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Genetically Engineered Mouse Models for Studying Inflammatory Bowel Disease

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Abstract

Inflammatory bowel disease (IBD) is a chronic intestinal inflammatory condition that is mediated by very complex mechanisms controlled by genetic, immune, and environmental factors. More than 74 kinds of genetically engineered mouse strains have been established since 1993 for studying IBD. Although mouse models cannot fully reflect human IBD, they have provided significant contributions for not only understanding the mechanism, but also developing new therapeutic means for IBD. Indeed, 20 kinds of genetically engineered mouse models carry the susceptibility genes identified in human IBD, and the functions of some other IBD susceptibility genes have also been dissected out using mouse models. Cutting-edge technologies such as cell-specific and inducible knockout systems, which were recently employed to mouse IBD models, have further enhanced the ability of investigators to provide important and unexpected rationales for developing new therapeutic strategies for IBD. In this review article, we briefly introduce 74 kinds of genetically engineered mouse models that spontaneously develop intestinal inflammation.

Keywords

epithelial barrier; ER stress; knockout mice; IBD susceptibility gene; immunoregulation; mouse models

Inflammatory bowel disease (IBD) is a chronic intestinal inflammatory condition that is classified into two major forms, Crohn's disease (CD) and ulcerative colitis (UC) [1,2]. Dr. Kirsner first introduced an experimental colitis model in 1957, which was induced in rabbits by sensitization to egg albumin together with rectal instillation of formalin [3], and different types of colitis models such as chemically induced colitis models, cotton-top tamarin model, spontaneous mutation models, and adoptive T cell transfer model have since been

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established [4,5,6]. Development of genetic engineering technology in animals made an important turning point in IBD research [7]. In 1993, three kinds of knockout (KO) mice deficient for interleukin (IL)-2 [8], IL-10 [9], or T cell receptor α chain (TCR α) [10] were reported as the first murine models of spontaneous colitis. IL-10 and its receptors have since been identified as susceptibility genes for IBD [11], and IL-2 and its receptor also represent IBD susceptibility genes [12,13]. In contrast, TCR α per se is not an IBD susceptibility gene, but several IBD susceptibility genes such as mucin 1 (Muc1) serve as protective factors in the colitis of TCR α KO mice [14]. So far, more than 74 kinds of genetically engineered mouse strains have been reported to develop colitis and/or ileitis spontaneously. In addition, more than 790 kinds of genetically engineered mice have been demonstrated to increase or decrease the susceptibility to chemically induced colitis and/or epithelial barrier dysfunction [7]. The alteration of inflammatory processes in so many kinds of genetically engineered mice suggests that IBD is mediated by much more complicated mechanisms than previously predicted [15]. Indeed, human genome-wide association studies have identified more than 160 susceptibility genes in IBD [12,13,15], and 20 kinds of genetically engineered mouse models carry the susceptibility genes identified in human IBD. Since there are so many kinds of genetically engineered mice associated with IBD, this review focuses on only 74 genetically engineered mouse models that develop intestinal inflammation spontaneously.

Genetically engineered murine IBD models

Genetically engineered mice for studying IBD are classified into 9 groups based on the gene construction strategies (Figure 1). Conventional transgenic (Tg) or knockout (KO) mice are genetically engineered to overexpress or lack a gene of interest in all cell types. Dominant negative transgenic (Dn) model is genetically engineered to overexpress a nonfunctional protein that interferes with the normal function of target protein by competing their ligand or receptor. Cell-specific Tg or KO model overexpresses or lacks a gene of interest in a specific cell type, respectively. Inducible knockout (iKO) models allow investigators to delete a gene of interest anytime during the adult life. Knock-in (KI) models carry a mutation in gene of interest for mimicking the polymorphism within the IBD susceptibility gene. Innate models lack a gene of interest in an immune deficient background. In this model, the single KO mice do not develop intestinal inflammation under immune sufficient conditions, but the development can be induced when they are further crossed with immune deficient mice such as RAG2 KO mice lacking both T and B cells. Mutagen-induced models carry a functional gene mutation that is randomly induced by N-ethyl-N-nitrosourea treatment.

In addition, mouse IBD models can be further classified into 4 groups depending on the organ that is affected by inflammation (Figure 2). Colitis models develop inflammation restricted to the colon, ileitis models develop inflammation restricted to small intestine, enterocolitis models develop inflammation in both colon and small intestine, and systemic models develop inflammation in multiple organs including the intestine.

AGR2

Anterior gradient 2 (*AGR2*) is a potential IBD risk gene [16], encoding an Endoplasmic Reticulum (ER)-resident disulphide isomerase involved in protein folding. *AGR2* KO mice spontaneously developed intestinal inflammation that was most severe in the terminal ileum, and to a lesser extent in the colon [16]. By utilizing inducible *AGR2* KO mice, defects in Paneth cells were shown as the primary causal factor of this ileitis.

