. Continued

| Model | Underlying Defect | Major Consequences | Significance |
|---|---|---|---|
| SAMP1/Yit mouse ¹⁵⁷ | The SAMP1/Yit strain was established by selective breeding of SAM (senescence accelerated mouse derived from AKR/J mice) P1 strain mice showing spontaneous skin ulcerations. | Spontaneous inflammation of the terminal ileum and caecum driven by a T _H 1 response; however, a T _H 2 response may develop at later stages of disease. Exhibit “skip lesions.” Inflammation originates from epithelial barrier defect. | One of the few models exhibiting severe inflammation of the terminal ileum, the primary location of Crohn’s disease lesions. |
| XBP1 model ^{158,159} | Epithelial cell-specific deletion of the transcription factor, XBP1, an important component of the ER stress response. | Spontaneous enteritis and enhanced susceptibility to DSS colitis. | Induction of ER stress through disruption of XBP1 causes small intestinal inflammation possibly due to absence of Paneth cell antibacterial mediators and reduction of goblet cells. |
| STAT3 Colitis ^{160,161} | Macrophage/neutrophil-specific deficiency of the transcriptional regulator STAT3. | Spontaneous chronic enterocolitis associated with a polarized T _H 1 response dependent on overproduction of IL-12p40. Defective IL-10 signaling in macrophages. | IL-10 signaling in macrophages and neutrophils is necessary to prevent abnormal regulation of responses to the normal microflora. |
| Related model (IL-22 deficiency) ¹⁶² | Targeted deletion of STAT3 in intestinal epithelial cells and associated IL-22 deficiency. | Enhanced susceptibility to DSS colitis. | In intestinal epithelial cells, STAT3 activity is critical for mucosal wound healing mediated by IL22 during acute colitis. |
| IL-7 transgenic mice ¹⁶³ | Systemic overexpression of IL-7. | Spontaneous chronic colitis associated with a T _H 1 response. | IL-7 acts as a critical survival factor for colitogenic CD4 ⁺ effector memory T cells. ¹⁶⁴ Bone marrow is the main source of IL-7 and a substantial population of colitogenic CD4 ⁺ memory T cells reside in the bone marrow. ^{165–168} |

Note: AOM, azoxymethane; DSS, dextran sulfate sodium; ER, endoplasmic reticulum; Foxp3, forkhead box P3; IBD, inflammatory bowel disease; IKK, I κ B kinase; IL, interleukin; MDR1a, multidrug resistance protein 1; Muc2, mucin 2; NEMO, NF- κ B essential modulating protein; NF- κ B, nuclear factor κ B; P-gp, P-glycoprotein; T_H, T helper cell; TLR, Toll-like receptor; TNF- α , tumor necrosis factor- α ; TSLP, thymic stromal lymphopoietin; STAT3, signal transducer and activator of transcription 3; TRUC, T-bet^{-/-} × RAG2^{-/-} ulcerative colitis; UC, ulcerative colitis.

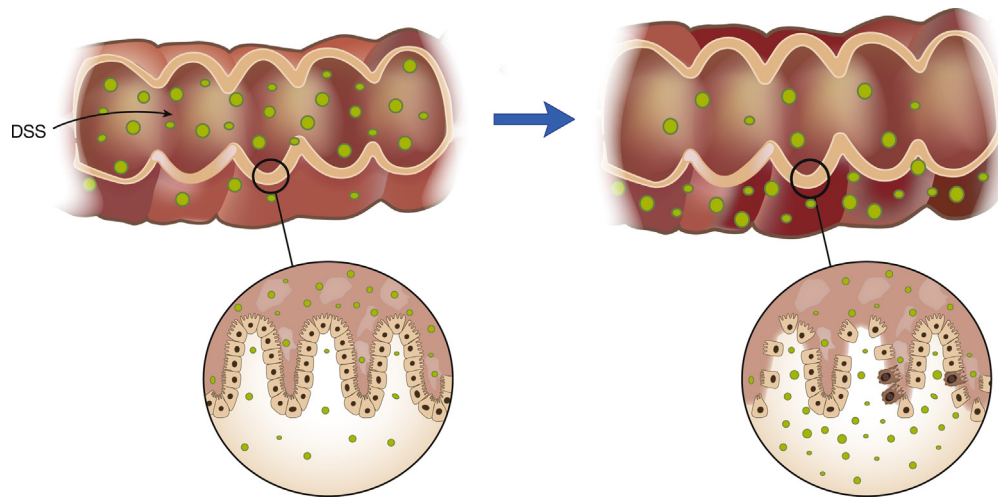


Figure 1. Mechanism of dextran sulfate sodium (DSS) colitis. DSS colitis is most essentially a colitis due to loss of epithelial barrier function and entry of luminal organisms or their products into the lamina propria. Such entry results in stimulation of innate and adaptive lymphoid elements and secretion of proinflammatory cytokines and chemokines. In addition, it results in the influx of cells with cytotoxic potential such as neutrophils and inflammatory macrophages.

A similar phenomenon occurs in dextran sulfate sodium (DSS) colitis, but in this case a sulfated polysaccharide that is directly toxic to colonic epithelium causes epithelial cell injury and the resulting immune responses alter mucosal barrier function throughout the colonic epithelium (Figure 1). Thus, administration of DSS to mice in their drinking water for a short period of time results in the induction of a very reproducible acute inflammation limited to the colon and characterized by erosions/ulcers, loss of crypts, and infiltration of granulocytes.^{2,3} Importantly, this inflammation develops in the absence of T cells mediating adaptive immunity, such as in SCID⁴ and *Rag1*^{-/-} mice,⁵ indicating that effector cytokines produced by innate cells are sufficient to cause the inflammation. On this basis, DSS colitis has become a useful model for the study of the innate immune mechanisms involved in the development of intestinal inflammation; indeed, multiple studies have highlighted the critical roles that innate cells such as macrophages and neutrophils play in mucosal inflammation.⁴⁻⁹ This has led to the insight that macrophages are important sources of proinflammatory cytokines as well as cytokines that regulate epithelial barrier function and proliferation and that neutrophils are important contributors to tissue damage.

