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Pharmacologic efficacy in inflammatory bowel disease models

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TABLE OF CONTENTS

1. ABSTRACT

The utility of inflammatory bowel disease (IBD) models in evaluating pharmacologic efficacy of novel drug candidates is reviewed. IBD models are generally classified into six groups based on the etiopathogenesis: chemically- and hapten-induced, spontaneously developed, T-cells, transgenic and immunoregulatory knockout models. The chemically- and hapten-induced models are the most widely utilized for evaluating pharmacologic efficacy of novel drug candidates because they are technically simple and rapid to induce gut pathology. In contrast, the T-cells adoptive transfer model is technically more complex to execute with longer study duration, resulting in the rare utility of this model in pharmacologic efficacy studies. Spontaneously developed, transgenic and immunoregulatory knockout IBD models gradually develop spontaneous colitis or ileitis as they age. In this critical review, the pathological and immunological characteristics of various IBD animal models, and the pharmacologic efficacy of current therapeutic agents and drug candidates for IBD in these animal models are compared. Moreover, perspectives on experimental conditions, and applicability to evaluation of prophylactic and therapeutic pharmacologic efficacy of drug candidates in drug discovery and development are discussed.

2. INTRODUCTION

Inflammatory Bowel Disease (IBD) is a complex, chronic inflammatory disease characterized by relapsing and remitting inflammatory conditions of gastrointestinal tract (GI). IBD is composed of two major forms, Crohn's disease (CD) and ulcerative colitis (UC), which are different from each other in pathogenesis and histopathological, immunological, and genetic characteristics. For example, CD can occur anywhere along the GI and is characterized by transmural (affecting all layers of the gut wall) inflammation and granuloma formation. While UC, the disease is limited to the colorectum region of the GI and inflammation is superficial (mucosa/submucosa). In addition, CD is driven by T helper 1 (Th1) cytokines, interleukin (IL)-12, tumor necrosis factor-alpha (TNF-alpha) and interferon-gamma (IFN-gamma), and is characterized by dense mixed cellular infiltration of lymphocytes and macrophages with granuloma formation in up to 60% of patients, ulceration, and fibrosis. UC, on the other hand, is driven by Th2 cytokines, IL-5, IL-13 and is characterized by loss of goblet cells, and infiltration of lymphocytes and granulocytes without granuloma formation and accompanied by ulceration and crypt abscesses. Current standard of care for IBD is principally administration of aminosalicylates,

corticosteroids, immunosuppressive agents, antibiotics, TNF-alpha inhibitors and/or alpha4-integrin inhibitors. Moreover, the role of prebiotics and/or probiotics is also getting noted as the treatment of IBD. However, there is still unmet medical need for IBD treatment, because it is difficult to bring maintenance of remission in CD and UC to each patient. Therefore, the current IBD therapeutic modalities strongly encourage pharmaceutical companies to develop novel IBD medicines.

In order to develop novel IBD medicines, it is essential that such drug candidates are appropriately evaluated in IBD models. IBD models are generally classified into six groups based on the etiopathogenesis: chemically- and hapten-induced, spontaneously developed, T-cells, transgenic and immunoregulatory knockout models. In the past decade, emergence of biologic drugs of various immunologic molecular targets provided lots of opportunities to investigate pathogenesis of IBD in animal models. This would certainly help accelerate development of novel IBD drugs through evaluation of drug candidates by using appropriate IBD models. Here, we critically review histopathological and immunological characteristics of major IBD animal models, and summarize prophylactic and therapeutic pharmacologic efficacy of current IBD medicines and drug candidates for IBD treatment in these models.