Ahr

Aryl hydrocarbon receptor (*Ahr*) plays an important role for the function of group 3 innate lymphoid cells (ILC3) that produce IL-22 to preserve epithelial barrier integrity. *Ahr* KO mice did not develop colitis, although spontaneous development of colitis became recognizable at 12–20 wk of age when haploinsufficiency of *ROR γ t* (a transcription factor for Th17 differentiation) was genetically introduced in these mice [17]; their colitis was characterized by fewer ILC3 cells and markedly more IL-17⁺ IFN γ ⁺ CD4⁺ T cells.

AP1B

Epithelial cell polarity is established by an epithelium-specific polarized sorting factor adaptor protein AP1B. Mice with epithelial cell-specific deletion of *ab1b* (AQ; Please check mouse gene abbreviation) spontaneously developed a Th17-dominant colitis at 8 wk of age [18]; the barrier function of epithelial cells was intact, but production of antibacterial peptides such as cathelicidin and defensin was significantly reduced. This colitis was treatable by antibiotics.

Atg5

Autophagy exerts diverse physiological functions ranging from microbial infections to antigen presentation [19]. The autophagy molecule Atg16L1 is produced by a major susceptibility gene of CD [12,13,20–22]. Atg16L1 KO mice were highly susceptible to a chemically-induced acute colitis, although they failed to develop colitis spontaneously [23]. In contrast, implantation of Atg5 KO thymi into nu/nu mice lacking T cells induced the development of multi-organ inflammation in the colon, liver, lung, and uterus [24].

Bach2

Bach2, which represents a susceptibility gene of CD, serves as a transcriptional repressor of Blimp-1 (an inducer of plasma cell differentiation) and of myeloid program to induce B cell development [25]. *Bach2* KO mice developed a progressive wasting disease from 3 mo of age due to severe pneumonia. Some of these mice developed a mild and incompletely penetrating inflammation in the small intestine and stomach [26] that was associated with lack of efficient formation of regulatory T cells (Treg).

Blimp-1

B lymphocyte induced maturation protein-1 (Blimp-1) encoded by *Prdm1* was initially identified as a B cell-specific transcription factor for plasma cell differentiation. However,

subsequent studies have identified the expression of Blimp-1 in different cell types such as memory T cells. *Prdm1* has been identified as a susceptibility gene for both CD and UC [12,13]. 20% of T cell-specific Blimp-1 KO mice developed a Th1-mediated colitis at 4 wk of age, and the penetration of colitis reached to 83% by 21 wk of age [27]. Treg cells were able to expand normally, but their regulatory function was impaired. In addition, T cells were more prone to differentiate into IL-17⁺ IFN γ ⁺ CD4⁺ T cell subset that is seen in the inflamed intestine of CD patients.

Caspase-8 (Caspase8, cFLIP)

Caspase-8 is a cysteine protease involved in regulating apoptosis. More than 80% of mice with epithelial cell-specific deletion of caspase-8 developed ileitis but not colitis [28]. This ileitis was characterized by a marked destruction of ileal architecture and by the induction of apoptosis-independent type of programmed necrosis so called necroptosis.

Cellular FADD-like interleukin 1 β -converting enzyme inhibitory protein (cFLIP) is an antiapoptotic protein through inhibition of caspase-8. Mice with epithelial cell-specific deletion of *cflip* died within one day after birth with massive intestinal bleeding due to abnormal epithelial homeostasis characterized by enhanced apoptosis that starts in the uterus (AQ: do you mean starts before birth?) [29].

CD51/integrin β 8

CD51 is the most unselective α subunit, associating with β 1, β 3, β 5, β 6, and β 8 subunits and participating in many cellular processes such as cell adhesion and cell survival. CD51 KO mice died before or shortly after birth from defects in brain vascular development. In contrast, mice with haematopoietic cell-specific deletion of *cd51* developed inflammation in multiple organs around 14 wk of age, including colon, caecum, peritoneum, liver, lungs, and nasal cavity [30]. This inflammation was characterized by aberrant Th1- and Th2-type responses due to the inability of phagocytic cells to remove apoptotic cells that serve as a source of self-antigens [31].

Integrin β 8 is associated with integrin α V subunit to form α V β 8 that is capable of activating the *TGF β* pathway. Mice with specific deletion of β 8 in both CD4⁺ T cells and DCs developed a progressive wasting disease from 4–5 mo of age, and all mice then developed colitis characterized by enhancement of both Th1 and Th2 responses by 10 mo of age [32].

