Chapter 3
Insights from Recent Advances in Animal Models of Inflammatory Bowel Disease

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Abstract Experimental animal models of intestinal inflammation that mimic inflammatory bowel disease (Nat Genet 2010, 42(4), 332–337) have become increasingly common over the last 2 decades. These include experimentally induced and genetically altered models as well as models of spontaneous intestinal inflammation. None of these models exactly replicate all features of Crohn's disease and ulcerative colitis, the two major subtypes of human IBD, but they have furthered our understanding and define the pathogenesis underlining intestinal inflammation. Of particular note is the recognition that enteric microorganisms play a central role in the maintenance of normal mucosal immune homeostasis and the development of pathologic innate and adaptive immune responses that lead to mucosal inflammation and disease. In this review, we summarize the data gathered from commonly utilized animal models of IBD. In addition, we review experimental models developed to define the role of recently identified human IBD susceptibility genes. These genes may regulate host responsiveness against microbial and environmental factors and control immune effector molecules during inflammation. These models not only promise to increase our understanding of the pathogenesis of IBD but also provide rationale for new therapeutic strategies.

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M. D'Amato and J.D. Rioux (eds.), *Molecular Genetics of Inflammatory Bowel Disease*,
DOI 10.1007/978-1-4614-8256-7_3,

Abbreviations

Ab	Antibody
APC	Antigen-presenting cell
ATG	Autophagy-related protein
BCR	B cell receptor
BMDC	Bone marrow-derived dendritic cells
BrdU	5-Bromo-2′-deoxyuridine
CD	Crohn's disease
cdcs1	Cytokine deficiency-induced colitis susceptibility-1
CECs	Colonic epithelial cells
DC	Dendritic cells
Defcr	Defensin-related cryptdin
DKO	Double knockout
DTH	Delayed-type hypersensitivity
ER	Endoplasmic reticulum
FM	Follicular mature
GF	Germfree
GWAS	Genome-wide association studies
HM	Hypomorphic
IBD	Inflammatory bowel disease
IEL	Intraepithelial lymphocytes
IFN	Interferon
Igμ	Immunoglobulin mu
IL or Il	Interleukin
KO	Knockout
LPS	Lipopolysaccharide
LRR	Leucine-rich repeat
LTi	Lymphoid tissue inducer
MAPK	Mitogen-activated protein kinase
MDP	Muramyl dipeptide
MHC	Major histocompatibility complex
MLN	Mesenteric lymph nodes
MyD88	Myeloid differentiation primary response gene 88
MZ	Marginal zone
NADPH	Nicotinamide adenine dinucleotide phosphate
NoD2	Nucleotide-binding oligomerization domain-containing protein 2
PD	Parkinson's disease
PKCθ	Protein kinase C theta
Rag, V(*D*) *J*	Recombination activation gene
SNP	Single-nucleotide polymorphism
SPF	Specific pathogen-free
STAT	Signal transducer and activator of transcription
TCRα	T cell receptor alpha-chain
TH	T helper
TLR	Toll-like receptor

TNBS	2,4,6-Trinitrobenzene sulfonic acid
TNF	Tumor necrosis factor
TRIF	TIR-domain-containing adapter-inducing interferon-β
TRUC	T-bet and Rag-2 DKO
UC	Ulcerative colitis
XBP-1	X-box-binding protein-1
γc	Common gamma chain
γδ	Gamma delta

Introduction

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is a group of intestinal chronic inflammatory conditions that affect individuals throughout life [1, 2]. Although the mechanisms involved in the pathogenesis of IBD are complex, continuous microbial stimulation resulting in pathogenic immune responses in a genetically susceptible host likely plays a key role in the development of IBD [3, 4]. Recent human genome-wide association studies (GWAS) have identified 140 risk loci/genes in CD and 133 risk loci/genes in UC [3, 5–7]. Polymorphisms in genes, which regulate intestinal homeostasis and immunity, including *IL10R* and its signaling components, are strongly associated with the pathogenesis of human IBD [3, 8–10]. Therefore, analysis of animal models that manipulate these IBD susceptibility identified by GWAS is likely to enhance our understanding of their role in IBD pathogenesis.

More than 60 different animal models of IBD have been established to study intestinal inflammation [11]. These include genetically manipulated animal models [conventional/conditional knockout (KO), knockin, or transgenic mice] that spontaneously develop colitis and/or ileitis [11–13]. Other models use chemical compounds or adaptive cell transfer to induce intestinal inflammation to investigate different phases of intestinal inflammation [14–16]. These experimental models have provided a way to dissect mechanisms that may underlie the pathogenesis of human IBD. Here, we summarize findings in commonly utilized as well as IBD susceptibility gene-manipulated animal models (Table 3.1) of intestinal inflammation and assess their value in human IBD.

Spontaneous Colitis Models

C3H/HeJBir Mouse Strain

C3H/HeJBir (C3Bir) mice, a substrain of inbred C3H/HeJ mice generated at the Jackson Laboratory, spontaneously develop inflammation in the cecum and right colon [17]. C3H/HeJ mice have a missense mutation in the third exon of the Toll-like receptor-4 gene (*Tlr4*) and therefore express a defective response to the biologic

Table 3.1 Categories of murine models of colitis

Category	Animal models	Sections in this chapter
Spontaneous colitis models	C3H/HeJBir	"*C3H/HeJBir* Mouse Strain"
	SAMP/Yit	"SAMP/Yit Fc Mouse Strain"
Induced models of colitis	Acetic acid	
	DSS	"DSS-Induced Colitis"
	TNBS	"TNBS-Induced Colitis"
	Oxazolone	"Oxazolone-Induced Colitis"
	CD45RBhi cell transfer	"CD45RB^{hi} Cell Transferred Model"
	CD3ε transgenic model	
	CD8 transfer	
	Anti-CD40 mAb-induced	"Anti-CD40 mAb-Induced Colitis"
Bacterial infectious colitis	*Salmonella*	
	Citrobacter	
	AIEC	
	ECOVA	
Genetically manipulated colitis (conventional KO only)	*Il-2* KO	"*Il2* KO and *Il2R* KO Mice"
	Il-2R KO	"*Il2* KO and *Il2R* KO Mice"
	Il-10 KO	"*IL-10* KO Mice"
	Tcrα KO	"*Tcra* KO Mice"
	Gαi2 KO	"*Gαi2* KO Mice"
	Tbet x *Rag* DKO (TRUC)	"*T-bet/Rag-2* DKO (TRUC) Mice"
	Wasp KO	"*Wasp* KO Mice"
	Integrin αV KO	
	Jak3 KO	
	A20 KO	
	Tgfβ KO	
	Mdr1a KO	
	Tlr5 KO	
	Muc2 KO	
	Ship1 KO	
	Keratin 8 KO	
	Gpx1/2 KO	
IBD susceptibility gene related models	*Il-10* KO	"*IL-10* KO Mice"
	Il-10R KO	
	Nod2 KO	"Nod2 KO Mice"
	Atg5 KO	"*Atg5* KO and *Atg16l1* Mutant Mice"
	Il23R KO	"IL23 receptor KO Mice"
	Stat3 KO	"Conditional *Stat3* KO Mice"
	Xbp1 KO	"*Xbp1* KO Mice"
	Lrrk2 KO	"*Lrrk2* KO Mice"
	P40phox KO	"*p40^{phox}* KO Mice"

Selected models of colitis (as noted above) have been described in the chapter

effects of lipopolysaccharide (LPS) [18, 19]. C3Bir mice also express this unresponsiveness [20]. The inflammation involving the right colon and cecum peaks at around 3–6 weeks of age with resolution by 12 weeks of age. However, a mild recurrence of colitis is sporadically observed after 1 year of age [17]. Using Western blot analysis, sera from colitic C3Bir mice show a unique reactivity to antigen extracts from cecal contents (bacteria), while sera from colitic C3H/HeJ mice do not show such reactivity [21].

Histologically, focal inflammation involving the cecum extends to the right colon with presence of mixed acute and chronic inflammation, colonic crypt hyperplasia, and submucosal scarring. In severe cases, crypt abscess formation is observed [22]. Enteric microflora plays a central role in the pathogenesis of intestinal inflammation in these mice [21]. Cell transfer studies with C3Bir $CD4^+$ T cells into combined immunodeficiency mice (*scid*, which have absent or atypical T cells and B cells) suggest that only cecal bacterial antigen-reactivated C3Bir $CD4^+$ T cells are the predominant pathogenic population to induce colitis [23].