3. CHEMICALLY- AND HAPTEN-INDUCED MODELS

3.1. Dextran sulfate sodium (DSS) model

DSS-induced colitis in mice was originally reported by Okayasu *et al.* in 1990 (1). This is an experimental UC model with many symptoms similar to those observed in human UC, such as diarrhea, bloody feces, body weight loss, mucosal ulceration and shortening of the large intestine. The DSS model is created by feeding DSS via drinking water, and is classified into the acute and chronic colitis models (2,3). The acute colitis occurs during the administration of DSS. Histopathological feature of the acute colitis is characterized by inflammatory cell infiltration, including polymorphonuclear leukocytes and multiple erosive lesions only in the large intestine. Occasionally, crypt abscess and regenerated epithelium are seen in the colonic mucosa (1). Considering the fact that the acute DSS colitis accompanied by an increase production of macrophage-derived cytokines in the lesions is observed in severe combined immunodeficient (SCID) mice given DSS, the trigger of acute colitis is suggested to be innate immune mechanisms (4). However, lymphocyte also plays an essential role in the acute colitis in the light of a marked upregulation in the pro-inflammatory Th1 cytokine (IL-12, IFN-gamma, IL-1, TNF-alpha) mRNA transcripts in colonic tissue (5). The chronic colitis can be induced by multiple cycles of DSS administration at intervals of normal water feeding. Histopathologically, severe inflammatory cell infiltrations including plasma cells, lymphocytes and swollen macrophages were seen in the colonic mucosa and submucosa. Moreover, scattered multiple erosions are also evident because of inflammatory changes, and formation of lymph follicles is frequently seen. These findings are particularly prominent on the left side of the large intestine and the transverse colon. Regenerative and dysplastic changes of the mucosal epithelia are also frequently observed (1). The chronic colitis is considered to be caused by lymphocytes that are activated by the cytokines secreted from the activated macrophages.

Intestinal mucosal leukocyte endothelial cell interaction is considered another crucial process in DSS colitis (6). In acute colitis, leukocyte migration to the colon observed 2 days after the beginning of DSS administration persists until 7 days after the end of DSS administration. On the other hand, in chronic colitis which is established after four cycles of DSS for 7 days followed by normal drinking water for 10 days each time, leukocyte migration persists until 4 weeks after the last cycle of DSS at the same level as in acute colitis. Combined with the fact that one cycle of DSS administration followed by drinking water results in extensive and relatively slow regeneration of the colonic epithelium after DSS injury (7), these results suggest that chronicity of DSS colitis is would be affected by timing and duration of DSS and water administration.

The immunological analyses of the expression pattern of serum cytokines in the DSS-induced colitis model shows that TNF-alpha, IL-6, IL-17 and chemokine (C-X-C motif) ligand 1 (CXCL1) increase in the acute DSS colitis, and on the other hand, Th2-mediated inflammatory response such as IL-4 and IL-10 becomes dominant being accompanied by concomitant decrease in TNF-alpha, IL-6, IL-17 and CXCL1 in the chronic DSS colitis (8). Increases in IL-1alpha and IL-1beta mRNA levels in the large intestine is also observed at 5 and 11 days in the acute phase of this model, and these cytokines are involved in the development of colitis (9). On the other hand, expression of IL-25 in the colon is found to be decreased in severe acute colitis in this model and exogenous addition of recombinant IL-25 can ameliorate the colitis, suggesting that IL-25 plays a protective role in the development of the acute colitis (10,11). In addition to these cytokines, a recent study has demonstrated that complement plays a role in the development of colitis in the acute DSS colitis model (12). As described above, the immunological response is quite distinct between the acute and chronic DSS colitis models. The acute DSS colitis shows the immunological response based on innate immunity. To the contrary, the chronic DSS colitis model is considered clearly to have Th2-dominant cytokine profiles.

Susceptibility to DSS-induced colitis is affected by inbred mouse strains, and C3H/HeJ and C3H/HeJBir are highly DSS susceptible in both cecum and colon (13). On the other hand, BALB/c and CBA/J treated with DSS have severe lesions predominantly in the transverse, descending and sigmoid colons, and rectum (1). C57BL/6J is relatively resistant in the cecum, but susceptible in the colon (13). Such a difference in susceptibility to DSS-induced colitis among inbred mouse strains is controlled by multiple genes (14). As is the case with susceptibility to DSS-induced colitis, difference in inbred mouse strains is also associated with chronicity of DSS colitis (15). Acute DSS colitis progresses to chronicity in C57BL/6 but not in BALB/c. In

addition to inbred mouse strains, the molecular weight of DSS is one of important factors that exert an influence on severity and/or regional localization of DSS-induced colitis (16). In mice given 5 kDa DSS, colitis is observed predominantly in the cecum and upper colon. In contrast, in mice treated with 40 kDa DSS, colitis is more predominant in the lower colon. The colitis induced by 40 kDa DSS is more severe than that induced by 5 kDa DSS. No colitis is observed in the mice given 500 kDa DSS.