In a related vein, DSS colitis has been used to explore the role of innate immune mechanisms in colitis induction such as those initiated by Toll-like receptors (TLR) and dectin receptors or inflammasome stimulation.^{6,7,10-16} An important finding here was the discovery that mice with gene deletions affecting TLR signaling such as *Tlr2*^{-/-}, *Tlr4*^{-/-}, and *Myd88*^{-/-} exhibit exacerbation of DSS colitis because the signaling in epithelial cells and macrophages that depends on these innate signaling components promotes epithelial proliferation and epithelial barrier restoration and limits bacterial translocation and inflammation.^{6,10,11} Therefore, the ability of these effectors to promote restoration of epithelial function is even more important to the

development of inflammation than their effect on production of effector cytokines. TLR4/MyD88 signaling is also required for neutrophil recruitment to the damaged mucosa, as DSS-treated TLR4/MyD88 knockout mice exhibit a decrease in neutrophil infiltrates due to reduced chemokine expression by macrophages and diminished chemotactic response by neutrophils.⁶ Furthermore, MyD88 signaling in macrophages has been found to support and promote proliferation and survival of colonic epithelial progenitors thereby facilitating efficient repair responses after DSS injury.⁷

Because the primary lesion in DSS colitis involves disruption of the epithelium, it lends itself to the study of factors that maintain or reestablish the integrity of the epithelium during or after injury. Many of these studies have centered on the effects of TLR and cytokine stimulation and have shown that factors derived from hematopoietic cells as well as factors derived from the epithelial cells themselves affect integrity. With respect to TLR stimulation, TLR2 ligand administration lessens the severity of DSS colitis whereas TLR2-deficiency enhances severity of DSS colitis. These observations can be explained in part by the effects of TLR2 ligand stimulation of the epithelial cell barrier function or epithelial cell apoptosis and in part by the ability of TLR2 to induce anti-inflammatory interleukin-10 (IL-10) production.¹¹ In addition, activation of epithelial TLR4 can lead to the release of factors that have positive effects on epithelial cell growth and reduce the severity of DSS colitis.¹⁷ This model has been used to show that granulocyte-macrophage colony-stimulating factor and IL-33 released by epithelial cells (and other nonhematopoietic cells) have positive and negative effects on epithelial cell renewal, respectively.^{18,19} Yet another focus of studies of epithelial reconstitution in DSS colitis is the role of various intracellular signaling pathways in such reconstitution. An interesting example of the latter type of study is one showing that inhibition of the

Wnt pathway by the Wnt antagonist Dkk1 favors epithelial cell renewal during DSS colitis.²⁰

Any discussion of the use of DSS colitis must also mention the series of studies in which this model has been applied to the evaluation of NLRP3 inflammasome responses because this TLR-activated innate immune mechanism results in the secretion of IL-1 β and IL-18, two cytokines with potential effects on epithelial cell integrity. However, the outcome of inflammasome responses in DSS colitis has proven to be unexpectedly complex. The reason this might be is that while IL-1 β is clearly a key proinflammatory cytokine in DSS colitis,²¹⁻²⁴ the function of IL-18 is somewhat ambiguous. Thus, whereas initial work concluded that IL-18 is required for induction of DSS colitis,^{21,25,26} later work showed that a deficiency in NLRP3 inflammasome activity and production of IL-18 as well as a defect in IL-18 signaling increases susceptibility to DSS colitis.^{13,14,27-29} In all likelihood, the basis of these findings is that, as noted herein, IL-18 has an important direct or indirect role in epithelial cell restitution and repair after injury.^{29,30}

The DSS colitis model has also been used extensively to study colon cancer developing in relation to colonic inflammation, such as that occurring in patients with longstanding ulcerative colitis.³¹⁻³³ For such study, mice are administered a single dose of azoxymethane, a genotoxic colonic carcinogen that gives rise to colon adenomas or carcinomas but only when such administration is followed by induction of DSS colitis or another type of chronic colitis.³⁴ The mechanism of neoplastic development in this model is not yet completely understood. However, it is likely that a simple initiator-promoter model, with azoxymethane serving as the initiator and the repair after cycles of DSS-induced injury serving as the promoter, is involved. One contributing factor to the development of cancer in this model may be that the neutrophils produce large amounts of IL-1 β , which leads to macrophage and dendritic production of IL-6, a cytokine that acts as a tumor promoter.³⁵ IL-18 also comes into the picture here because *IL18*^{-/-} and *IL18r1*^{-/-} mice display increased susceptibility to DSS colitis neoplasia.²⁹ This effect of IL-18 may be channeled through IL-22 and IL-22bp (binding protein), the latter a decoy protein that neutralizes IL-22. This possibility arises from the fact that, on the one hand, the interplay between IL-22 and IL-22bp has been shown to impact epithelial cell growth and repair and thus control tumorigenesis in the colon, and, on the other hand, these factors are regulated by IL-18 and the NLRP3 or NLRP6 inflammasomes.³⁰

Finally, and more recently, DSS colitis has provided a platform for the investigation of the effects of the gut microbiome on colitis development. This is exemplified by studies demonstrating that the NOD2 abnormalities give rise to mice that develop changes in susceptibility to DSS colitis and that the latter is associated with (or perhaps caused by) changes in the gut microbiome.^{22,36} In addition, it has been shown that mice deficient in NLRP6, an inflammasome protein produced exclusively in epithelial cells, results in more severe DSS colitis, because NLRP6

inflammasome-induced IL-18 production in the epithelial cell microenvironment controls the growth of *Prevotellaceae* species within the crypts. Interestingly, such susceptibility to colitis is also manifest in coreared normal mice without NLRP6 abnormalities.¹⁵ Similarly, lack of NLRP6 leads to more severe colorectal cancer, a phenotype that is also transmissible through microbiota transfer to wild-type mice.³⁷⁻³⁹

Even though DSS colitis is caused primarily by disruption of the epithelium and activation of macrophages and neutrophils the absence of adaptive immunity, it should be noted that T-cell responses can aggravate the inflammatory response when both innate and adaptive immunity are intact. A polarized T_H1 response has been observed in acute colitis whereas a mixed T_H1/T_H2 response was found in a chronic form of DSS colitis, achieved by repeated cycles of DSS administration.⁸ These T-cell responses result in effector cytokine production that contributes to the high levels of tumor necrosis factor- α (TNF- α) and IL-6 produced by macrophages in both forms of DSS colitis.

As apparent from this discussion, DSS colitis has been put to good use in the study of many of the attributes of intestinal inflammation. It should not be assumed, however, that this model is an accurate mimic of human IBD, as massive epithelial cell damage and wholesale invasion of the lamina propria by microbes is unlikely to be a primary mechanism of the human disease. This difference between DSS colitis and human IBD is also reflected in the fact that chronic DSS colitis is characterized by a mixed T-cell proinflammatory cytokine response, whereas chronic human IBD is characterized by polarized T-cell responses. Particularly in the study of primary epithelial cell defects as a mechanism of human IBD initiation, DSS colitis must be used with caution as it causes a more widespread and indiscriminate lesion than is likely to occur in human disease.