The ll-10-deficient C3Bir (*C3Bir.Il10* KO) mice developed much more severe colitis in the cecum and colon as early as 4 weeks of age, as compared to *B6.Il10* KO mice [24]. Severe inflammation with ulceration, epithelial hyperplasia, and exuberant exudates can be seen in the colons of *C3Bir.Il10* KO [25]. Genetic analysis revealed that *Cdcs1* (cytokine deficiency-induced colitis susceptibility-1) on chromosome 3 has a strong linkage to the control of multiple colitogenic subphenotypes in C3Bir mice. The C3Bir *Cdcs1* susceptibility allele confers a reduced responsiveness to Tlr2, Tlr5, Tlr9, and Nod2-mediated bacteria sensor signaling. Compensatory mechanisms of adaptive immune responses in turn induce the hyperactivation of nuclear factor-kappa B (NF-κB) signaling in macrophages [25].

SAMP/YitFc Mouse Strain

A colony of the senescence-accelerated mouse (SAM1–10) was developed at Kyoto University after an unintentional outcross between a non-AKR strain and AKR/J strain [26]. Subsequently, a SAMP/Yit substrain was generated from the SAMP1 line that exhibited severe intestinal inflammation (restricted to the ileum and cecum with a skip pattern) and skin lesions [27]. A distinct substrain (SAMP1/YitFc) displays unique features of intestinal inflammation including perianal disease with fistula formation in a subset of mice (approximately 5 %). These mice developed ileitis as early as 10 weeks of age with elevated interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) levels. The incidence of skin lesions was inversely correlated with the occurrence of ileitis [28, 29]. By 30 weeks of age, both SAMP/Yit and SAMP1/YitFc mice develop transmural and discontinuous inflammation in the terminal ileum with 100 % penetrance [27, 28]. In SAMP1/YitFc mice, the earliest histological change entails a dramatic expansion of epithelial cells of the secretory lineage (e.g., Paneth, goblet, and intermediate cells) [30]. In addition, global expansion of the crypt epithelial stem cell population can be seen as

disease severity progresses [30, 31]. In inflamed areas, SAMP mice show an increased number of apoptotic intestinal epithelial cells (IRCs), which are normalized after anti-TNF treatment [32]. These epithelial alterations are considered to be the primary defects that trigger ileitis in this model [33]. The mice also develop *Helicobacter*-negative, immune-mediated gastritis [34].

The spontaneous ileitis in SAMP1/YitFc mice resembles human Crohn's disease [35]. In a genome-wide scan performed in two cohorts of F2 mice (SAMP1Yit/Fc x C57Bl/6J), a single SAMP-derived susceptibility locus (*Ibdq1*) was identified on chromosome 9 (Chr9) [35]. Both IL-10 receptor alpha (*Il10ra*) and IL-18 (*Il18*) genes, which regulate inflammatory responses, are located on *Ibdq1*. Comparative sequencing detected two single-nucleotide insertions at positions 732 and 3098 of introns 1 and 3 of the *Il10ra* gene in SAMP/YitFc strain as compared to AKR/J or C57Bl6/J mice, but no polymorphisms were detected for *Il18* among the three mouse strains [35]. However, recent studies have identified association of polymorphisms in the promoters of *Il18* [36] and the *Il18* gene haplotype-2 [37], suggesting the involvement of an undetected alteration in the *Il18* locus of the SAMP1/YitFc mice [35].

Both Th1 and Th2 responses are exaggerated in SAMP/YitFc mice. In particular, Il13 and Il5 expression levels are significantly (>25-fold) increased in the inflamed mucosa between 9 and 16 weeks of age [38, 39]. The Th2 pathway appears to contribute to the amplification of the inflammatory process during the chronic stage in SAMP mice [38] as the administration of anti-IL-4 monoclonal antibody after 15 weeks of age (with established disease) significantly decreases ileitis. Administration of anti-IL-15 antibody also ameliorates SAMP ileitis [39]. SAMP B cells also contribute to ileitis by producing immunoglobulins that specifically recognize antigenic epitopes resulting in immune complex formation in the gut mucosa [40]. A variety of experimental treatments including anti-TNF [32, 41] have been shown effective in treating the ileitis in SAMP mice studies.

Commonly Utilized Induced Models of Colitis

These models include colitis induced by the administration of chemicals or by the transfer of cells or antibodies. Although these models do not represent the spontaneous nature of human IBD, the induced intestinal inflammation may reflect various pathophysiological aspects of human IBD (Table 3.1). Furthermore, these models are often used to reveal susceptibility to mucosal injury and inflammation in genetically altered mice that may not develop spontaneous intestinal inflammation. Selected induced models of colitis are summarized in this chapter.

DSS-Induced Colitis

The dextran sodium sulfate (DSS; $C_6H_7Na_3O_{14}S_3$)-provoked colitis is one of the most widely used chemically induced colitis models. Approximately 2–5 % DSS

(molecular weight of approximately 40 kDa) is orally administered in the drinking water to induce acute or chronic colitis in mice by inducing direct hyperosmotic damage to epithelial cells [42]. One cycle of 3–5 % DSS administration for 5–7 days, followed by regular drinking water intake, results in extensive colonic injury with complete crypt depletion in the acute phase and subsequently regeneration of epithelial cells in the recovery phase followed by regeneration of epithelial cells in the recovery phase. The C57Bl/6 strain is relatively resistant to this colitis, while the C3H/HeJ strain is highly susceptible [43].

After 5–10 % DSS administration for 8–9 continuous days, Balb/c mice gradually develop weight loss and diarrhea with presence of occult blood. The colon shows multiple mucosal erosive lesions with inflammatory cell infiltration of mononuclear cells (lymphocytes, macrophages) and polymorphonuclear cells [44]. Occasional crypt abscesses and regenerative epithelium are also seen in the colonic mucosa. After termination of DSS treatment, the colonic mucosa recovers gradually from the acute colonic injury. Therefore, DSS-induced colitis has been used for studying epithelial wound healing and regulatory immune cells during the recovery phase [45]. Chronic colitis can be induced by treating mice with repeated (3–5) cycles of DSS; each cycle consists of DSS administration for 7 days followed by drinking water for the subsequent 10 consecutive days [44]. In chronic DSS colitis, lymphoid follicular formation accompanied by regenerative and dysplastic changes of the mucosal epithelium is frequently seen in the left side of the large intestine and the transverse colon [44]. Long-term DSS administration with repeated cycles produces colorectal dysplasia and/or adenocarcinoma, which is reported to be similar to human IBD-associated colon cancer [43].

Acute DSS colitis can be induced in the absence of acquired immunity as *scid* mice are also susceptible. In the inflammatory lesions of DSS-treated *scid* mice, abundant proinflammatory cytokines such as IL-1β, TNF-α, and IL-6 are detected. These cytokines are mainly produced by activated macrophages after exposure to luminal bacterial components or products. Since the colitis is ameliorated by treatment with selected antibiotics (metronidazole, or vancomycin-imipenem combination) [46], an antibiotic-sensitive component of luminal bacteria is involved in the pathogenesis of DSS-induced colitis. However, mice kept in a germfree (GF) facility or treated with wide-spectrum antibiotics (mixture of vancomycin, neomycin, metronidazole, and ampicillin) develop more severe and even lethal DSS-induced colitis accompanied by massive intestinal bleeding [47, 48]. This finding is supported by the observation that *MyD88* KO and *Tlr4* KO mice developed lethal colitis after DSS administration [48]. Therefore, signaling through TLRs, in particular TLR2, TLR4, and TLR9, is important in maintaining epithelial homeostasis and protection from direct epithelial injury induced by DSS. This is accomplished by directly inducing the expression of several factors, including heat shock proteins, TNF, IL-6, keratinocyte-derived chemokine-1 (KS), and/or type I IFN, which are involved in tissue repair and cytoprotection [48, 49]. The concentration of DSS, treatment periods, genetic background of mice, and microbial environment of facilities can influence the results of the experiments.

TNBS-Induced Colitis

TNBS (2,4,6-trinitrobenzene sulfonic acid; $C_6H_3N_3O_9S$) is a nitroaryl oxidizing acid. Simultaneous rectal administration of TNBS (between 0.5 and 6 mg per mouse) and ethanol (between 35 and 50 %) induces colonic inflammation [50]. A high dose of TNBS causes an acute necrotizing enterocolitis in mice [51]. C3H/HeJ, SJL/J, and Balb/c mice are highly susceptible to TNBS as compared to C57Bl/6 and DBA/2 mice [52, 53].