DSS-induced colitis is one of the most commonly used IBD models for efficacy evaluation of drug candidates in a pharmaceutical setting. This is because colitis in this model can be easily induced by just administrating DSS via drinking water and the model offers a high throughput advantage in pharmacologic studies (Table 1). Endpoints employed for efficacy evaluation in this colitis model are body weight, colon length, disease activity index composed from body weight loss, stool consistency and occult-gross bleeding, and/or histopathological examination of the intestine (Figure 1). Consequently, most of medicines currently prescribed for IBD patients have been evaluated in this UC model, therefore; 5-amino salicylic acid (5-ASA) is often used as a positive control in pharmacologic evaluation efficacy studies (Table 2). Sulphasalazine (oral administration, 30 or 100 mg/kg/day in drinking water, concomitant with DSS treatment) and olsalazine (oral administration, 10, 30 or 100 mg/kg/day in drinking water, concomitant with DSS treatment) which are both a 5-ASA derivative are shown to be efficacious in chronic DSS colitis model through the release of 5-ASA as an active moiety (17). Steroids like dexamethasone have been shown to reduce colonic inflammation in chronic DSS-induced colitis (intraperitoneal administration, 0.25 mg/kg, days 1-5 after completion of the 4 cycles of DSS treatment), whereas they were not efficacious in the acute model (intraperitoneal administration, 3 mg/kg, days 3-7 after the initiation of DSS treatment; subcutaneous administration, 0.06 mg/day, five days before and during DSS treatment for 7 days) (18,19). Cyclosporine, an immunosuppressive agent used to treat IBD, ameliorates body weight loss, mucosal destruction and epithelial apoptosis in DSS-induced colitis, when administered intraperitoneally at the dose of 20 mg/kg/day 1 day before DSS treatment followed by the intraperitoneal injection of 10 mg/mL of anti-transforming growth factor-beta (TGF-beta) monoclonal antibody (mAb) (20). In addition, the other type of major immunosuppressants which has the activity to inhibit mammalian target of rapamycin (mTOR) can prevent the acute colitis of this model (21).