Trinitrobenzene Sulfonic Acid Colitis

Intrarectal administration of the haptenating agent 2,4,6-trinitrobenzene sulfonic acid (TNBS) renders colonic proteins immunogenic to the host immune system and thereby initiates a mucosal immune response that drives colitis in susceptible mouse strains.⁴⁰ Intrarectal TNBS administration to SJL/J or C57BL/10 mice induces a transmural colitis mainly driven by a T_H1-mediated immune response and characterized by infiltration of the lamina propria with CD4⁺ T cells, neutrophils, and macrophages as well as development of severe diarrhea, weight loss, and rectal prolapse.⁴¹ As some of these characteristics resemble features of Crohn's disease, TNBS colitis has been widely used in the study of immunologic aspects relevant to this disease, including cytokine secretion patterns, mechanisms of oral tolerance, and effects of potential immunotherapies.

It is now well established that cytokine responses are key elements that control the inflammatory mechanisms underlying IBD. Indeed, it was noted early on that interferon- γ (IFN- γ) synthesis, in particular, is a characteristic

feature of Crohn's disease that might be responsible for the inflammation observed in this disease.⁴² Thus, it was highly significant that inflammation in TNBS colitis was associated with elevated levels of IFN- γ and that prevention of IFN- γ production by anti-IL-12p40 treatment completely abrogated both nascent and established disease.⁴¹ It was this finding and those derived from subsequent related studies^{43,44} that paved the way for the development of a humanized anti-IL-12p40 antibody for treatment of patients with Crohn's disease that is reported to be highly effective in controlling this disease in phase II and III clinical trials, even in patients who are unresponsive to anti-TNF- α .^{45,46}

Although IL-12p40-neutralizing antibody was originally intended to block the pathogenic contributions of IL-12, it was later found that the p40 receptor subunit for IL-12 couples with p19 to form the receptor for IL-23, and that the latter is essential to the stability of the T_H17 response.⁴⁷ Several studies were therefore conducted to define the role of IL-23 in experimental colitis and to determine whether IL-12 or IL-23 drives the dominant effector response in these models.^{48,49} With respect to TNBS colitis, p19^{-/-} mice, which lack IL-23, were unexpectedly found to develop even more severe colitis than wild-type mice.⁴⁸ This correlated with the fact that mice with TNBS colitis lacking IL-23 express increased amounts of IL-12⁴⁸ and that IL-17A production can suppress the induction of T-bet, a major transcriptional regulator of IFN- γ production.⁵⁰ Thus, in TNBS colitis the T_H1 response appears to be the dominant effector response, and the T_H17 responses seem to play both effector as well as regulatory roles in the inflammation. As we will discuss, a somewhat different situation may be obtained in other models such as adoptive transfer colitis, where the T_H17 responses appear to be dominant although both T_H1 and T_H17 responses result in cells producing IFN- γ (see the further discussion herein).

In a recent study, TL1a (TNFSF15) was also shown to be involved in the pathogenesis of TNBS colitis, in that treatment of mice with anti-TL1a completely blocked colitis induction.⁵¹ Because abrogation of T_H1 cytokine responses also blocks colitis induction, this finding implies an underlying relation between TL1a and T_H1 responses; however, at the moment, this link is undefined. One possibility based on the fact that TL1a acts as a T-cell costimulation factor is that TL1a costimulation is necessary for the generation of the T_H1 response. Another possibility based on TL1a signaling via DR3 (TNFSFR15) and its capacity to induce IL-1 β via the NLRP3 inflammasome is that IL-1 β production is a necessary antecedent of the T_H1 response. Further work will be necessary to decide between these as well as other possibilities.

As alluded to earlier, susceptibility to TNBS colitis and the type of cytokine response that is induced by TNBS administration varies significantly among mouse strains. In BALB/c mice, for instance, TNBS colitis has been associated with T_H2-mediated hypertrophy of colonic patches, a phenotype that is accentuated in the absence of IFN- γ .⁵² A T_H2 component was also observed in C57BL/6 mice deficient in IL-12 and IFN- γ .⁵³ Similarly, BALB/c mice are the

preferred strain for induction of chronic TNBS colitis, in which repeated administration of low doses of TNBS results in a less intense and more sustained inflammation than obtained in the single, high-dose acute TNBS colitis described in SJL/J mice. This chronic model has shown that cytokine expression patterns are dynamic in that they change as the inflammation evolves. Thus, in this setting inflammation is characterized by sequential responses, with the initial T_H1 cell response accompanied by high levels of IL-12 and IFN- γ that is subsequently replaced by a persistent T_H17 cell response accompanied by persistent IL-13 and transforming growth factor- β (TGF- β) secretion.⁵⁴ Such evolution may also be characteristic of human IBD, at least in part.

Chronic TNBS colitis in BALB/c mice is also associated with persistent lamina propria fibrosis. This model has therefore been used to study the potential mechanisms underlying the intestinal fibrosis that accompanies human IBD. As shown in the initial report of chronic TNBS colitis, this model is characterized by increased collagen deposition as well as production of factors associated with fibrosis including insulin-like growth factor I (IGF-I), tissue inhibitor of metalloproteinase-1 (TIMP-1), and TGF- β .⁵⁵ Subsequent studies explored the mechanism of such fibrosis and provided evidence that TGF- β production induced by IL-13, acting via IL-13R α 2, was an initiator of a fibrotic program that involved early growth response protein 1 (Egr-1), IGF-I, and myofibroblast production of collagen.⁵⁶ Other studies have provided evidence that fibrosis in this model is induced by substance P acting via the neurokinin-1 receptor⁵⁷ and can be opposed by the antimicrobial peptide cathelicidin.⁵⁸ Interestingly, fibrosis in chronic TNBS colitis may also be induced by TL1a, as it has been shown that this factor also induces fibrogenic factors and it is said to have direct collagen-inducing effects on fibroblasts in vitro.⁵⁹