Acute colitis induced by rectally administered TNBS/ethanol leads to massive transmural inflammation associated with loose stools, rectal prolapse, and weight loss. After 2–3 days of TNBS administration, discrete foci of acute necrosis and mucosal inflammatory cell infiltration with focal basal cryptitis are observed. The acute inflammation is followed by mononuclear cell infiltration, which lasts for a variable amount of time [22]. The inflammation, which peaks a few days after TNBS administration, is mainly mediated by Th1-type immune responses with increased production of Th1 cytokines (e.g., IFN-γ, IL-2) and IL-12p70 (IL-12p40/p35) in the affected colon. However, the colitis is mediated by Th2 cytokine (e.g., IL-4) in Balb/c strain [54]. Generally, mice are sacrificed in the first or third week after TNBS administration depending on the stage of inflammation to be evaluated [55]. Chronic TNBS-associated colitis, induced by several weekly intrarectal administrations of TNBS in ethanol, is associated with IL23 (IL-12p40/p19) and IL-17 production by lamina propria mononuclear cells. Pathogenic IL23, but not IL-12p70, is associated with this type of colitis [56]. Zhang et al. demonstrated the importance of IL-17 during the development of chronic inflammation in TNBS-induced colitis, by utilizing IL-17 receptor A (*Il17ra*) KO mice [57]. Furthermore, administration of IL-17RA IgG1 fusion protein ameliorated colitis. IL-13 (a Th2-type cytokine), which triggers TGF-β-dependent tissue fibrosis, is also involved in this model [58].

By sensitizing mice with TNBS 6–7 days before the rectal administration of TNBS, the role of delayed hypersensitivity (DTH) reaction on colitis development can be studied [51]. The reaction is mediated by "hapten-modified self-antigen" followed by a local immune response through the activation of T cells and macrophages [59].

The TNBS-induced colitis model has been widely utilized for drug screening and examination of the effect of IBD susceptibility genetic variants in animal models [22, 56]. The relevance of the TNBS colitis model to CD is reflected in the experiments showing that *Nod2* (a key CD susceptibility gene) protects mice from TNBS colitis [60]. CD-associated frameshift-mutated *Nod2* in mice leads to increased sensitivity to colitis, compared to control mice carrying wild-type *Nod2* [61].

Oxazolone-Induced Colitis

Oxazolone (4-ethoxymethylene-2-phenyloxazol), a haptenating agent, has been used to elicit intestinal inflammation in mice. Intrarectal administration of 6 mg of oxazolone in 150 μL of 50 % ethanol in SJL mice leads to rapid onset of colitis

marked by diarrhea, weight loss, and development of hemorrhagic colitis in the distal colon with patchy ulceration, submucosal edema, and presence of mucosal inflammatory infiltrate (lymphocytes and neutrophils) [62]. The inflammatory process is resolved by 10–12 days. Anti-IL-4, but not anti-IL-12, antibody leads to amelioration of colitis. In C57Bl/10 mice, pre-sensitization with 3 % oxazolone by skin painting 5 days before rectal challenge with 1 % oxazolone leads to marked edema of the colonic wall with mucosal ulceration and dense infiltration of superficial mucosa by neutrophils [63]. IL-13 but not IL-4 increases in the inflamed tissues, and mice treated with IL-13Rα2-Fc are protected from colitis. NK T cells are essential for the induction of colitis. Interestingly, lamina propria T cells from UC patients may produce more IL-13 than cells from healthy controls with a clear expansion of IL-13-producing CD161+ nonclassical NK T cells [64].

Studies in *Ifng* KO and *Il4* KO mice indicate that both Th1 and Th2 cytokines play a crucial pathogenic role in oxazolone colitis in C57BL/6 and BALB/c mice [65]. Recent studies indicate that IL-6 is also essential for the induction of oxazolone colitis; IL-6 production is regulated by the nuclear factor of activated T cells [66]. Oxazolone colitis can be suppressed by TNF-α by promoting local glucocorticoid synthesis [67].

CD45RB^{hi} Cell Transferred Model

CD4+ T cell population can be subdivided by CD45 antigen expression in the rat, mouse, and human [68]; naïve CD4$^+$ T cells are included within the CD45RB^{high} fraction, whereas antigen-primed CD4$^+$ memory T cells are in the CD45RB^{low} fraction. These two populations have direct lineage relationship [69]. Adoptive transfer of CD4$^+$ CD45RB^{high} T cells (from the spleen of WT mice) into *scid* mice results in colitis [70, 71]. Severe colitis is found approximately 6–12 weeks after reconstitution of mice with $1–5 \times 10^5$ CD4$^+$ CD45RB^{high} T cells. The characteristic features of colitis include transmural mononuclear cell infiltration, crypt hyperplasia, and goblet cell depletion. The induction of colitis is strain independent and can also be seen in nonobese diabetic mice [72], *Rag1* KO mice, *Cd3* KO mice, or athymic nude rats [73].

This model allows examination of the earliest immunological events associated with the induction/perpetuation of colitis [74]. Reconstitution of immunodeficient mice with CD4$^+$ CD45RB^{high} T cells shows polarized Th1 cells with increased production of IFN-γ and TNF-α in the inflamed colon. The colitis is inhibited by treatment of CD45RB^{high} cells with anti-IFN-γ or anti-TNF-α monoclonal antibodies [71, 75]. In addition to Th1 cells, Th17 cells may also have a colitogenic effect in this model [76, 77]. However, other studies have shown that Th17 cells may not play any role in IBD [78–80]. In mice with colitis, CD4$^+$ T cells isolated from the spleen, mesenteric lymph nodes (MLN), and colonic lamina propria produce both Th1 and Th17 types of cytokines [74, 81]. IL-6 trans-signaling is also required for the development of colitis [82] as IL-6 inhibits the generation of inducible regulatory T cells

from naïve T cells [83]. IL-4 plays a role in the pathogenesis of CD45RB^{hi} colitis by blocking the generation of TGF-β-induced Foxp3^{+} Treg cells and by inducing a population of IL-9^{+} IL-10^{+} Foxp3(−) Th9 cells, which have no regulatory function despite producing abundant IL-10 [84].

This model has been very useful in the identification of regulatory T cell subsets involved in the suppression of colitis development. *Scid* mice that received CD45RB^{high} and CD45RB^{low} fractions together do not show severe colitis because of the presence of regulatory T cells within the CD45RB^{low} fraction [70, 71]. The regulatory effect of CD45RB^{low} cells is inhibited by anti-TGF-β, suggesting the critical role of TGF-β-producing CD4^{+} T (Th3) cells in the regulation of this form of colitis [85]. IL-10 drives the generation of a CD4^{+} T regulatory cell 1 (Tr1), which produce high levels of IL-10, low levels of IL-2, and no IL-4. Tr1 cells actively suppress pathogenic immune responses in this model in an antigen-dependent manner [86]. Furthermore, IL-10 produced by CD11b^{+} myeloid cells is a critical regulator since Treg cells could not maintain Foxp3 expression and regulatory activity in IL-10-deficient *Rag1* KO mice [87]. However, this observation is challenged by a recent finding that Foxp3 expression can be preserved in IL-10R-deficient Treg cells [88].

Anti-CD40 mAb-Induced Colitis

CD40 is a type I membrane glycoprotein and is expressed in many cell types including B cells, dendritic cells, monocytes/macrophages, fibroblasts, and activated endothelial cells [89–92]. The ligation of CD40 and CD154 (CD40L) activates the pathogenic signals in intestinal inflammation including TNBS-induced colitis [93] and CD45RB^{high} T cell transferred into *scid* mice [94, 95]; inhibition of CD40-CD40L interactions prevents or ameliorates the colonic inflammation. By generating a positive feedback loop, CD40^{+} APCs and CD40L^{+} T cells are further activated after CD40-CD40L ligation [92, 96].

A new colitis model was developed by injecting agonistic anti-CD40 monoclonal antibody FGK45 into *Rag1* KO or *scid* mice leading to body weight loss, diarrhea, and anal inflammation within the first 4 days after antibody treatment [97]. On day 7, the mice showed splenomegaly, hepatopathy, and lymphadenopathy (MLN) with colonic wall thickening. The colitis is characterized by epithelial hyperplasia with goblet cell depletion, epithelial cell damage, and inflammatory cell infiltration in the lamina propria. The colitis starts resolving by day 10 with complete resolution by day 21. Therefore, activation of CD40-CD40L pathway in the innate immune cells is sufficient to induce colitis in the absence of T and B cells and is mediated by TNF-α, IL-12p40, and IL23 (p19). Interestingly, IL23p19 controls CD40-induced colitis, and IL-12p35 is involved in the systemic manifestation of this colitis, including wasting disease and serum proinflammatory cytokine production. IL-12 (p40/p35) and IL23p19 initiate the release of proinflammatory cytokines by dendritic cells [TNF-α, IFN-γ, and IL-6] and by NK cells [IFN-γ] [97]. The major source of

IL23 is CD11c$^+$ DCs after anti-CD40 or bacterial stimulations [98–100]. Human and murine dendritic cells and macrophages not only produce IL23 but also express IL23R, which support the activation of autocrine loop within the innate immune response [97, 101, 102]. Thus, IL23 is a critical cytokine which initiates a primary response in the mucosal inflammatory cytokine cascade in this model.