Neutralization of cytokines brings complex outcomes in treating colitis in the DSS model. In acute colitis, blocking of IL-1 and TNF-alpha by intraperitoneal injection of 100 mcg of anti-IL-1 receptor type I (IL-1RI) mAb from day 0 to day 7 and 100 mcg of anti-TNF-alpha mAb from days 3-7 after the initiation of DSS treatment, respectively, leads to aggravation of colitis. By contrast, treatment of mice that have chronic colitis with intraperitoneal dosing of 50 mcg of anti-IL-1beta mAb on days 1, 3 and 5 or 50 mcg of soluble IL-1RI (sIL-1RI) on days 1-5 failed to show significant efficacy, whereas 10 or 100 mcg of anti-TNF-alpha mAb 5 days after completion of the 4 cycles of DSS treatment significantly reduced the colitis (18). However, another group shows that intraperitoneal dosing of 25 mcg of anti-TNF-alpha mAb at the beginning of DSS treatment can ameliorate acute colitis in mice treated by 5-day DSS feeding followed by 12-day water feeding, and this therapeutic outcome can be enhanced when combined with oral dosing of a TNF-alpha release inhibitor of pentoxifylline (100 mg/kg/day) (22). These controversial effects of anti-TNF-alpha mAb would be explained as follows. In the acute colitis, little leukocyte migration is observed and expansion of inflammation is insufficient. Under such a condition, the concentration of TNF-alpha would be still within or close to its physiological level in which TNF-alpha stimulates proliferation of intestinal epithelial cells (23), suggesting that neutralization of TNF-alpha leads to aggravation of colitis. On the other hand, in the chronic colitis and the acute colitis in mice treated by 5-day DSS feeding followed by 12-day water feeding, immunopathological analyses of neutralization of TNF-alpha is carried out at the timing when inflammation expands at colitis sites. In this situation, lymphocytes are activated by the cytokines including TNF-alpha, the concentration of which is evidently exceeds the physiological level, demonstrating that neutralization of TNF-alpha leads to amelioration of colitis. These would be the major reasons for the controversial effects of anti-TNF-alpha mAb between acute and chronic DSS colitis. Efficacy of neutralization of other cytokines is evaluated in both acute and chronic colitis models. Anti-IL-12p40 treatment (100 mg/mouse, intraperitoneal) of acute colitis attenuates local cytokine levels and improves clinical symptoms, although no major histological improvement is observed (24). To the contrary, anti-IL-12p40 treatment (100 mg/mouse, intraperitoneal) of chronic colitis is partly effective with a significant reduction in plasma and/or colonic levels of several cytokines and a small improvement in inflammatory score and histology. Moreover, neutralization of IL-17 by intraperitoneal administration of 100 mcg of anti-IL-17 mAb at the beginning of the study and every 48 hours during the study aggravates acute colitis in the DSS model accompanied by the increase in mucosal mRNA of TNF-alpha, IFN-gamma, IL-6, regulated upon activation, normal T cell expressed and secreted (RANTES), and IP-10 (25). Neutralization of IL-18 by intraperitoneal dosing of 400 mcL of anti-IL-18 antiserum at days 0, 4 and 8 after the beginning of DSS treatment prevents development of DSS-induced acute colitis by reducing severity of inflammation and IFN-gamma and TNF-alpha production (26).

Leukocyte trafficking is one of therapeutic targets in IBD. Actually an anti-alpha4 integrin mAb, natalizumab, which inhibits lymphocyte transendothelial migration by blocking the binding of alpha4 integrin to its ligand, has been approved for the treatment of IBD in the US. Among the target molecules in leukocyte trafficking, mucosal addressin cell adhesion molecule-1 (MAdCAM-1) which is a ligand for alpha4beta7 integrin is expected to be a promising target for IBD treatment in the light of its exclusive expression in gastrointestinal tracts. So far the effects of blocking of MAdCAM have been evaluated in