Despite continued administration of TNBS to BALB/c mice, chronic intestinal inflammation ultimately resolves as does production of proinflammatory IL-17 driving the inflammation. Extensive studies of this resolution of inflammation have determined that persistent IL-13 secretion and accompanying signaling via signal transducer and activator of transcription 6 (STAT6) results in changes in glycogen synthase kinase-3 β (GSK-3 β) phosphorylation and that this in turn induces expression of IL-10, a regulatory cytokine. This set of observations suggests that the spontaneous waning of human Crohn's disease inflammation may also be due to glycogen synthase kinase-3 β induction of IL-10.⁶⁰

Under normal circumstances, when both innate and adaptive immunity are present, T-cell-derived cytokines, particularly IFN- γ , are critical in TNBS colitis as described earlier. Studies in SCID and *Rag1*^{-/-} mice, however, have shown that innate immunity is also involved in the development of TNBS colitis because in response to TNBS these mice develop a colitis that is superficially similar to that observed in their wild-type counterparts.⁶¹ In addition, production of enabling cytokines such as IL-12 resulting from stimulation of innate cells by various innate stimuli

such as TLR ligands is necessary for the initiation and maintenance of the T-cell responses operative in the inflammation. Indeed, inhibition of innate responses by the NOD2 ligand muramyl dipeptide has been shown to prevent TNBS colitis.⁶²

The TNBS colitis model has also been used to explore mechanisms of mucosal homeostasis and the role of NOD2 in the maintenance of such homeostasis. In part, these studies centered on effects of NOD2 on expansion of regulatory T cell (Treg) in the lamina propria-bearing latency-associated protein (LAP), a molecule that binds to TGF- β . These Treg proliferate in response to the entry of luminal bacteria or their products into the lamina propria and thus quell inflammation.⁶³ Because NOD2 is an intracellular bacterial sensor that recognizes the peptidoglycan constituent muramyl dipeptide,⁶⁴ it was initially assumed that abnormal Nod2 function would result in uncontrolled invasion of microorganisms into the lamina propria and associated inflammatory responses. On the contrary, NOD2-deficient mice do not develop spontaneous colonic inflammation and are resistant to TNBS colitis. This may reflect increased intestinal permeability to luminal bacteria or their bacterial products and expansion of regulatory LAP⁺ T cells.⁶⁵ This interpretation is supported by the observation that adoptive transfer of lamina propria T cells from NOD2-deficient mice to NOD2-deficient recipient mice—but not adoptive transfer of LAP⁺ T-cell-depleted lamina propria T cells—provided protection from TNBS colitis. Overall, these studies demonstrated that NOD2 deficiency may paradoxically protect an individual from inflammation by maintaining gut homeostasis under conditions in which proinflammatory influences arising from other factors are relatively mild.

It is often forgotten that the TNBS colitis model has contributed to our understanding of the relation of oral tolerance mechanisms and to the concept that intestinal inflammation can result from a loss of immune tolerance. In particular, it was shown early on that oral administration of TNBS-haptenated colonic proteins before intrarectal instillation of TNBS suppressed colitis and that this suppression was dependent on TGF- β -producing regulatory T cells.⁶⁶ Furthermore, it was shown that the development of such Tregs depended on the production of IL-10, thereby establishing the latter as a key anti-inflammatory cytokine.⁶⁷ It was also shown that the feeding of TNBS-haptenated colonic proteins “cross-protected” mice from the development of oxazolone colitis (another form of hapten-induced colitis), albeit to a lesser extent than it protected mice from TNBS colitis.⁶⁸ This protection was associated with cells that exhibited a regulatory phenotype. Overall, these studies were the first to link oral tolerance with the development of antigen independent Tregs that are capable of controlling gut inflammation.

As our discussion of TNBS colitis has indicated, this induced model of colitis, while differing in important ways from human IBD, has served as an important source of information about the cytokines driving human IBD and the way to therapy of the human disease. In addition, it remains a highly useful platform for studying several aspects of

human IBD such as the factors guiding its spontaneous resolution and fibrosis.

Oxazolone Colitis

Intrarectal administration of a haptenating agent other than TNBS was initially expected to give rise to a colitis similar to TNBS colitis. It therefore came as a surprise that administration of oxazolone (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one), a haptenating agent that, like TNBS, induces a delayed skin reaction, elicits colonic inflammation that differs markedly from that caused by TNBS and that resembles many features of ulcerative colitis rather than Crohn's disease.^{69–71} In particular, administration of a single dose of oxazolone leads to acute superficial inflammation of the mucosa in the distal colon. This is characterized by infiltration of lymphocytes and neutrophils, lamina propria edema, and ulcerations.⁶⁹ A similar but longer-lasting colitis can be achieved by subcutaneous sensitization with oxazolone before a single-dose intrarectal challenge.⁷⁰ As in the case of TNBS colitis, oxazolone colitis is most easily elicited in SJL/J or C57BL/10 mice whereas C57BL/6 mice are more resistant to this colitis. Chronic colitis can also be induced in BALB/c mice by weekly administration of low oxazolone doses.

The cellular and cytokine responses underlying oxazolone colitis differ markedly from those in TNBS colitis. Oxazolone colitis is characterized by robust IL-13 production originating from lamina propria CD4⁺ natural killer T (NKT) cells rather than by conventional CD4⁺ T cells producing IFN- γ (Figure 2). Importantly, both IL-13 and NKT cells are involved in oxazolone colitis, as IL-13 blockade by an IL-13R α 2-Fc fusion protein prevented colitis, and oxazolone failed to induce colitis in NKT cell-deficient mice.⁷⁰ These observations may be explained by data showing that NKT cells are cytotoxic to epithelial cells bearing NKT cell targets and this cytotoxicity can be augmented by IL-13.⁷² In contrast, IL-13 reduced epithelial barrier function by increasing epithelial cell apoptosis as well as tight junction permeability via claudin-2 and tricellulin.^{73–77} This detrimental effect of IL-13 on epithelial barrier function is abetted by IL-9, another T_H2-like cytokine shown to be produced during oxazolone colitis.⁷⁸

The origin of the IL-13 response in oxazolone colitis is as yet poorly understood although there is some evidence that it is induced by IL-33 originating from damaged epithelial cells.⁷⁹ IL-25 produced by epithelial cells may also contribute.⁷⁹ Alternatively, IL-9 may be produced as a result of T-cell stimulation by IL-13 and TGF- β , both of which are abundant in oxazolone colitis.