CD86

CD86 is a costimulatory molecule expressed by antigen presenting cells (APCs) to interact with CD28 for T cell activation and with CTLA4 for immune regulation. Mice with liver-specific overexpression of soluble CD86 Ig Fc fusion protein developed transmural colitis by 8–10 wk of age [33]. This colitis was mediated by IFN- γ -producing T cells activated via CD28.

CD154

CD154 is expressed on T cells to interact with CD40 on APCs to provide bidirectional co-stimulatory signals between T cells and APCs for activation of both humoral and cellular immune responses. Mice with T cell-specific overexpression of *Cd154* developed inflammation in multiple organs, including colon, stomach, kidney and lung at 3–6 weeks of age [34]. The colitis was characterized by lethal, transmural, granulomatous inflammation. Although B cells do not express CD154, except in some patients with systemic lupus erythematosus (SLE), mice with ectopic overexpression of *Cd154* on B cells developed colitis, ileitis, and glomerulonephritis at 8–15 weeks of age [35]. In addition, mice with skin epidermis-specific overexpression of *Cd154* developed dermatitis and systemic autoimmune diseases [36].

Enteric glia

Mice that were genetically engineered to express herpes simplex virus thymidine kinase under the control of glial fibrillary acidic protein (GFAP), lost the GFAP-positive glial cells of the small intestine after administration of Ganciclovir, an antiviral medication. This inducible ablation of enteric glial cells resulted in a fulminating ileitis with necrosis and haemorrhage through disrupting the intestinal epithelial barrier [37,38]. This necrotic ileitis was not improved by antibiotic treatment [37].

FASyn

Fatty acid synthase (FASyn) is an insulin-regulated enzyme that synthesizes saturated fatty acids for cell membranes, energy stores, and signalling molecules. Tamoxifen-induced epithelial cell-specific deletion of FASyn during adult life induced body weight loss from 5 d after tamoxifen treatment [39]. Spontaneous development of inflammation particularly in the caecum and to a lesser extent, colon was then observed. This colitis was treatable by antibiotics and characterized by a disruption of intestinal barrier due to impaired secretion of Muc2.

Gai2

Gai2 belongs to a family of GTP-binding protein involved in a wide variety of transmembrane signalling systems. All Gai2 KO mice on a 129/sv background developed a lethal, pan-colitis at 16–20 wk of age and 30% developed adenocarcinoma [40]. In contrast, Gai2 KO mice on a C57BL/6 background were relatively resistant to colitis development. Reduced production of IL-10 by DCs was seen in 129/sv, but not C57BL/6, Gai2 KO mice [41].

Gimap5

GTPase of immunity-associated protein (Gimap), which shares a GTP-binding AIG homology domain, is expressed predominantly by lymphocytes to regulate their survival. *Gimap5* is a susceptibility gene for SLE. Through screening of N-ethyl-N-nitrosourea-mutagenized mice, spontaneous development of colitis and hepatitis was identified in mice

carrying a mutation in *Gimap5* [42]. The colitis was seen from 4 wk of age, and severe colitis was established by 10 wk of age. This colitis was treatable by antibiotics and characterized by lymphopaenia with a functional defect of B cells.

GPX

Glutathione peroxidase (GPX) is a selenium-dependent hydroperoxidase-reducing enzyme to reduce H_2O_2 and fatty acid hydroperoxides. Mice deficient for both, but not either, *Gpx1* and *Gpx2* spontaneously developed colitis and ileitis with perianal ulceration by 24 d of age. Approximately 40% of these mice died before 36 d after birth [43]. Interestingly, a cholesterol diet exacerbated this colitis through reduction of ER stress responses.

IL-1Rn

IL-1 receptor antagonist (IL-1RA), encoded by the *Il1rn* gene, serves as an endogenous IL-1 inhibitor. An association of *Il1rn* polymorphism with CD in a population in India has been reported. Mice with deletion of *Il1rn* on an immune deficient background (RAG2 KO mice lacking T and B cells) spontaneously developed colitis with high mortality [44]; 80% of mice developed rectal prolapse at 12 wk of age.