Anti-CD40 monoclonal antibody-mediated colitis cannot be induced in mice lacking the orphan nuclear receptor RORγt. The RORγt-deficient mice do not have LTi (lymphoid tissue inducer) cells, lymph nodes, or intestinal lymphoid clusters [103, 104]. A subsequent study revealed that RORγt-deficient NKR (natural killer cell receptor)-expressing LTi cells, which produce IFN-γ in IL23-dependent manner and upregulate perforin and granzyme B expression, are required for colitis development in anti-CD40 monoclonal antibody-treated *Rag1* KO mice [105]. In contrast, RORγt$^+$ NKR-LTi cells produce IL-22, which may be involved in repairing tissue damage. The expression of RORγt in innate lymphoid cells determines their distinct function.

All in all, anti-CD40 monoclonal antibody-mediated colitis is a useful model to analyze the role of innate immune system in intestinal inflammation.

Genetically Manipulated Colitis Models

Il2 KO and Il2R KO Mice

IL-2 is a key cytokine in the regulation of immune and inflammatory responses and is necessary for the development of Treg population [106, 107]. IL-2 specifically binds with an IL-2 receptor complex consisting of IL-2Rα (CD25), IL-1Rβ (CD122), and common gamma chain (γc) (CD132) and subsequently activates important signaling (e.g., PI3K/Akt, Ras/MAPK, JAK/STAT) cascades for maintaining the immune system [108]. *Il2* KO mice [109] develop chronic colitis within 10–25 weeks of age when raised under SPF conditions [110]. Approximately 50 % of *Il2* KO mice die before 9 weeks of age with severe splenomegaly, lymphadenopathy, and anemia preceding colitis, and the remaining 50 % of mice developing colitis with 100 % penetrance [110]. Clinical signs of severe colitis in *Il2* KO mice include enlarged colon, rectal prolapse, splenomegaly, and lymphadenopathy. The chronic inflammation in the colon is characterized by crypt hyperplasia and transmural mononuclear cellular infiltration of lymphocytes and plasma cells [110]. T cells, but not B cells or autoantibodies, are necessary for the development of this disease [111]. *Il2* KO mice raised under GF conditions continue to develop splenomegaly, lymphadenopathy, and mild and focal colitis, manifested by mild infiltration with mononuclear cells and the loss of goblet cells, despite absence of clinical signs of disease (e.g., diarrhea, rectal prolapse, weight loss) or mortality up to 46 weeks of age [112]. When mono-colonized with *E. coli* mpk, *Il2* KO mice raised in GF conditions develop chronic colitis when mono-colonized with

E. coli mpk [113]. In contrast, mice colonized with *Bacteroides vulgatus* mpk remain healthy by promoting differentiation of semi-mature dendritic cells in the colonic lamina propria [114].

IL-2 specifically binds to the high-affinity IL-2 receptor (IL-2R), composed of three component chains, IL-2Rα, IL-2Rβ, and IL-2Rγ [115–117]. Both IL-2Rβ and IL-2Rγ chains form a low-affinity IL-2 receptor and induce signal transduction upon ligation with IL-2. In contrast, IL-2Rα cannot function independently but forms high-affinity IL-2R associated with the other two chains [118]. Both *Il2ra* KO [119] and *Il2rg* KO [120] mice develop colitis spontaneously. Colitis, characterized by colonic shortening, mucosal hypertrophy, diarrhea, rectal bleeding, and rectal prolapse, in *Il2rg* KO mice manifest within 4 months of age. Histologically, colonic epithelial hyperplasia, loss of goblet cells, crypt distortion, and macrophage/lymphocyte infiltration in colonic lamina propria are observed [120]. *Il2ra* KO mice develop massive enlargement of MLN and the spleen around 4–6 weeks of age due to the expansion of $CD4^+/CD8^+$ T cells and IgM^+B220^+ B cells. Approximately 25 % of *Il2ra* KO mice die due to a severe anemia at approximately 8–20 weeks of age. The majority of the surviving mice begin to develop severe colitis at 12–16 weeks of age. The colitis is characterized by a marked thickening of the colonic mucosa and epithelial destruction with mucosal ulceration, infiltration by lymphocytes/neutrophils, and presence of crypt abscesses [119].

IL-10 *KO Mice*

IL-10 is an important anti-inflammatory, immunoregulatory cytokine that is mainly produced by monocytes/macrophages, T cells, B cells, thymocytes, and keratinocytes upon activation [121, 122]. IL-10 inhibits antigen-specific T cell responses by downregulating the MHC (major histocompatibility complex) class II molecule on the surface of monocytes in an autoregulatory manner [123, 124]. *Il10* KO mice in a conventional facility demonstrate growth retardation, anemia, and chronic enterocolitis with normal development of B and T cells [125]. After 3 months of age, underweight *Il10* KO mice develop chronic enterocolitis involving the entire intestinal tract with marked regenerative crypt hyperplasia in the colon [125]. The colitis in *Il10* KO mice of C57Bl/6 background is much milder than the colitis seen in mice of C3H or Balb/c background. As the colitis can be accelerated after administration of nonsteroidal anti-inflammatory drugs (NSAID) such as piroxicam [126], NSAID treatment is useful in inducing rapid and reproducible colitis in *Il10* KO mice.

Under SPF conditions, the inflammation in *Il10* KO mice is in the proximal part of the colon but not in the small intestine. Development of colitis in *Il10* KO mice has been shown to be dependent on *Helicobacter* species [127]. T cells, in particular $TCR\alpha\beta^+CD4^+CD8\alpha^-$ and $CD4^+$ $CD8\alpha^+$ T cells, but not B cells, mediate chronic colitis in *Il10 KO* mice [128].

The chronic colitis in *Il10* KO mice is initiated by the IFN-γ-producing Th1 cells driven by IL-12 produced by antigen-presenting cells (APCs). Early treatment with

anti-IFN-γ mAb or anti-IL-12p40 mAb (which neutralizes both IL-12 and IL23) prevents colitis [129, 130]. In contrast, anti-IL-12p40, but not anti-IFN-γ mAb, reverses ongoing colitis in *Il10* KO mice as well as *Rag*-KO mice reconstituted with IL-10 KO CD4+ T cells [130]. IL23 (IL-12p19/p40), not IL-12p70 (IL-12p35/p40), is essential for the development of chronic colitis in *Il10* KO mice, and the colitis is initiated by a unique subset of tissue-homing memory T cells, specifically activated by the proinflammatory mediators IL-17 and IL-6 [131]. IFN-γ has an anti-inflammatory effect in the initiation phase of this colitis as IFN-γ-deficient *Il10* KO mice demonstrate significantly increased colonic inflammation as compared to *Il10* KO mice [132]. IFN-γ exerts its regulatory function by targeting the colonic $CD11b^+$ cells, which are a primary source of IL23 during the development of colitis in *Il10* KO mice. Although earlier studies suggested that the colitis in *Il10* KO is "Th1 mediated," more recent studies indicate that the colitis is Th17 mediated [56]. Interestingly, Th1 (IFN-γ) may play a protective role during the development of chronic colitis in *Il10* KO mice [56].

TLR signaling in effecters $CD4^+$ T cells plays distinct roles depending on the nature of TLR ligand/TLR interaction in *Il10* KO mice. IL-10-deficient *Tlr9* KO mice do not develop colitis by 8 months of age. In contrast, IL-10-deficient *Tlr4* KO mice develop accelerated colitis as early as 8 weeks of age, suggesting that TLR4-mediated signaling has a protective role in the colitis of *Il10* KO mice [127, 133]. LPS stimulation of TLR4-expressing $CD4^+$ T cells inhibits MAPK (mitogen-activated protein kinase) p42/p44 activation upon subsequent TCR stimulation by interacting with MAP kinase phosphate 3, suggesting that TLR4 signaling plays an inhibitory role in TCR-dependent colitogenic $CD4^+$ T cell responses independent of TLR4 expression on innate immune cells [133]. However, chronic colitis in *Il10* KO mice seems to be partially mediated by MyD88 (myeloid differentiation primary response gene 88)-dependent TLR signaling since the presence of colitogenic $CD11c^+$ MHC class II high cells in the MLN is completely abolished upon MyD88 deficiency in Il10 × *MyD88* double knockout (DKO) mice [134, 135].