The importance and value of the oxazolone colitis model is that it resembles human ulcerative colitis not only with respect to morphology but also with respect to immunopathogenesis. Ulcerative colitis is characterized by increased production of IL-13 and IL-9 and has a lamina propria infiltrate containing NKT cells capable of producing IL-13.^{72,78} One important difference between oxazolone colitis and ulcerative colitis, however, is that in the latter inflammation the NKT cells are so-called type II NKT cells,

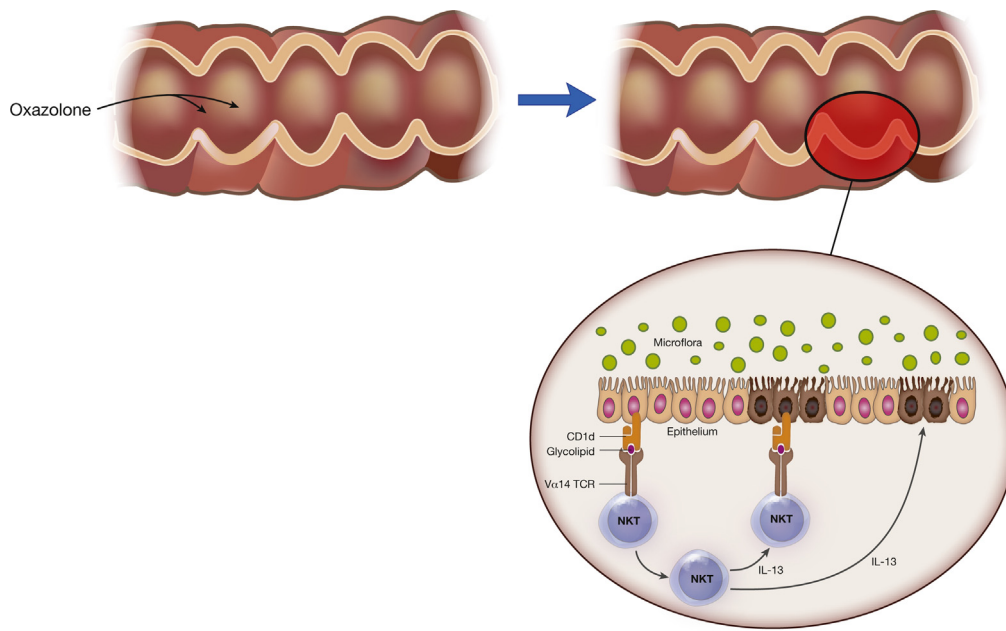


Figure 2. Mechanism of oxazolone colitis. Oxazolone colitis is driven by natural killer T (NKT) cells that originate in the cytokine milieu of epithelial cells subjected to damage by oxazolone as well as hematopoietic cells in the lamina propria. As shown in the figure, the NKT cells are activated by glycolipids presented to them in the context of CD1 and then mediate toxicity via their direct cytotoxic activity directed at target epithelial cells bearing glycolipid antigen. Alternatively, the NKT cells mediate tissue damage via their production of interleukin-13, a cytokine that has been shown to affect tight junction proteins and to thereby affect epithelial barrier function.

which do not have the invariant T-cell receptors characteristic of the more familiar type I NKT cells. It has been shown that the type II NKT cells in ulcerative colitis recognize and respond to sulfatide glycolipids.⁸⁰ This suggests that ulcerative colitis may be driven by the epithelial-cell release of a sulfatide glycolipid that induces NKT cells and IL-13.

Adoptive Transfer Colitis

A major step forward in the development of murine models of intestinal inflammation came with the discovery that adoptive transfer of naïve CD4⁺ T cells (CD4⁺CD45RB^{high} T cells) from donor mice into syngeneic immunodeficient (lymphopenic) SCID or *Rag1*^{-/-} recipient mice causes a wasting disease and a primarily colonic inflammation that develops 5 to 10 weeks after treatment. In contrast, transfer of mature CD4⁺CD45RB^{low} T cells or cotransfer of both naïve and mature T cells into the recipient mice did not cause colitis⁸¹⁻⁸⁴ (Figure 3). This initially curious and unexplained finding was then shown to be due to the fact that the mature T-cell population contains Treg cells that expand and suppress inflammation whereas the naïve T-cell population lacks such cells. These Treg cells were first characterized as CD4⁺CD25⁺ T cells in studies showing that transfer of CD4⁺CD45RB^{low} T cells depleted of CD4⁺CD25⁺ cells into recipient mice induced disease.⁸³ More recently, however, similar studies have shown that the regulatory cells are the now familiar forkhead box P3⁺ (Foxp3⁺) Treg cells. Given the crucial role of Treg cells in

this model of colitis and the importance of understanding the mechanisms that control intestinal immune homeostasis, the cell transfer model has positioned itself as one of the most widely used models of experimental colitis.

It is now widely accepted that Foxp3⁺ Treg cells exert their suppressive function and antagonize effector T cells in the gut through multiple mechanisms, including the production of the cytokines IL-10, TGF- β , and IL-35.^{85,86} Early studies highlighted an immunoregulatory role of IL-10 by showing that administration of anti-IL-10 receptor antibodies or transfer of CD4⁺ T cells deficient in IL-10 to *Rag1*^{-/-} hosts resulted in severe colitis.^{87,88} Later studies probing the mechanism of IL-10 regulatory activity in the transfer colitis model showed that Foxp3⁺ cells deficient in the IL-10 receptor subunit β (IL-10R β) failed to protect recipient mice from colitis and lost their ability to express Foxp3, suggesting that IL-10R signaling in regulatory cells is important for the maintenance of their function.⁸⁹

The role of IL-10 in regulatory T-cell function does not exclude a role for TGF- β . The latter was demonstrated by studies showing that protection from colitis by Treg cells did not occur when TGF- β -deficient CD4⁺CD25⁺ T cells were cotransferred into recipient mice and by the observation that systemic administration of anti-TGF- β antibody blocked the ability of CD4⁺CD25⁺ T cells to attenuate colitis.^{90,91} In addition, TGF- β expressed on the surface of CD4⁺CD25⁺ T cells in association with LAP mediated CD4⁺CD25⁺ T-cell suppression in vitro and CD4⁺LAP⁺, but not CD4⁺LAP⁻, T cells protected recipient mice from colitis.⁹² Finally, and perhaps most importantly, naïve T cells