IL-2 pathway (IL-2, IL-2R α , IL-2R β , and Common γ)

IL-2 binds to a heterotrimer receptor composed of IL-2R α , IL-2R β , and Common γ chains to induce activation-induced cell death and elicit the function of Treg cells. Importantly, *Il2ra* encoding IL-2R α is a CD susceptibility gene and the *il2* gene is located within a susceptibility locus for UC [12,13]. IL-2 KO mice spontaneously developed a systemic autoimmune disease characterized by colitis, gastritis, hepatitis, pneumonia, pancreatitis, nephritis, and haemolytic anaemia [8]. In addition, IL-2R α KO, IL-2R β KO, and Common γ chain KO mice all developed similar autoimmune phenotypes [45–47]. Furthermore, deficiency of Janus family tyrosine kinase (JAK) 3, which is an essential transducer of Common γ chain, caused spontaneous development of colitis in mice [46]. A unique feature of the IL-2 KO model was the dependence of colitis on an autoimmune mechanism, as indicated by the spontaneous development of colitis in germ-free condition [48]. The CD4⁺ TCR $\alpha\beta$ ⁺ T cells but not CD8⁺ TCR $\alpha\beta$ ⁺ T cells, TCR $\gamma\delta$ ⁺ T cells or B cells are required for the development of this colitis [49].

IL-7

IL-7 plays a major role in T cell homeostasis at various developmental stages, and its receptor encoded by *Il7r* gene has been identified as a candidate gene associated with UC [12,13]. IL-7Tg mice spontaneously developed colitis between 4 and 12 wk of age [50]. Systemic, but not intestinal, IL-7 contributed to the perpetuation of this colitis by generating long-lived colitogenic memory CD4⁺ T cells that reside in the bone marrow [51].

IL-10

The gene for IL-10, a regulatory cytokine, is a susceptibility gene not only for adult IBD but also for early onset of IBD in children [11–13]. IL-10 KO mice spontaneously developed a

colitis sharing some features with CD after 3 mo of age [9]. A more severe colitis was observed when they were backcrossed to C3H or Balb/c backgrounds as compared to C57BL/6. Development of colitis was inhibited when IL-10 KO mice were maintained under germ-free conditions [52], and many studies focusing on enteric bacteria and probiotics have used this model [53].

CX3CR1⁺ macrophages have been proposed to represent a major source of IL-10 in the inflamed colon. However, CX3CR1-specific IL-10 KO mice failed to spontaneously develop colitis. In contrast, CX3CR1-specific IL-10Ra KO mice developed inflammation most prominently in the caecum and distal colon [54], suggesting that macrophages are a major responder to, rather than producer of, IL-10 in the inflamed colon.

IL-15

IL-2 and IL-15 bind to heterotrimeric receptors that have two receptor subunits in common, but these two cytokines have distinct roles in adaptive immune responses. Mice overexpressing *Il15* in the thymus and intestinal epithelial cells under a control of T3b promoter spontaneously developed inflammation with an expansion of CD8 α β ⁺ Natural Killer (NK) T cells in proximal small intestine [55]. This inflammation was recognized from 3 mo of age with 100% penetration at 6 mo of age.

IRE1 α

Inositol Requiring Enzyme (IRE) 1 α is an ER transmembrane protein that serves as a major sensor of ER stress. Mice with epithelial specific deletion of *Ire1 α* spontaneously developed a colitis with goblet cell loss and epithelial barrier dysfunction [56]. Rectal bleeding was observed from 16–24 wk of age, and female mice were more susceptible.

K8

Keratin 8 (K8) serves as a structural protein for single layered epithelial cells. K8 KO mice on a C57BL/129 background suffered embryonic lethality, but K8 KO mice on an FVB/N background survived and developed colorectal, but not small intestinal, hyperplasia with rectal prolapse at 9 wk of age [57].

Mdr1a

Multiple drug resistance (Mdr) 1 protein pumps small amphiphilic and hydrophobic molecules across membranes for detoxification. *MDR1* is a susceptibility gene for the late onset of UC in Japanese population [15]. 20–25 % of Mdr1a KO mice on an FVB background at one year of age developed a colitis that was improved by antibiotic treatment [58].

Mucus pathway (Muc2, C1galt1)

Muc2 is a component of the mucus layer, which serves as a lubricant and a physiological barrier between luminal contents and mucosal surfaces. Muc2 KO mice were initially established as a cancer model with spontaneous adenocarcinoma in the colon at 6 mo of age

[59]. A subsequent study identified that colitis, with mild inflammatory cell infiltration, was present primarily in the distal colon of Muc2 KO mice from 5 wk of age [60].

Intestinal mucus carries large numbers of O-glycan chains, moieties? AQ: please clarify), which account for approximately 80% of the mucus molecule's mass. O-glycan synthesis in epithelial cells is altered in approximately 30% of UC patients [61]. A glycosylation enzyme termed core 1 β 1,3-galactosyltransferase (C1galt1) is required for the initiation of O-glycan synthesis on mucus molecules. All mice with epithelial cell-specific deletion of *c1galt1* spontaneously developed inflammation in the distal colon at 12 weeks of age [61]. The colitis still developed in the absence of T and B cells, and antibiotics treatment improved this colitis. In addition, inducible deletion of *c1galt1* in epithelial cells during adult life rapidly elicited the development of colitis within 10 d after initiation [61].