Recent GWAS have identified *Il10* as a key susceptibility gene for both UC and CD [3, 5, 6, 136]. Of note, the *Il10* gene is associated with other immune-mediated disorders (e.g., sarcoidosis, Behçet's disease, type 1 diabetes mellitus, systemic lupus erythematosus) and is part of the "shared loci," which are enriched for many genes, including *IL23*, *IL21*, *IL7R*, and *IFNG*, involved in Th1 and Th17 cell differentiation [5]. Furthermore, recent reports show that loss of IL-10 signaling with IL-10 and/or IL-10R deficiency is associated with early onset of IBD and allogeneic hematopoietic stem cell transplantation can induce remission in patients with IL-10R deficiency [137, 138]. In summary, IL-10 is a critical cytokine capable of suppressing inflammatory immune responses.

Tcra *KO Mice*

T cell receptor (TCR) is a specific receptor expressed on the surface of T cells and is responsible for recognizing antigens presented by APC via MHC restriction. The

majority of T cells express α and β chains, while a small number of T cells express γ and δ chains. *Tcra* KO mice spontaneously developed Th2-mediated colitis after 4–5 months of age, and about 60 % of the mice developed chronic colitis at 6 months of age under SPF conditions [139, 140]. The colitis is strain dependent; C57Bl/6 strain mice are more susceptible as compared to C3H/HeJ or Balb/c strains [140]. Like UC, the inflammation is restricted to the colonic mucosa with elongation of crypts, goblet cell depletion, and a mixed cellular infiltration into the lamina propria [140–142]; occasional crypt abscesses are present in severe colitis. Unlike UC, mucosal ulceration or erosion is not generally observed in *Tcra* KO mice [141]. However, ulceration becomes detectable in CD1d-deficient *Tcra* KO mice [143]. Interestingly, the lack of both IL-4 and B cells in *Tcra* KO mice resulted in the development of granulomatous inflammation in the mucosa and submucosa of the colon and in the ileocecal junction at approximately 24 weeks of age [144].

Several factors are involved in the pathogenesis of colitis in *Tcra* KO mice [56]. *Tcra* KO mice develop unique $CD4^+$ TCR $\alpha^-\beta^+$ T cells, which express TCRβ chains without TCRα chain and primarily recognize superantigens. These unique T cells are immunologically functional and actively produce IL-4, which results in spontaneous colitis development [139, 145, 146]. The colitis is associated with the presence of restricted diversity of $V\beta8.2^+$ T cell subsets, which are characterized by a specific TCR motif [147]. These T cells can survive under Th2 conditions with colonic epithelial cells (CECs), suggesting the requirement of self-antigen(s) for the survival of pathogenic Th2 cells in *Tcra* KO mice. In addition, TCRVα7.2 chain transgenic *Tcra* KO mice developed rapid onset of colitis as compared to *Tcra* KO mice. The TCRVα7.2 chain may have a chaperone function that extends the half-life of the newly synthesized TCRβ chain of pathogenic T cells, which clonally expand in *Tcra* KO mice [148]. Protein kinase C theta (PKCθ) is an important component in the intracellular signaling cascade and plays a fundamental role in *Tcra* KO mice; *Tcra* x *Prkcq* DKO mice develop a milder form of colitis, as compared to *Tcra* KO mice [149] with reduced proliferation and production of IL-17 as well as Th2 cytokines (e.g., IL-4, IL-13) but unaltered apoptosis. Gamma delta (γδ) T cells may play a pathogenic role in colitis development in *Tcra* KO mice [150]. TNF-α/TNFR2 [151], IL-6 [151], IL-7 [152, 153], IL-1 [142], lipoteichoic acid [154], galectin-4 [155], and chitinase 3-like 1 [156] are also involved in the pathogenesis of chronic colitis in *Tcra* KO mice.

Tcra KO mice fail to develop colitis under GF conditions [157]. Interestingly, the development of colitis is suppressed when *Tcra* KO mice are maintained for several generations in a conventional facility, as compared to a SPF facility [158, 159]. In contrast, B cell-deficient *Tcra* KO mice continue to develop severe colitis in conventional facilities, suggesting that B cells, in particular B-1 B cells, contribute to the suppression of colitis [159]. There is no evidence for primary epithelial barrier disruption, as determined by mannitol transmural flux, in *Tcra* KO mice between 6 and 25 weeks of age [160]. Similar to the *Il10* KO mouse model, treatment with piroxicam, which directly induces epithelial cell apoptosis and weakens the mucosal barrier function [161], accelerates the development of colitis in *Tcra* KO mice presumably by facilitating the invasion of luminal bacteria into the colonic mucosa

[162]. Of note, *Helicobacter hepaticus* infection is not necessary for intestinal disease in *Tcra* KO mice although this infection is sufficient to cause chronic proliferative colitis in *Tcrb* KO mice [163]. Interestingly, *Helicobacter* species infection shifts the cytokine profile of *Tcra* KO mice from Th2- to Th1-dominant responses [164]. In addition, *Tcra* KO mice maintained in SPF conditions develop spontaneous left-side colon, while *Helicobacter*-infected *Tcra* KO mice develop typhlitis (inflammation in cecum).

The colitis in *Tcra* KO mice can be regulated by several factors. In *Tcra* KO mice, resection of the cecal patch (equivalent to human appendectomy) before 3 weeks of age results in decreased number of MLN cells and significantly lower incidence of colitis (<3.3 %) at 6–7 months as compared to the sham-operated mice (>80 %) [165]. This suggests that the appendix lymphoid follicle (cecal patch) may be the priming site for pathogenic cells leading to the development of colitis in *Tcra* KO mice. Recent studies show that cigarette smoke (carbon monoxide) and heme oxygenase (HO-1) induction ameliorates active colitis in *Tcra* KO mice by suppressing the colonic production of IL-1β, TNF, and IL-4 [166]. Of interest, elemental diet-fed *Tcra* KO mice showed no pathogenic features of colitis, with reduced production of Th2-type cytokine, low incidence of *Bacteroides vulgatus* infection, and diversification of Vβ usage of $TCR\alpha^{-}\beta^{+}$ T cell population, as compared to regular diet-fed *Tcra* KO mice [167]. Oral administration of small-size (less than 10 μm in diameter) chitin, a polymer of *N*-acetylglucosamine, suppresses the development of *Tcra* KO mice [168]. Furthermore, regulatory B cells (Breg), which produce large amounts of IL-10 or IL-12p70, contribute to the suppression of colitis in these mice [143, 169]. Local delivery of Il-22 (an IL-10 cytokine member) gene in the colon enhanced STAT3 activation specifically within CECs and ameliorated mucosal inflammation by enhancing the restitution of mucus-producing goblet cells [170]. This result strongly suggests that the local *Il22* gene-delivery system could be a useful therapeutic strategy for treating UC.

Gαi2 *KO Mice*

G proteins are important signal transducing factors, which couple a large family of receptors to effectors, including phospholipase C, adenyl cyclase, and ion channels. They are composed of αβγ (alpha, beta, gamma) heterotrimers that are referred to by their α-subunits [171]. The α-subunit of G_{i2} (so-called Gαi2) has been listed as a potential IBD susceptibility gene based on linkage studies in human IBD [172, 173]. Rudolph et al. generated *Gαi2* KO mice by homologous recombination in embryonic stem cells, in which *Gαi2* was disrupted at the *Nco*I site in exon 3 [173]. After 16–20 weeks of age, every *Gαi2* KO mouse on 129/Sv background develops chronic active inflammation of the colon with mixed inflammatory cellular infiltration in the lamina propria [173]. Of note, nonpolypoid adenocarcinoma resembling neoplastic change seen with UC develops in approximately 30–40 % of *Gαi2* KO mice [173]. Studies so far suggest that dysfunction of Th1-polarized T cells

potentially contributes to the development of chronic colitis as well as colonic adenocarcinoma in *Gαi2* KO mice [173]. Furthermore, *Gαi2* KO mice on a 129SvEv [125] background developed earlier and more severe colitis as compared to those on a C57Bl/6 background, accompanied by greater levels of IFN-γ, IL-6, IL-12p40, IL-17, and TNF-α in the colon [174]. The difference can be explained by the distinct signaling pathways in bone marrow-derived dendritic cells (BMDCs) between these two strains. BMDCs in the 129Sv *Gαi2* KO mice displayed increased MAPKp38 signal activation followed by TLR9 ligand (CpG) stimulation and less anti-inflammatory IL-10 production than the C57Bl/6 *Gαi2* KO mice. This result also supports previous observations that colitis in *Gαi2* KO mice is mediated mainly by Th1- but not Th2-type cytokines [175, 176]. Under both conventional and SPF conditions, *Gαi2* KO mice developed severe colitis and colonic adenocarcinoma, suggesting that the disease was mediated by normal flora. In addition, *Gαi2* KO mice have impaired TGF-β responses in peripheral T cells via decreased phosphorylation of Smad2 and Smad3 [177].