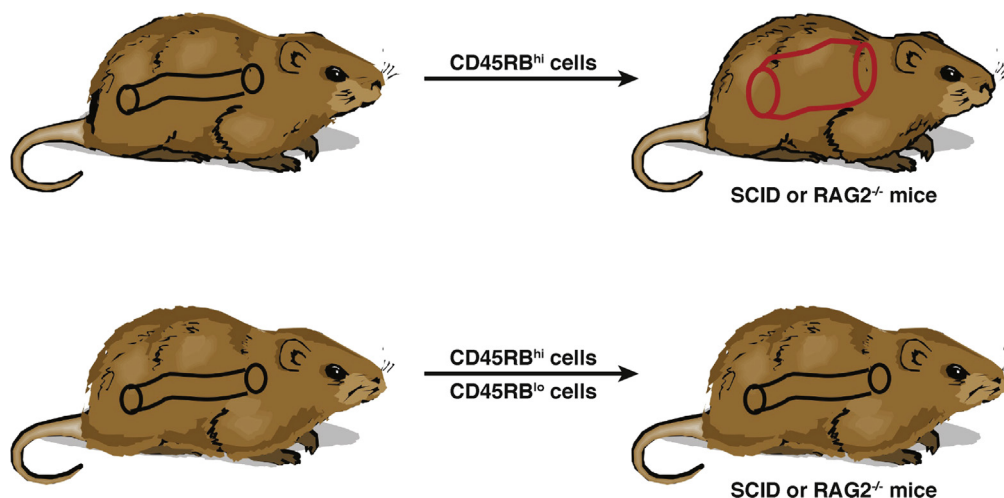


Figure 3. The cell transfer colitis model. Cell transfer colitis is initiated in lymphopenic (SCID or $Rag^{-/-}$) mice that are the recipients of naïve $CD45RB^{high}$ T cells, a cell population that cannot be induced to generate regulatory T cells in a timely fashion to prevent the expansion of effector T cells that mediate inflammation. However, if the naïve T cells are transferred in the company of mature $CD45RB^{low}$ T cells that already contain regulatory T cells, the latter prevent the development of inflammation. This model thus provides a way of isolating the function of effector cells and regulatory cells.

made unresponsive to $TGF-\beta$ via expression of a dominant negative $TGF-\beta$ receptor type II could not be suppressed by Treg cells.⁹³

Although extensively studied, the precise nature of the inflammatory response that regulatory cells suppress in the transfer colitis model has, at least until recently, been challenging to define. The area of controversy concerned the relative importance of the contributions of the T_H1 and T_H17 effector T-cell responses to the colonic inflammation. The earliest studies considered transfer colitis to be a T_H1 -mediated model, as it resulted in the development of a large population of T cells producing $IFN-\gamma$ and $TNF-\alpha$ in the lamina propria of recipient mice.⁸² In addition, anti- $IFN-\gamma$ treatment of the transferred mice ameliorated colitis,⁸² and transfer of naïve T cells lacking T-bet, which are unable to produce $IFN-\gamma$, failed to induce colitis.⁹⁴

The discovery of the fact that T cells can differentiate into T_H17 cells under the influence of IL-23 raised the possibility that IL-17 (IL-17A and IL-17F) and other cytokines also associated with the T_H17 differentiation pathway such as IL-23, IL-21, and IL-22 might be the main players driving inflammation in transfer colitis rather than $IFN-\gamma$. Evidence supporting this possibility quickly became available and included studies showing that (1) $Rag1^{-/-}$ recipient mice also deficient in IL-23p19 did not develop colitis upon transfer of naïve T cells;⁴⁹ (2) colitis was not induced by transfer of naïve T cells lacking the IL-23 receptor;⁹⁵ (3) transfer of IL-17A-producing (previously polarized T_H17) antigen-specific T cells into SCID mice caused severe colitis that could be prevented by an anti-IL-23p19 antibody;⁹⁶ and (4) naïve T cells deficient in $ROR\gamma t$, the master regulator of T_H17 cells, failed to induce colitis in lymphopenic hosts whereas systemic administration of recombinant IL-17A restored susceptibility to colitis.⁹⁷ These rather convincing studies establishing the T_H17 response as the major effector pathway in transfer colitis ran up against

the fact that the number of T cells producing $IFN-\gamma$ is far greater than those producing IL-17 in the lamina propria of mice with transfer colitis.^{98,99} Thus, in spite of strong evidence to the contrary, the T_H1 response still appeared to be the main effector T-cell response underlying this model of colitis. A similar argument can be made in Crohn's disease.¹⁰⁰

Recent studies have resolved this apparent contradiction by showing that T_H17 responses that generate IL-17-producing T_H17 cells are inherently plastic and can also give rise to T_H1 cells producing $IFN-\gamma$. This was initially evident in studies demonstrating the accumulation of an IL-17⁺ $IFN-\gamma$ ⁺ T-cell subpopulation in transfer colitis.^{95,101} More importantly, IL-17F reporter mice were used to show that, in the absence of $TGF-\beta$, IL-23 and IL-12 convert T_H17 cells into “ T_H1 -like” $IFN-\gamma$ -producing cells, and that transfer of previously polarized IL-17F⁺ T_H17 cells to $Rag1^{-/-}$ mice resulted in the development of colitis accompanied by the progressive disappearance of IL-17⁺ cells and the appearance of $IFN-\gamma$ ⁺ cells in the inflamed mucosa.¹⁰² Finally, linear transformation from T_H17 to apparent T_H1 cells via intermediate T_H17/T_H1 cells was observed in studies using $ROR\gamma t$ reporter mice in which the initial population of transferred cells could be unequivocally identified as T_H17 cells.¹⁰¹ Overall, then, current data support the view that transfer colitis is in fact largely mediated by T_H1 cells producing $IFN-\gamma$, but the latter have an atypical origin in that for the most part they originate indirectly from a T_H17 differentiation pathway depending initially on IL-23 rather than directly from a T_H1 differentiation pathway depending only on IL-12.