N-cadherin

N-cadherin mediates homophilic adhesive interactions between epithelial cells. Chimeric mice that were generated from ES cells to carrying a dominant negative mutation of N-cadherin only in small intestinal epithelial cells developed transmural inflammation in the jejunum by 3 mo of age [62].

NFATc2/RAG

The nuclear factor of activated T cells, cytoplasmic 2 (NFATc 2) plays redundant functions in gene regulation for T cell differentiation. Although NFATc2 KO mice did not develop colitis, the offspring of crosses with RAG2 KO mice (lacking T and B cells) developed a spontaneous colitis around 15 wk of age [63]. Polyclonally-activated regulatory B cells participated in the suppression of this colitis. (AQ: please check that your original meaning has been retained)

NF κ B1 pathway (NEMO, IKK1, IKK2, RelA, A20)

The nuclear factor (NF) κ B1 pathway is activated via a trimer composed of I κ B kinase (IKK)1, IKK2 and NF κ B essential modulator (NEMO, also known as IKK γ). Although inhibition of NF κ B1 in adaptive immune cells has been shown to improve colitis [64], mice with specific deletion of *nemo* in epithelial cells spontaneously developed colitis with epithelial apoptosis at 6 wk of age [65]. Absence of both, but not either, IKK1 and IKK2 in epithelial cells also led to spontaneous development of colitis [65]. In addition, 10–15% of epithelial cell-specific RelA (also known as NF κ Bp65) KO mice exhibited diarrhoea within 3 d after birth and died within 25 d [66]. Crypt structures in the small intestine were completely lost in these mice.

A20, also known as TNF α inducible protein 3 (TNFIP3), is an ubiquitin-editing enzyme involved in the termination of NF κ B1 activation. A20 KO mice developed inflammation in multiple organs including the liver, kidney, intestine, and joints at 3–6 wk of age [67]. The colitis still developed in the absence of T and B cells. Myeloid lineage cells were primarily responsible for the development of this colitis [68]. Although epithelial cell-specific A20

KO mice failed to spontaneously develop colitis, both epithelial cell- and myeloid cell-specific A20 KO mice developed multi-organ inflammation [69].

Notch (RBP-J, Pofut1)

Notch receptors undergo proteolytic cleavage to release the Notch intracellular domain (NICD). These translocate into the nucleus to form a transcriptional activator complex with recombination signal binding protein for Ig κ J region (RBP-J), and intestinal epithelial cells, this complex activates Notch target genes. A Th17-dominant colitis developed in 50% of mice with epithelial cell-specific deletion of *rbpj*. Interestingly, this colitis was characterized by an increased production of defensin and by goblet cell hyperplasia at 20–26 wk of age [70]. This colitis was improved by antibiotics treatment.

Protein O-fucosyltransferase 1 (Pofut1) is an enzyme that is required for Notch ligand binding. Mice with epithelial cell-specific deletion of *Pofut1* exhibited goblet cell hyperplasia with enhanced expression of Muc2. Intestinal inflammation was evident from 4 wk of age, and all mice developed enterocolitis with transmural inflammation by 36 wk of age [71].

PI3K pathway (p110 δ , PDK1, Cbl-b)

Phosphatidylinositol 3-kinase (PI3K) is required for second messenger signalling from antigen receptors. A PI3K p100 subunit, p110 δ is expressed predominantly in leukocytes. Knock-in (KI) mice carrying a point mutation in the P110 δ gene locus at position 910 (D>A) spontaneously developed a focal inflammation restricted to the rectum and caecum [72]. The colitis of p110 δ KI mice is mediated by impaired activation of Foxp3⁺ Treg [73].

Phosphoinositide-dependent kinase 1 (PDK1) is a key downstream effector of PI3K pathway. Mice with CD4-specific deletion of *Pdk1* spontaneously developed colitis at 8 wk of age [74]. Interestingly, this colitis was characterized by an expansion of IL-17A expressing TCR $\gamma\delta$ T cells in the intraepithelial compartment.

Casitas B-lineage lymphoma b (Cbl-b) proteins function as E3 ubiquitin ligases and molecular adaptors to promote ubiquitin conjugation to the p85 regulatory subunit of PI3K. Cbl-b acts as a negative regulator of T cell activation. Cbl-b KO mice spontaneously developed multi-organ inflammation (intestine, salivary glands, pancreas, liver, lung, heart, bladder, and connective tissues) with massive infiltration of activated T cells and B cells from 3 mo of age [75].