Dysregulated B cell subpopulations, including regulatory B cells, may be associated with the development of chronic colitis in these mice [178]. In particular, IL-10 producing CD1d ^{hi}, CD23^{hi}, and CD21^{intermediate} B cells, which are also observed in other inflammatory disorders [179], may be altered or diminished in *Gαi2* KO mice, suggesting an important role of Gαi2 protein in the development of immunoregulatory B cell populations.

T-bet/Rag-2 *DKO (TRUC) Mice*

T-bet protein, encoded by the *TBX21* gene in humans, is a member of the T-box transcription factor family, which orchestrates adaptive and innate immune systems by initiating proinflammatory Th1 lineage development from naïve T helper precursor cells [180, 181]. However, the precise role(s) of T-bet protein in autoimmunity or neoplastic diseases is not well understood [182, 183]. Garrett et al. examined T-bet deficiency in the innate immune compartment by generating *T-bet* and *Rag-2* DKO (named TRUC) mice [184]. Although *T-bet* KO mice do not develop spontaneous colitis, TRUC mice spontaneously developed colitis that resembles human UC after 4 weeks of age, characterized by rectal prolapse and continuous inflammation of the rectum/left-side colon with mucosal inflammatory infiltrate and ulceration [184]. Interestingly, the colitis in TRUC mice is transmittable to T-bet-sufficient wild-type mice both vertically (by cross fostering) and horizontally (by cohousing). In culture of colon explants from 4-week-old TRUC mice, TNF-α production was significantly higher as compared to those of *Rag-2* KO control mice, while there were no apparent difference in the levels of other inflammatory cytokines, including IFN-γ, IL-1α, IL-1β, IL-6, IL-10, IL-12, IL-13, or IL23 [184]. The major source of TNF-α in the colon was from the CD11c$^+$ dendritic cells. T-bet may control the mucosal immune system by downregulating TNF-α production negatively in colonic

dendritic cells at the initial stage of the colitis development. Since intestinal dendritic cells are constantly interacting with abundant microbes in the colonic lumen and maintaining intestinal homeostasis by controlling the clearance of entero-invasive pathogens [185, 186], perhaps T-bet deficiency in dendritic cells allows for the growth of potentially pathogenic organisms. Therefore, different antibiotics regimens were examined for potential therapeutic benefit. It was found that a combination of vancomycin, metronidazole, neomycin, and ampicillin, as well as selective treatment with metronidazole alone, significantly ameliorated colitis. This suggests that colitis in TRUC mice is dependent on the presence of certain microbes, in particular anaerobic pathogenic bacteria. A later study by the same group, utilizing 16S rRNA-based analysis in fecal samples, showed that *Klebsiella pneumoniae* and *Proteus mirabilis*, both gram-negative facultative organisms, correlate with the development of colitis in TRUC mice [187] and can induce disease given to wild-type recipients.

Interestingly, over 96 % of TRUC mice spontaneously develop colonic high-grade dysplasia and rectal adenocarcinoma by 6 months of age as a consequence of MyD88-independent intestinal inflammation [188]. Restoration of T-bet expression in colonic dendritic cells in TRUC mice reduces colonic inflammation and prevents colonic neoplastic development [188].

Wasp *KO Mice*

A model of IBD had been developed by deletion of gene that encodes for the Wiskott–Aldrich syndrome protein (WASP). Although this model is not widely utilized, we have included it as one of the Th2 type of colitis models in this chapter given its unique characteristic of being one of a few models with a human correlate. *Wasp* encodes a cytoplasmic protein involved in regulating actin cytoskeleton [189], which is defective or absent in patients with Wiskott–Aldrich syndrome, a subset of whom suffer from colitis. *Wasp* KO mice also develop T cell-mediated colitis by 6 months of age with markedly thickened colons seen grossly and crypt hyperplasia and a mixed lymphocytic and neutrophilic infiltrate seen histologically [190]. Lamina propria lymphocytes from *Wasp* KO mice secrete exaggerated levels of IFN-γ, IL-4, and IL-13 without much difference in IL-6 as compared to controls; there were no difference in IL-17 levels [190]. The colitis in *Wasp* KO mice is ameliorated by treatment with antibody against IL-4, but not to IFN-γ. Interestingly, *Wasp* KO mice have a decreased number of naturally occurring $CD4^{+}CD25^{+}Foxp3^{+}$ cells [88]. These Treg cells were found to be markedly defective in their ability to ameliorate colitis using $CD45RB^{hi}$ transfer model [191]; the initiating pathogenic deficiency appears to lie in the innate immune cell population. *Wasp* KO mice represent a model of colitis with a human correlate, potentially mediated by Th2 cytokines and associated with altered innate immune cell and regulatory T cell defects.

Genetically Manipulated Models Associated with the IBD Susceptibility Genes

Nod2 KO Mice

NOD2 (nucleotide-binding oligomerization domain 2) is a member of the NOD-leucine-rich repeat (LRR) protein family. *Nod2* gene codes an intracellular receptor of muramyl dipeptide (MDP), which is a moiety of bacterial cell wall peptidoglycan. In 2001, it was reported that *Nod2* variants confer an increased risk for development of CD [192, 193] and Blau syndrome, which is an autosomal dominant syndrome characterized by familial granulomatous arthritis, uveitis (iritis), and skin granulomas [194]. A mouse model, which carries a *Nod2*-2939insC ($Nod2^{2839ic}$) frameshift mutation similar to human CD-associated *Nod2*-3020insC ($Nod2^{3020ic}$) frameshift mutation, shows significantly more severe DSS-induced colitis with ulceration and increased infiltration of F4/80-positive macrophages, as compared to wild-type controls [195]. The DSS-induced acute injury in Nod2-deficient mice is exacerbated under GF conditions as compared to SPF conditions, suggesting that the *Nod2* gene plays a pivotal role in commensal flora-mediated immunoregulatory function during recovery from acute injury [48]. To assess the immunobiological function of Nod2, other groups examined *Nod2* KO mice containing deletion of exon 1 [196] or exon 3 [197]. In humans, these *Nod2* mutations show defective NF-κB activation after cell stimulation with bacterial products, including LPS and MDP [193, 198, 199]. In APCs of *Nod2* KO mice, IL-12p70 production was significantly increased in response to TLR2 ligation, suggesting that Nod2 signaling inhibits a potentially inflammatory Th1 response mediated by TLR2 signal [196]. The *Nod2* KO mice, which were generated by the deletion of exon 3, are healthy and fertile and demonstrate a normal lymphoid architecture and development in the thymus and spleen [197]. Since *Nod2* plays a pivotal role in the innate immune responses against host/microbial interactions, handling of *Listeria monocytogenes*, a gram-positive intracellular bacteria, was examined in wild-type and *Nod2* KO mice [197]. There was no significant difference survival between the wild-type and *Nod2* KO mice injected intravenously or intraperitoneally with *L. monocytogenes*. In contrast, *Nod2* KO mice challenged with *L. monocytogenes* via intragastric route showed a significantly increased bacterial burden in the spleen and liver, but not in Peyer's patches, as compared to wild-type mice. Furthermore, expression of antibacterial peptides [defensin, defensin-related cryptdin 4 (Defcr4), and Defcr-related sequence 10 (Defcr-rs10)], preferentially produced in intestinal Paneth cells, was significantly reduced in bacterial infected *Nod2* KO as compared to wild-type mice. This suggests that Nod2 plays a protective role specifically in Paneth cell, but not Peyer's patch-dependent route of bacterial infection in intestine. The expression of *Nod2* is dependent on the presence of commensal bacteria, since wild-type mice raised in GF conditions expressed significantly less Nod2 expression, with restoration of expression after infection with commensal bacteria [200]. Interestingly, this

Nod2 KO mouse strain is susceptible to granulomatous inflammation restricted to the ileocecal region in the context of *Helicobacter* infection [201]. In summary, the studies in *Nod2* KO mice support that mutations in the *Nod2* gene are important genetic risk factors in a subset of patients with CD.