Table 1. Comparative immunopathological aspects of IBD models

IBD model	Human IBD model	Clinical signs	Immunity type	Major cytokines	Histopathology
Acute DSS	UC	Diarrhea, gross rectal bleeding, body weight loss, bloody faces	Innate Th1	Serum: TNF-alpha, IL-6, IL-17 and CXCL1 (I)[1] Large intestine: IL-1alpha and IL-1beta (I), IL-25 (D)[2]	Colon and cecum shortening, inflammatory cell infiltration, multiple erosive lesions, crypt abscess and regenerated epithelium in colonic mucosa, ulceration
Chronic DSS	UC	Diarrhea, occult blood in the feces, body weight loss	Th2	Serum: IL-4 and IL-10 (I), TNF-alpha, IL-6, IL-17 and KC (D)	Colon shortening, inflammatory cell infiltration including plasma cells, lymphocytes and macrophages, scattered multiple erosions, formation of lymphoid follicles, regenerative and dysplastic changes of the mucosal epithelia
Acute TNBS	CD	Diarrhea, body weight loss, rectal prolapse	Th1-Th17	Serum: IL-12, IL-17, IFN-gamma and MIP-1alpha (I) Large intestine: TNF-alpha (I)	Inflammation in the colon, lymphocytic infiltrates, thickening of the colon wall, ulceration, loss of goblet cells
Chronic TNBS	CD	Diarrhea, body weight loss after initial dosing of TNBS	Th1-Th17 (DTH)[3]	Serum: IL-12 and IL-17 (I) Large intestine: IL-18 (I) NF-kappaB activities in colon (I)	Intestinal fibrosis, inflammation of the colonic lamina propria, thickening of the colon wall, collagen in the subepithelial and in deeper layers of the colonic lamina propria
Oxazolone	UC	Diarrhea (free of diarrhea by 10-12 days after oxazolone administration), body weight loss (increased slowly 4-7days after oxazolone administration)	Th2	Lamina propria cells in colon: IL-4, IL-5, IL-13 and TGF-beta (I) Hepatic mononuclear cell: IL-13 (I) Mesenteric lymph node cell: IL-13 (I) Splenocyte: IL-13 (I)	Hemorrhagic colitis in the distal 50% of the bowel, epithelial cell loss, patchy ulceration, pronounced depletion of mucin producing-goblet cell, reduction of the density of the tubular glands, lymphocytes and granulocytes infiltration into the bowel lumen, edema of submucosal layer
SAMP1/Yit	CD	Skin lesions in the dorsal part of the skin and eyelid	Th1	Mesenteric lymph node: TNF-alpha, IFN-gamma, IL-5 (I)	Thickening of the intestinal wall of the terminal ileum, discontinuous and transmural inflammation at the distal part of the jejunum, ileum and cecum, infiltration of macrophages, neutrophils and lymphocytes into the lamina propria, transluminal neutrophils in the necrotic crypts
$CD4^+CD45RB^{high}CD25^-$ -transferred model	CD	Soft stool, body weight loss	Th1	Colon: gene expression of CCR1, CCR2, CCR5, CXC chemokine receptor 3, their ligands, TNF-alpha, IFN-gamma and IL-6 (I)	Inflammation from the cecum to the rectum with infiltration of macrophages accompanied by moderate numbers of activated $CD4^+$ lymphocytes, mucin depletion and epithelial hyperplasia resulting in glandular elongation and mucosal thickening, $CD40^+$ and $CD40L^+$ cells in inflamed mucosa (I)
IL-7 TG	UC	Chronic: Diarrhea, body weight loss, rectal prolapse, remittent intestinal bleeding	Chronic: Th1	Acute: IL-7 (I) Chronic: Colonic mucosa: IFN-gamma and IL-2 (I)	Acute: Infiltration of neutrophils and lymphocytes ($CD4^+$ and T cell receptor gamma/delta) Chronic: Erosion and neutrophil infiltration in the anal ring, inflammatory cell infiltration and goblet cell depletion throughout entire colon (most prominent in the rectum), crypt abscess, paneth cell metaplasia, infiltration of eosinophils
IL-10 KO	CD	Body weight loss, anemia	Th1-Th17	IL-23 is essential for spontaneous induction of colitis followed by production of IL-17 and IL-6	Inflammation in duodenum, proximal jejunum and proximal colon, variable pattern of mucosal inflammation associated with either hyperregenerative or degenerative lesions of the intestinal epithelia, excessive regenerative hyperplasia of the mucosa leading to a marked thickening of the intestinal wall. Formation of abnormal crypt and villus structures
IL-2 KO	UC	Diarrhea, rectal prolapse	Th1	Colon: mRNA of IFN-gamma, IL-1 and TNF-alpha (I), mRNA of IL-4 and IL-10 (D), IL-1beta (I), IL-10 (D)	Lesions in the mucosa and submucosal tissue of the large bowel with pronounced thickening of the bowel wall, prominent ulcerations and frequent crypt abscesses, loss of goblet cells, infiltration of granulocytes, lymphocytes and plasma cells at mucosa (100-fold T cell counts compared with control mice), elevation of IgA- and IgG1-secreting cells, detection of anti-colon antibodies, a large numbers of $CD4^+$, $CD8^+$, TCR-alpha $beta^+$ and TCR-gamma $delta^+$ T cells, and macrophage, dendritic cells and $MAdCAM\text{-}1^+$ endothelial cells are observed in the caecum and colon
TCRalpha KO	UC	Diarrhea, anorectal prolapse	Th2	Mesenteric lymph node: IL-2 (D), IL-4 and IFN-gamma (I) Colonic mucosa: mRNA of IL-1alpha and IL-1beta (I)	Thickening of the intestinal wall, colitis, loss of goblet cells, elongation of crypts, crypt microabscesses and inflammatory cell infiltrate in the lamina propria, the presence of autoantibodies
dnKO	UC	Fail to thrive by 3-4 weeks, rapid body weight loss	Th1	Serum: TNF-alpha, IFN-gamma and IL-6 (I) Colon: TNF-alpha and IFN-gamma (I)	Disruption in the gross morphology of the entire cecum, descending colon and rectum; extensive epithelial hyperplasia, diminished goblet cells and crypt number, erosion of surface epithelial cells, numerous crypt abscesses, and mixed leukocyte infiltrates in both the mucosa and submucosa of cecum, descending colon and rectum; T cells, immature myeloid / monocytes, neutrophiles, NK / NKT, and B cells in the cecum, descending colon, and rectal regions (I)