Protein C

Protein C (PC) pathway represents a key coagulation system. Since PC KO mice died soon after birth, PC KO mice were further crossed with PC transgenic mice (PCKO/PCtg) to allow them to express low levels of PC. Interestingly, the PCKO/PCtg mice spontaneously developed mild colitis, recognized by histological and endoscopic examinations at 6–8 wk of age [76].

PP4

Protein phosphatase 4 (PP4), encoded by *Ppp4c*, is a serine/threonine phosphatase. Approximately 60% of mice with CD4-specific deletion of *ppp4c* developed colitis with rectal prolapse by 15 wk of age [77]. Development of functional Tregs was impaired in this model.

Runx3

Runx3, which serves as a context-dependent transcription factor involved in thymopoiesis and dendritic cell maturation, has been reported as a susceptibility gene for UC in Dutch and Chinese populations [78]. All Runx3 KO mice spontaneously developed colitis, with an enhancement of both Th1 and Th2 responses by 4 weeks of age, and 20% of these mice featured extension of inflammation to the small intestine [79]. This intestinal inflammation was associated with impaired activation of Treg and CD8⁺ T cells [80].

SHIP

The Src homology 2 (SH2)-containing inositol-5 phosphatase (SHIP), which is activated in response to various growth factors as well as TCR and BCR ligations, is located within an IBD association locus at 2q37. Over 90% of SHIP KO mice developed transmural, segmental ileitis with granulomas (28%) at 6–8 wk of age [81]. Interestingly, inducible deletion of SHIP during adult life also led to the rapid development of ileitis within 5 wk after gene deletion. Granulocyte-monocyte lineage cells, but not T cells or NK cells, are primarily responsible for this ileitis.

SHP2 (PTPN11)

Src homology 2-containing protein tyrosine phosphatase 2 (SHP2), encoded by *Ptpn11*, is a ubiquitously expressed cytoplasmic protein. *Ptpn11* was a susceptibility gene for UC in a Japanese population [82]. Mice with epithelial cell-specific deletion of *Ptpn11* developed colitis at 3 wk of age, and some of them died by 10 wk of age [83].

STAT3 pathway (STAT3, SOCS3, gp130)

The signal transducer and activator of transcription (STAT) 3, which serves as a master transcriptional factor to control a broad spectrum of adaptive and innate immune responses such as Th17 differentiation and epithelial regeneration, represents a susceptibility gene for CD and UC [12,13]. Although activation of STAT3 in CD4⁺ T cells induced the development of colitis [84,85], macrophage/neutrophil-specific Stat3 KO mice spontaneously developed colitis at 20 wk of age [86]. Development of colitis in macrophage/neutrophil-specific Stat3 KO mice was abolished in the absence of T and B cells [87]. In contrast, specific deletion of *Stat3* in epithelial cells failed to produce spontaneous colitis [88]. These findings suggest a pathogenic role for STAT-3-mediated activation of adaptive immunity and a protective role of STAT3-mediated activation of innate immunity in colitis. Interestingly, inducible deletion of *Stat3* during adult life led to rapid development of colitis [89]. Since the deletion occurred in all cell types, the data from

inducible Stat3 KO mice tend to suggest that the overall function of STAT3 in colitis depends more heavily on innate, rather than adaptive, immune cells.

Activation of STAT3 is regulated by suppressor of cytokine signaling (SOCS) protein. Approximately 40% of mice with T cell-specific overexpression of *Socs1* developed colitis after 15 wk of age when they were kept in conventional, but not SPF, conditions [90].

IL-6 interacts with gp130 receptor to activate STAT3. Mice carrying a truncating mutation within *Gp130* spontaneously developed ulcerations at the gastric pylorus and anorectal region at 4 mo of age when maintained under SPF conditions [91]. The distal ulceration region extended to the caecum when they were kept under conventional conditions. Arthritis was also recognized in some of these mice.

STAT4

STAT4, a transcription factor that promotes Th1 development, is a susceptibility gene for UC in Caucasians [92]. Mice that were genetically engineered to overexpress *Stat4* when the cytomegalovirus (CMV) promoter is activated, developed transmural colitis within 7–14 d after immunization with DNP-KLH/CFA to activate the CMV promoter [93]; this colitis was mediated by CD4⁺ Th1 T cells.

T-bet/GATA3

Recent studies found the expression of T-bet (a transcription factor for Th1) and GATA3 (a transcription factor for Th2) in Treg. To test the role of these transcriptional factors in Treg function, mice with Treg-specific deletion (under control of Foxp3 promoter) of *Tbet* and/or *Gata3* were generated. Interestingly, these mice started to display splenomegaly and lymphadenopathy from 6–8 wk of age and then developed inflammation in multiple organs, including colon, small intestine, liver, lung, and kidney [94].