As already discussed in relation to TNBS colitis, IL-17 production in transfer colitis can paradoxically suppress inflammation by blocking the development of T_H1 cells producing $IFN-\gamma$, which has for greater proinflammatory potential. Consistent with this, transfer of naïve T cells from

IL-17A KO mice to *Rag1*^{-/-} mice induces a more aggressive wasting disease with earlier onset and higher levels of IFN- γ relative to disease following transfer of wild-type naïve T cells.⁵⁰ These findings were explained by showing that T_H1 cells express IL-17 receptors, and that IL-17A signaling through those receptors decreases T_H1 differentiation by inhibiting production of T-bet, a key transcriptional regulator of IFN- γ production. Interestingly, this interference between potentially colitogenic T_H1 and T_H17 cells may be bidirectional. Studies using parabiotic mice in whom T_H1 and T_H17 colitis was induced separately, before surgery and effector cells mixing, showed that colitogenic responses mediated by T_H1 cells or T_H17 cells are in competition and down-modulate one other.⁹⁸

The T_H17 T-cell differentiation pathway—or, more specifically, a major component of this pathway, IL-23—has also been shown to negatively influence regulatory T-cell responses. In initial studies supporting this idea, it was demonstrated that IL-23p19-deficient mice exhibit an increased number of Treg cells in the colon;¹⁰³ in addition, transfer of naïve T cells to *Rag1*^{-/-} mice deficient in IL-23p19, which might be expected to not induce colitis because of lack of IL-17, does in fact induce colitis in situations where recipient mice could not mount a regulatory T-cell response due to lack of IL-10, TGF- β , or CD4⁺Foxp3⁺ T cells.¹⁰³ Finally, and most convincingly, the numbers of Foxp3⁺ Treg cells in the colon of *Rag1*^{-/-} recipient mice reconstituted with IL-23 receptor-deficient T cells are increased.⁹⁵ Taken together, these findings demonstrate rather unequivocally that IL-23 production favors the development of inflammation by mediating T_H17 effector cell responses and by inhibiting Foxp3⁺ regulatory T-cell differentiation.

Recent studies have provided data supporting the idea that the described negative effect of IL-23 on regulatory T-cell development is not unopposed. In particular, IL-33, a cytokine constitutively expressed by epithelial cells in response to tissue damage, enhances regulatory T-cell stability and function in transfer colitis.¹⁰⁴ Moreover, Treg cells that lack the IL-33 receptor (ST2) lost their Foxp3 expression over time and were unable to protect mice from adoptive transfer colitis.¹⁰⁴ Consistent with previous observations establishing an important role for the transcription factor GATA-3 in regulatory T-cell function,^{105,106} it was shown that ST2-expression in Tregs was largely dependent on GATA-3.¹⁰⁴ Overall, then, Treg activity in transfer colitis is under the *yin* of IL-23 and the *yang* of IL-33, which operate in opposing fashions to regulate inflammation. A similar regime may govern Crohn's disease because IL-33 has also been found in inflamed tissues of IBD patients.^{107–111}

Whereas the main effect of IL-23 in relation to the induction of T_H17 T-cell differentiation is to stabilize and ensure the survival of IL-17 cells initially induced by TGF- β and IL-6 or IL-1 β ,^{112–114} this cytokine may also have other effects that influence inflammation in transfer colitis. Along these lines, there is some evidence that IL-23 induces IL-17-producing cells with more proinflammatory potential (such as T_H17 cells) that do not coproduce IL-10.^{114–116} In

addition, there is emerging evidence that transfer colitis, presumably as a result of IL-23 production, is accompanied by increased granulocyte-macrophage colony-stimulating factor¹¹⁷ and IL-5 production (personal communication from F. Powrie), both cytokines that can profoundly affect the nature of the inflammatory infiltrate in transfer colitis.

Finally, it is important to mention that although it is primarily linked to T_H17 T-cell development, IL-23 induces innate lymphoid cells, a recently discovered population shown to express IL-17, IL-22, and IFN- γ and to mediate intestinal inflammation in models of innate colitis, particularly when they produce IFN- γ .¹¹⁸ There is also some evidence that small numbers of these cells accumulate in the inflamed mucosa of Crohn's disease patients, and it has been postulated that these cells play a role in the initiation of Crohn's disease.¹¹⁹

As in most other colitis models,^{120–123} adoptive transfer colitis does not develop in germ-free mice.¹²⁴ This important fact suggests that, as in DSS colitis, transfer colitis is marked by a change in epithelial barrier function and that it is the entry of either commensal bacteria or bacterial components into the lamina propria that then drives the inflammation. However, there is no evidence that a particular subset of bacterial organisms serve as colitogenic organisms in transfer colitis (or indeed in human IBD); instead, it is likely that a large and diverse subset of organisms with particular ability to penetrate an impaired epithelial layer act together to induce inflammation.¹²⁵ It should also be noted that commensal bacteria are thought to contribute to intestinal immune homeostasis and confer protection from the development of inflammation. This is nicely demonstrated by the fact that transfer colitis conducted in immunodeficient mice colonized with *Helicobacter hepaticus* results in a severe colitis that is abrogated by co-colonization with *Bacteroides fragilis*.¹²⁶ The observed protective activity of *B. fragilis* requires the molecule polysaccharide A (PSA); co-colonization with a mutant *B. fragilis* lacking PSA failed to ameliorate colitis whereas oral administration of PSA reduced the disease. Colonization of mice with *B. fragilis* was later shown to facilitate Treg differentiation and IL-10 production.¹²⁷ Other commensal bacteria from clusters IV and XIVa of the *Clostridium* genus have also been shown to induce accumulation of Treg in the colon and confer resistance to colitis in mice.¹²⁸ There is some evidence that this occurs via bacterial secretion of short-chain fatty acids that induce epigenetic changes favoring Foxp3⁺ T-cell development.¹²⁹

This overview of the cell transfer model reminds us of the extraordinary contributions of this model to the study of intestinal inflammation. In particular, it has been used with great effect to define the role of regulatory cells in such inflammation and to thus establish the fact that mucosal homeostasis depends on a balance between mucosal proinflammatory effector function and anti-inflammatory regulatory function. This said, it cannot be assumed that a model that depends on cell expansion in a lymphopenic host mimics human IBD. Further, it is yet to be proven that a qualitative or quantitative defect in regulatory cell

development is present in IBD patients and is a contributor to disease pathogenesis.¹³⁰

Interleukin-10 Knockout Mice

One of the earliest models of intestinal inflammation was that identified in mice with IL-10 deficiency.¹³¹ This model continues to have great value given the important anti-inflammatory function of this cytokine, already discussed in relation to other experimental models of colitis. In addition, it is now known that genetic polymorphisms at the IL-10 locus confer increased risk of both ulcerative colitis and Crohn's disease^{132,133} and, perhaps more importantly a familial form of early onset Crohn's disease has been identified that is due to homozygous mutations in subunits of the IL-10 receptor: *IL10RA* and *IL10RB*.¹³⁴