Atg5 *KO* and Atg16l1 *Mutant Mice*

An autophagy gene, *ATG16L1*, has been identified as a susceptibility allele for CD by GWAS [202–204]. There was a significant increased risk for CD risk between markers rs22141880 (a SNP coding for T300A) in the *ATg16L1* gene and the established *NOD2* susceptibility variants. ATG16L1 protein is an essential component of autophagy, which is the major intracellular degradation system of a cell's own components (autophagy) [205, 206]. Autophagy is involved in the clearance of intracellular components such as apoptotic bodies and organelles as well as microbes (xenophagy), which results in protection against infectious intracellular pathogens [207]. In addition to ATG16L1, ATG5 is another essential autophagy protein, which is important for the biological functions of antibacterial peptide-containing Paneth cells.

Cadwell et al. generated two mouse lines with hypomorphic (HM) for the expression of the ATG16L1 protein (ATG16L1^{HM1} and ATG16L1^{HM2}) and a third-line knocking out *Atg5* expression in IECs specifically (*Atg*$^{flox/flox}$ *villin-Cre* mice) [8]. Abnormalities in both *Atg16l1*^{HM} and *Atg5* conditional KO mice were confirmed to only Paneth cells with a lack of lysosome staining in the mucus and disorganized/decreased numbers of granules [8]. Even with *L. monocytogenes* infection by oral gavage, the lack of any changes in the spleen, liver, and MLN indicate that Atg16l1 and Nod2 have a distinct function in maintaining the integrity of Paneth cells. Electron microscopic analysis also revealed that Paneth cells in *Atg16l1*^{HM} mice showed significantly increased numbers in cytoplasmic vesicles; a similar abnormality is present in CD patients [8]. Dendritic cells obtained from CD patients with *NOD2* or *ATG16L1* mutation showed functional defects in autophagy, bacterial processing/handling, and antigen presentation [208]. However, recent observations strongly suggest that intracellular sensors of NOD are critical for the autophagic responses [209–211].

Akira's laboratory generated *Atg16l1* mutant mice [212], which express deleted forms of the ATG16L1 protein, lacking the entire coiled-coil domain, that is essentially required for processing autophagy [206]. ATG16L1-deficient macrophages showed increased production of IL-1β at the posttranscriptional level, as compared to wild-type control, while both message and protein levels of TNF-α, IL-6, and IFN-β in response to LPS stimulation show no obvious difference between the two groups. The increased IL-1β production was due to the TRIF (Toll/IL-1 receptor domain-containing adaptor inducing IFN-β)-dependent activation of caspase 1. Interestingly, chimeric mice with ATG16L1-deficient hematopoietic cells are highly susceptible to DSS-induced colitis with severe ulceration, increased inflammatory

cell infiltration, and increased serum levels of IL-1β/IL-18 and 100 % mortality, which can be ameliorated by neutralizing these cytokines [212]. Cadwell et al. elegantly demonstrated that murine norovirus-infected *Atg16l1* KO mice have aberrant DSS-induced changes with increased productions of TNF-α and IFN-γ as compared to wild-type mice [208]. Therefore, ATG16L1 is an essential factor in controlling endotoxin- as well as virus-induced inflammatory immune responses.

In summary, ATG16L1 and ATG5 play a central role in the secretion of granules in Paneth cells that may alter/exclude intestinal microorganisms efficiently. However, how *Atg16l1* polymorphisms affect the biological function of differentiated Paneth cells is still unclear [8].

IL23 Receptor KO Mice

IL23 is a heterodimeric cytokine comprising of p40 and p19 subunits [101]. The IL23 receptor is also composed of two subunits, IL-12Rβ1 and IL23R, and is mainly expressed on T cells and innate immune cells [102]. IL23 is produced by dendritic cells and macrophages in response to pathogenic bacteria such as *Mycobacterium tuberculosis* [213], *Streptococcus pyogenes* [214], and *Klebsiella pneumoniae* [215]. IL23 stimulates macrophages to produce TNF-α and serves as a maintenance factor of Th17 T cells producing IL-17A and IL-17F.

The ligation of IL23 receptor is involved in the pathogenesis of many inflammatory disorders, including those involving the joints [216], brain [217], and intestine [131, 218, 219]. Therefore, IL23 is a key cytokine in several autoimmune diseases. Recent GWAS have demonstrated that polymorphisms of the *IL23* receptor are negatively associated with the development of both UC and CD [220]. By analyzing 14,500 non-synonymous SNPs from 1,000 cases of autoimmune disorders and breast cancer, Burton et al. identified that the *IL23R* locus has an initial association with ankylosing spondylitis. This disease shows occasional strong association with CD development [221]. Following high-throughput re-sequencing of DNA pools, the protective effects of low-frequency coding variants (p.Arg381Gln, p.Gly149Arg and p.Val362Ile) against IBD were confirmed [10]. This piece of data added to the already known fact that *IL12B* (IL-12p40) has been identified as an IBD-associated gene [222].

Recently, Powrie et al. showed that IL23R signaling in intestinal T cells suppresses the IL-10 production by T cells as well as generation of FoxP3+ cells induced by adaptive transferring the $CD4^+CD45RB^{hi}$ (naïve) T cells (isolated from wild-type or *Il23R* KO mice) into *Rag1* KO mice [223]. The majority of mice reconstituted with *Il23R* KO $CD4^+$ T cells, developed milder colitis than those transferred with wild-type $CD4^+$ T cells. Interestingly, IL23R-deficient T cells do not accumulate in the colon after the adaptive transfer into *Rag1* KO mice. In contrast, these cells accumulate in the spleen and liver, suggesting that IL23 signaling in T cells is specifically necessary for effector T cell accumulation in the colon rather than systemic lymphoid organs [223]. Interestingly, *IL23R* and *NOd2* genes can encode

truncated variants that inhibit their signaling pathways [224, 225]. Th17 cells isolated from subjects with an *IL23R R381Q* gene variant show reduced production of IL-17A in response to IL23, suggesting the importance of IL23-related pathways in both CD and UC [226].

Conditional Stat3 *KO Mice*

STAT3 (signal transducer and activator of transcription 3) is a member of the STAT protein family, which acts as transcription activators after being phosphorylated by receptor-associated kinase [227]. STAT3 was the first molecule identified as a member of this family, which is efficiently activated by IL-6 family cytokines [227, 228] and is known to be involved in cell survival and proliferation [229]. STAT3 proteins form either a homodimer or heterodimer when combined with STAT1 and rapidly translocate into the cell nucleus after activation [230, 231]. STAT3 is involved in a broad spectrum of innate and adaptive immune functions, including epithelial regeneration and Th17 differentiation. The importance of STAT3 in IBD is recognized after recent human GWAS as one of the genes associated with increased susceptibility to both CD and UC [5, 6, 220, 232].

Since conventional *Stat3* KO mice are embryonically lethal [233], the function of *Stat3* gene has to be analyzed in a cell or tissue-specific gene KO systems. Takeda et al. generated mice, in which STAT3 is deficient specifically in macrophages and neutrophils (*LysMcre*/*Stat* $^{flox/-}$) where cells have one floxed *Stat3* allele and one disrupted *Stat3* allele [234]. These deficient mice exhibited mortality to endotoxin shock with increased production of proinflammatory cytokines including TNF-α, IL-6, IL-1, and IFN-γ within 24 h after the injection of a small amount of LPS (20 μg), while the wild-type mice survived over 4 days after the injection. Furthermore, *LysMcre*/*Stat* $^{flox/-}$ mice spontaneously developed leukocytosis, anemia, and colitis at the age of 20 weeks. The markedly thickened colonic wall showed reduced crypts, goblet cell depletion, regenerative epithelium, mixed cellular infiltration in the lamina propria, frequent crypt abscesses, and occasional mucosal ulcers. The development of colitis is strongly associated with a decreased production of IL-10 by macrophages and enhanced production of IFN-γ by Th1 cells. Interestingly, the conditional *Stat3* KO mice do not develop colitis when crossed with *Rag-1* KO mice [235], suggesting the requirement of intact STAT3 signaling in the adaptive immune compartment.

To analyze the effect of STAT3 activation in multiple cell types by triggering type I IFN, mice carrying a *STAT3floxed* allele were crossed with *MX-Cre* transgenic mice expressing Cre recombinase under the control of IFN-responsive *Mx1* promoter (*MX+*; *STAT3*$^{fl/fl}$) [236]. In *MX+; STAT3*$^{fl/fl}$ mice, most of the enteric epithelial STAT3 signals disappeared. These mice developed a severe wasting syndrome with an aggressive form of colitis within 2–3 weeks after the injection of synthetic double-stranded RNA (pIpC), which almost completely deleted STAT3 expressions in liver, bone marrow, and adipose tissues. These mice produced high

amounts of IL-6, IL-12p40, IFN-γ, and IL-10 as compared to *MX-; STAT3*$^{fl/fl}$ mice. Treatment with the anti-IL-12p40 antibody, but not neutralization of CD4$^+$ or NK cells, or treatment with oral antibiotics (2.5 mg/mL streptomycin/bacitracin in drinking water 7 days prior to pIpC injection) prevented the development of colitis, suggesting a crucial role of STAT3 in the maintenance of intestinal homeostasis. In addition, Pickert et al. showed that specific deletion of *STAT3* in epithelial cells increases susceptibility of mice to DSS-induced colitis [45], suggesting that STAT3 activation in innate versus adaptive immune responses plays distinctly different roles in the pathogenesis of colitis.