T-bet/RAG

T-bet is a T-box transcription factor involved in the differentiation of Th1 T cells. T-bet KO mice did not develop colitis, although colitis did develop when they were crossed with RAG2 KO mice lacking T and B cells. A continuous colitis resembling that of UC was observed from 4 wk of age [95]. Interestingly, the colitis was transmissible to wild type mice both vertically (maternally from mother) and horizontally (between neighbours).

TCR α

The T cell receptor (TCR), which is composed of TCR α and TCR β chains, is required for the recognition of antigens by T cells. Approximately 60% of TCR α KO mice spontaneously developed a Th2-mediated colitis (sharing some features with human UC) by 6 mo of age [10]. Mice with Balb/c and C3H/HeJ backgrounds were relatively resistant to this colitis as compared with C57BL/6. As for the majority of IBD models, TCR α KO mice failed to develop colitis in a germ-free facility [96,97]. In contrast to other IBD models, the development of colitis in TCR α KO mice was abolished when exposed to wide spectrum of microorganisms in conventional facilities [98,99]. In addition, IL-10-producing regulatory B

cells (termed Breg), which contribute to the suppression of colitis, were first identified in this model [100,101]. The existence of Breg in a subset of UC patients is supported by the exacerbation/induction of UC after B cell-depletion therapy [102–104].

TCR24 $\alpha\beta$

24 $\alpha\beta$ transgenic mice carry TCR from a CD1d-reactive NKT cell line with expression of TCRV α 3.2 and V β 9. When 24 $\alpha\beta$ Tg mice were crossed with CD1d Tg mice to activate the NKT cells, 70% of them developed colitis at 6 mo of age [105].

TGF β pathway (TGF- β 1, TGFRII, TAK1, SMAD4)

Transforming growth factor (TGF)- β 1 contributes to immune regulation and Treg differentiation. TGF β 1 KO mice developed necrotic inflammation in multiple organs and majority of them died before 3–4 weeks of age [106]. In contrast, CD4⁺ T cell-specific dominant negative TGF β receptor type II transgenic mouse survived and spontaneously developed inflammation at 3–4 mo of age, with increased expression of both Th1 and Th2 cytokines in multiple organs, including the colon, liver, stomach, duodenum, pancreas, and kidney [107]. A protective role of B cells was demonstrated in this model [108]. Unlike CD4⁺ T cell-specific suppression of TGF β signal, epithelial cell-specific suppression of TGF β signalling failed to induce spontaneous development of colitis [109].

Mice with a DC-specific deletion of TGF- β receptor type II developed multi-organ inflammation from 4 wk of age, including hepatitis, pancreatitis, gastritis, and colitis. Only 30–40% of mice survived at 12 wk of age. Interestingly, abnormal expansion of CD25⁺ Foxp3⁺ CD4⁺ T cells was seen in these mice [110].

TGF β activated kinase 1 (TAK1) participates in the activation of innate immune pathways. Severe epithelial damage in the small intestine was seen in epithelial cell-specific TAK1 KO mice by 3 days after birth [111]. In addition, inducible epithelial specific deletion of *Tak1* gene promptly led to intestinal epithelial damage within 3 d after deletion of TAK1.

Mothers against decapentaplegic homolog 4 (SMAD4) is a central mediator of TGF β signalling. T cell-specific SMAD4 KO mice and CD4⁺ T cell-specific SMAD4 KO mice both developed inflammation throughout the gastrointestinal tract (gastritis, ileitis, colitis) at 3 mo of age [112]. Rectal prolapse (25%) and development of cancer throughout the gastrointestinal tract was also observed. In contrast, epithelial cell-specific SMAD4 KO mice did not develop inflammation or cancer.

TLR5

TLRs play important roles in innate immunity through recognizing different microbial products. TLR5 serves as a receptor for bacterial flagellin that has been shown to represent a dominant pathogenic antigen in CD [113]. In contrast, a polymorphism in *tlr5* was proposed to be protective for CD [114]. Approximately 30% of TLR5 KO mice developed rectal prolapse and colitis restricted primarily to the cecum and proximal colon at 8–12 wk of age [115].

TNF

Neutralization of TNF- α activity has been widely used for the treatment of IBD, and CD-specific susceptibility variants have been identified within *TNF* in Japanese population [15]. The involvement of TNF- α in the pathogenesis of CD has been well supported by spontaneous development of ileitis in mice that exhibit an enhanced TNF- α activity due to deletion of adenosine-uracil multimers (AU-rich elements, ARE) responsible for the *Tnf* mRNA destabilization [116]. The inflammation in TNF(ARE) mice was localized primarily to the terminal ileum and occasionally to the proximal colon. This ileitis was mediated by CD8⁺ T cells, IL-12p40, TNF- α , and IFN- γ [117,118].