Mice with targeted deletion of IL-10 (*Il10*^{-/-}) develop spontaneous inflammation of the colon characterized by the presence of an inflammatory infiltrate made up of lymphocytes, macrophages, and neutrophils.¹³¹ The inflammation is initially driven by a proinflammatory T_H1 T-cell response and as such is ameliorated by systemic administration of anti-IL-12p40, and to a lesser extent anti-IFN- γ .¹³⁵ However, for as yet unexplained reasons, the production of IL-12 and IFN- γ decreases over time and is to some extent superseded by progressive increases in the production of the T_H2 cytokines IL-4 and IL-13.¹³⁶ *Blimp1*^{-/-} and *Il10rb*^{-/-} mice, which also have a defect in IL-10 production or responsiveness, recapitulate the phenotype of *Il10*^{-/-} mice and thus support the presumption that lack of IL-10 production is directly responsible for the intestinal inflammation in such mice.^{137,138} In addition, deletion of IL-10 in all T cells or specifically in Foxp3⁺ Treg cells also results in spontaneous colitis, indicating that IL-10 derived

from these cells is important to the maintenance of gut homeostasis.^{139,140}

Stimulation of the mucosal immune system by commensal microflora is critical for the development of colitis in IL-10-deficient mice, as the latter do not develop colitis in a germ-free environment but do develop colitis several weeks after transfer from a germ-free environment to a pathogen-free environment. Such stimulation is most likely dependent on the sensing of commensal microbes by Toll-like receptors, as MyD88 deficiency in colonic mononuclear phagocytes completely prevents colitis.^{121,141,142} It should be noted, however, that onset of inflammation in IL-10-deficient mice can be initiated by administration of piroxicam, a nonsteroidal anti-inflammatory drug that induces oxidative stress.¹⁴³ The mechanism of this effect is unknown, but it could be associated with activation of the NLRP3 inflammasome, which is sensitive to such stress.

A related observation is that mice lacking both IL-10 and PTEN (phosphatase and tensin homolog on chromosome 10), a phosphatase that regulates cell proliferation and receptivity to TLR stimulation, develop colitis more rapidly and with greater severity than *IL-10*^{-/-} mice and also form colonic tumors.¹⁴⁴ This greater susceptibility to colitis is reversed in mice lacking the ability to respond to TLR4 stimulation, indicating that the lack of PTEN is operating, at least in part, by increasing responsiveness to TLR stimulation. An interesting additional observation is that PTEN/IL-10 deficient mice have a fecal microbiome that contains increased amounts of *Bacteroides* organisms, suggesting that the lack of PTEN may also influence disease pathogenesis via effects on the microbiome.

The finding that TLR ligand stimulation is critical for induction of colitis in IL-10-deficient mice suggests that

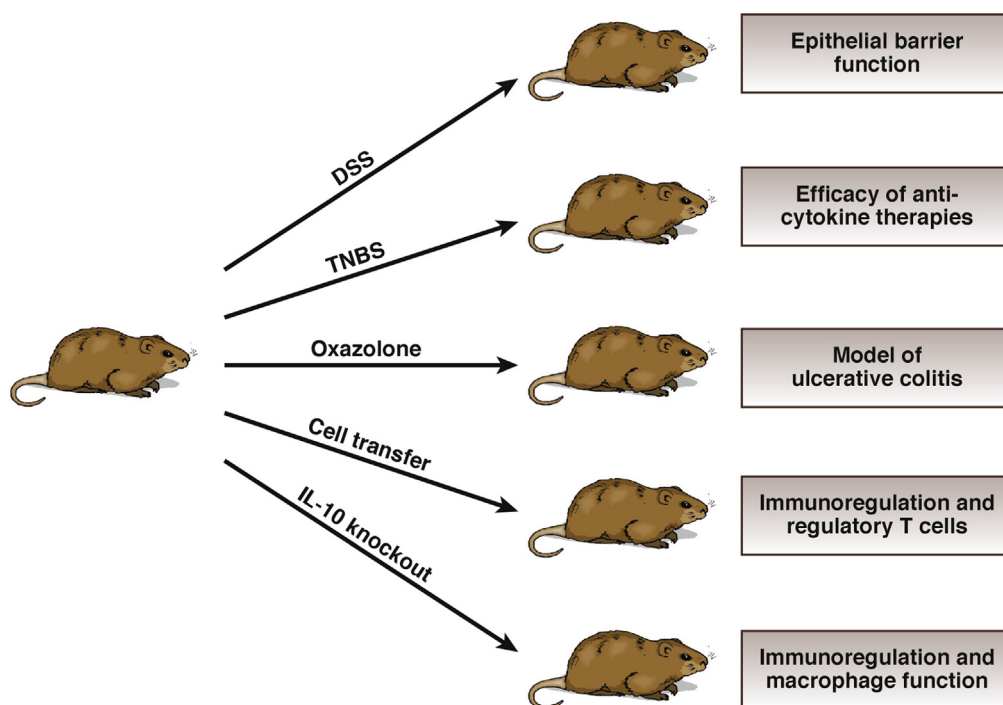


Figure 4. The five experimental models of inflammation discussed in the text are grouped here in relation to their varying inducing conditions. To the right of the individual mouse models are the major mucosal immune functions/therapies/diseases that are best addressed using these respective models.

macrophage dysfunction associated with the absence of IL-10 is a major cause of inflammation in such mice. This possibility is supported by a recent study showing that failure of intestinal resident macrophages to respond to IL-10 (because of lack of IL-10 receptor expression) led to the development of a proinflammatory macrophage migratory profile essential for the development of spontaneous colitis.¹⁴⁵ In addition, a related report showed that IL-10 sensing by anti-inflammatory macrophages was important for their ability to establish mucosal homeostasis.¹⁴⁶ Finally, monocyte-derived macrophages from early onset Crohn's disease patients harboring loss of function mutations in *IL10RA* and *IL10RB* also exhibit impaired differentiation and function.¹⁴⁶ However, these studies should not be interpreted to indicate that macrophage dysfunction is the only abnormality accounting for inflammation in IL-10 deficiency, as Treg cells also depend on IL-10 and thus Treg cell dysfunction likely plays an important role.

Conclusions

The five models of intestinal inflammation we have discussed (Figure 4) provide a survey of the extensive and sometimes complex mechanisms operative in the genesis of intestinal inflammation and pathogenesis of IBD. Table 1 briefly describes a number of additional models; at our present level of understanding, these models usually focus on particular rather than general abnormalities and mechanisms of gut inflammation. Finally, it is worth noting that even though the study of models of inflammation is no longer in its infancy, an enormous body of knowledge has yet to be harvested from the application of this biologic methodology.

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Conflicts of interest

The authors disclose no conflicts.

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