To further dissect the functional role of STAT3 on T cells during the development of colitis, Durant et al. utilized the CD45RB^{high} CD4$^+$ T cell transfer into *Rag-2* KO colitis mouse model. The recipient *Rag-2* KO mice were reconstituted with naïve CD4$^+$ T cells from control (*Stat3*$^{flox/flox}$) or *Stat3* KO (*cd4 Cre; Stat3*$^{flox/flox}$) mice. *Rag-2* KO recipient mice that received *Stat3* KO T cells had no colonic inflammation, while the mice that received control T cells developed marked colitis at 9 weeks of age [237]. Interestingly, chromatin immunoprecipitation and massive parallel sequencing analysis revealed that STAT3 directly binds to multiple survival gene promoters, including *Bcl2*, *ler3*, *Fos*, *Jun*, and *Fos12*, suggesting that STAT3 directly regulates genes that are involved in the survival as well as proliferation of CD4$^+$ T cells.

Xbp1 *KO Mice*

The transcription factor XBP-1 (X-box-binding protein-1) is a key component for the stress response in the endoplasmic reticulum (ER) and is required for ER expansion, the development of highly secretory cells, and adaptation of tumor cells to stressful (e.g., low glucose, hypoxia) conditions [238–240]. ER stress is known to be increased in the IECs isolated from patients with IBD, and inflammation-induced ER stress is efficiently inhibited by IL-10 in vivo [241]. XBP-1 is expressed in the IECs and *Xbp1* deletion results in increased ER stress as well as the exacerbation of DSS-induced colitis [242]. To further analyze the role of XBP-1 on colonic epithelial homeostasis, intestinal epithelial-specific *Xbp1* KO (*Xbp*$^{flox/flox}$ *villin-Cre*) mice were generated [243]. About 60 % of *Xbp1* KO mice and 30 % of *Xbp1*+/− mice spontaneously developed small intestinal inflammation in association with ER stress, suggesting a pivotal role for mono-allelic expression of *Xbp1* in inducing the organ-specific inflammation [244]. The small intestinal inflammation was characterized by polymorphonuclear infiltration in the lamina propria with occasional crypt abscesses and mucosal ulceration. Paneth cells were completely absent and goblet cells were reduced in the small intestine. In contrast, the morphology and function of absorptive epithelium and enteroendocrine cells were intact [244] and the colon did not demonstrate any abnormalities. Examination of a German patient cohort (1103 controls, 550 CD, and 539 UC patients) found that

the *Xbp1* variant rs35873774 had the strongest association with both CD and UC among the 20 candidate SNPs studied [244]. In summary, XBP-1 is one of the susceptibility factors for IBD, and its abnormality in IECs is associated with spontaneous ileitis development.

Lrrk2 *KO Mice*

LRRK (leucine-rich repeat kinase 2) is a large (285 kDa) protein, which contains a Ras of complex GTPase domain, a C-terminal of Ras of complex domain and an MAPK kinase domain. Point mutations in *Lrrk2* are the most common genetic cause of both familial and apparently sporadic forms of Parkinson's disease (PD) [245, 246]. A PD-related G2019S substitution in the kinase domain of LRRK2 enhances the phosphorylation of putative protein kinases ezrin, radixin, and the moesin (ERM) family proteins, which links the actin cytoskeleton with membrane proteins [247, 248]. The G2019S substitution likely increases kinase activity in LRRK2 [249, 250]. In addition, *LRRK2* PD-associated mutations induce an alteration of cell death and autophagy function [251, 252]. Of note, LRRK2 forms a protein complex with heat shock protein 90 (HSP90). Since inhibition of the Hsp90 chaperone function dramatically decreases the stability of LRRK2, Hsp90 may suppress the accumulation of mutant *LRRK2*, which is strongly related to pathogenic activities in neurons [253]. Tong et al. generated *Lrrk2* KO mice to analyze its physiological role in vivo. *Lrrk2* KO mice appear normal, but gross morphological abnormalities in the kidney (altered size, weight, color, and texture) become evident at 3–4 months of age [254]. Increased accumulation of autofluorescent granules in proximal renal tubules becomes obvious in *Lrrk2* KO mice with increasing age, although kidney filtration function is intact [255]. The impaired autophagy function in *Lrrk2* KO kidneys is observed by accumulation of lipofuscin granules, altered levels of LC3-1/II, a reliable marker for autophagy, and increased number of apoptotic cells [254, 255].

A recent GWAS identified a single-nucleotide polymorphism (SNP) rs11175593 as a risk factor for CD. The *LRRK2* gene is located downstream from this SNP [232]. *LRRK2* is mainly expressed in immune cells including B cells, monocytes, and dendritic cells, based on the microarray data using *LRRK2* probes from the Genomics Institute of the Novartis Research Foundation and RIKEN data sets [256]. The expression of *LRRK2* is significantly upregulated in the lamina propria cells of inflamed intestinal tissues obtained from CD patients, as compared to the cells from noninflamed areas from the same CD patients or from inflamed tissues from UC patients [256]. The same group also demonstrated that *LRRK2* is an IFN-γ target gene upregulated during bacterial infection, and it is one of the activators of the NF-κB pathway: LRRK2-induced NF-κB activation is IKK dependent but is independent of LRRK2 kinase activity. These results suggest that LRRK2 might be involved in the regulation of mucosal immune responses by activating the NF-κB

signaling pathway, which is relevant to CD pathogenesis. Utilization of *Lrrk2* KO mice in chemically induced or bacterial infectious colitis models could provide further knowledge regarding the role of LRRK2 on regulation of the immune response to pathogen or epithelial injury.

p40^{phox} *KO Mice*

In phagocytes, reactive oxygen species (ROS), generated by NADPH oxidase, play a pivotal role in regulating proinflammatory signaling and in killing pathogens by phagocytosis. The NADPH oxidase complex is composed of five subunits including gp91^{phox}, p47^{phox}, p22^{phox}, p67^{phox}, and p40^{phox} [257]. Recent genetic studies have revealed an association between increased susceptibility for CD and *NCF4* (which encodes p40^{phox}) and *NCF2* (which encodes p67^{phox}) polymorphism [203, 258]. Functional studies have confirmed that impaired p40^{phox} promotes intestinal inflammation with impaired ROS production [259]. In addition, neutrophils isolated from *P40^{phox}* KO mice have severe defects in NADPH oxidase regulation as well as oxidant-dependent in killing of *Staphylococcus aureus,* both in vitro and in vivo [260]. The *p40^{phox}* KO mice showed enhanced intestinal inflammation during the acute and recovery phases of DSS colitis through upregulation of the chemokine receptor 1 and downregulation of enzymes for glycan modifications: these results were obtained by using an integrative bioinformatics approach [261].

Conclusions/Future Prospective

Although the intestinal inflammation seen in animal models does not exactly replicate human IBD, studies utilizing these models have provided important insights into the pathogenesis of IBD. One principle concept is the central role of the intestinal microbiota in the development of the innate and adaptive immune responses that can result in intestinal inflammation. Migratory patterns of immune cells and the effects of mucosal immune dysregulation and alteration of mucosal epithelial barrier during initiation and maintenance of intestinal inflammation can also be learned from these models. Furthermore, the development of animal models that incorporate variants of human IBD susceptibility genes has provided a means to directly examine the role of these genes in intestinal inflammation. Application of new technology will refine and extend our current knowledge thus far obtained from these models.

Acknowledgement The authors are grateful to Terry Danford Lott, Cindy W. Lau, and Doris A. Regal for their excellent secretarial assistance in preparing this manuscript and Drs. Daren Low and Deanna Nguyen for critically reading this manuscript.

Grant Supports: This work has been supported by the National Institute of Health and grants from the Eli and Edythe L. Broad Medical Foundation, the American Gastroenterological Association Foundation, and the Crohn's and Colitis Foundation of America.

Financial Disclosure: All authors have no disclosures in regard to any potential conflicts that may be relevant to this manuscript.

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