TNFSF14

TNF superfamily 14 (TNFSF14), which interacts with two receptors, herpes virus entry mediator and lymphotixin β receptor to control the response of T cells, is encoded by *TNFSF14*, another susceptibility gene for UC [12,13]. Mice with T cell-specific overexpression of human *TNFSF14* developed hepatitis and an ileitis characterized by villus atrophy [119].

TNFSF15

TNFSF15, which binds to a death domain receptor 3 (TNFRSF25), is encoded by *TNFSF15*, a gene associated with CD and UC [12,13]. Mice with T cell-specific overexpression of *Tnfsf15* spontaneously developed transmural inflammation in the small, but not large, intestine with 100% penetration after 6 weeks of age [120]. CD4⁺ T cells and IL-13 were primarily responsible for the development of this ileitis. In addition, mice with CD11c⁺ cell-specific overexpression of *tntsf15* developed ileitis, but the severity was milder as compared to T cell-specific TNFSF15 Tg mice.

TSC1

Tuberous sclerosis-1 (TSC1) is an upstream negative regulator of mammalian target of rapamycin (mTOR). Mice with CD4-specific deletion of *Tsc1* spontaneously developed inflammation characterized by large lymphoid aggregates in the colon and liver around 6 mo of age [121]. The absence of Tsc1 reduced the expression of Foxp3 and induced the expression of IL-17 in Treg cells.

WASP

Wiskott-Aldrich syndrome protein (WASP) is an organizer involved in the remodelling of actin cytoskeleton to induce cell movement, cell signalling, and cell division. All WASP KO mice developed a UC-like colitis spontaneously at 6 mo of age [122]. The colitis was mediated by IL-4-expressing CD4⁺ T cells [123] and by impaired development of functional Treg [124].

XBP1

X-box-binding protein (XBP1) is required for unfolded protein response induced by ER stress. An association of XBP1 variants with CD and UC has been identified [125]. Although XBP1 plays an important role in the terminal differentiation of B cells into plasma cells, mice with specific deletion of *Xbp1* in intestinal epithelial cells spontaneously developed ileitis with absence of Paneth cells that secrete defensins [125].

Yipf6

Through an environmentally sensitized genetic screen of approximately 6,000 mice that were treated with N-ethyl-N-nitrosourea to randomly induce genetic mutations, Yipf6 mutant mice were found to spontaneously develop ileitis and colitis at 16 mo of age [126]. Yipf6 is a Yip gene family member that regulates Rab-protein-mediated ER-to-Golgi membrane transport.

Uhrf1

Epigenetic regulation plays an important role in controlling the expression of many genes. Uhrf1 (ubiquitin-like, with pleckstrin-homology and RING-finger domains 1), which is encoded by *Np95* in mice and *ICBP90* in humans, serves as an epigenetic regulator. Mice with CD4-specific deletion of *Np95* spontaneously developed colitis by 10 wk of age, and almost all mice succumbed to death before 24 wk of age [127]. The colitis was characterized by a defect in proliferation of colonic Treg.

Conclusion

Spontaneous development of intestinal inflammation in more than 74 kinds of genetically engineered mouse strains, as well as presence of more than 160 IBD susceptibility genes in humans certainly indicate a much more complex mechanism of IBD than previously predicted. This raises a possibility that IBD includes more diverse disease conditions than the currently recognised two forms, CD and UC. In addition, data from these mouse models highlight the critical involvement of dysregulated immune responses and impaired epithelial defence in the pathogenesis of IBD (Figure 3).

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Figure 1. Gene targeting strategies

Genetically engineered mouse models of IBD are here classified into 9 groups based on the strategy used for the gene targeting, including conventional transgenic (TG), conventional knockout (KO), inducible KO (iKO), cell-specific TG, cell-specific KO, dominant negative (Dn); knock in (KI); Mutagen-induced; and innate models. For cell-specific genetic engineering, epithelial cells (epi), T cells (T), B cells (B), CD4⁺ T cells (CD4), dendritic cells (DC), macrophages (M ϕ), Treg cells (Foxp3) are targeted.

Figure 2. Classification of IBD models depending on the organ affected by inflammation
 74 genetically engineered mouse models of IBD are further classified into four groups; colitis models, ileitis models, enterocolitis models developing colitis and ileitis, and systemic model developing inflammation in multiple organs including intestine. Mice carrying human IBD susceptibility genes are indicated by *Italic Bold*.

Figure 3. Major pathways involved in the pathogenesis of IBD

Genetically engineered mouse IBD models highlight immune response and epithelial defence for the pathogenesis of IBD. The pathways, which are primarily responsible for the development of intestinal inflammation in genetically engineered models, are indicated.