[1]I: increase, [2]D: decrease, [3]DTH: delayed-type hypersensitivity

Table 2. Pharmacologic efficacy of therapeutic agents in various IBD models

IBD model	Aspect of evaluation	Medicine or drug candidate	
		Efficacious	**Inefficacious**
DSS	Prophylactic effect in acute model	calcineurin inhibitor (cyclosporine: 20 mg/kg/day, ip[1], ref[2] 20) mTOR inhibitor (P2281: 15 mg/kg/day, ip, ref 21) TNF-alpha inhibitor (25 mg, ip, ref 22) anti-IL-18 antiserum (400 mcL, ip, ref 26) anti-IL-12 mAb (100 mg/mouse, ip, ref 24) NF-kappaB inhibitor (tetrandrine: 40 mg/kg/day, po[3], ref 30; decoy oligonucleotide: 25 nmol, ic[4], ref 31) PDE4 inhibitor (rolipram: 5 mg/kg/day, bid[5], ip; mesopram: 10 mg/kg/day, qd[6], po or ip, ref 33-35) PPARgamma agonist (rosiglitazone: 30 mg/kg/day, qd, po; pioglitazone: 30 mg/kg/day, qd, po; troglitazone: 200 mg/kg/day, qd, po, ref 36) HMG-CoA reductase inhibitor (pravastatin: 1 mg/kg, qd, ip, ref 37)	steroid (dexamethasone: 3 mg/kg, ip, ref 10; 0.06 mg/day, sc[7], ref 19) anti-IL-1RI mAb (100 mg, ip, ref 18) anti-TNF-alpha mAb (100 mg, ip, ref 18) anti-IL-17 mAb (100 mg, ip, ref 25) anti-MAdCAM mAb (1 mg/kg, ip, ref 26; 200 mg/mouse, ip, ref 27) p38 MAPK inhibitor (FR167653: 30 mg/kg/day, ip, ref 32)
	Therapeutic effect in chronic model	5-ASA derivative (sulphasalazine: po, 30 or 100 mg/kg/day in drinking water; olsalazine: po, 10, 30 or 100 mg/kg/day in drinking water, ref 17) steroid (dexamethasone: 0.25 mg/kg, ip, ref 18) anti-TNF-alpha mAb (10 or 100 mcg, ip, ref 18) anti-IL-12 mAb (100 mcg/mouse, ip, ref 24) anti-MAdCAM mAb (40 mcg/mouse, ip, ref 28) PDE4 inhibitor (rolipram: 5 mg/kg/day, bid, ip; mesopram: 10 mg/kg/day, qd, po or ip, ref 34,35)	anti IL-1beta mAb (50 mcg, ip, ref 18) sIL-1RI (50 mcg, ip, ref 18)
TNBS	Prophylactic effect in acute model	anti-TNF-alpha mAb (100 mcg, ip, ref 45) anti-IL-12 mAb (1 mg, ip, ref 38) anti-IL-12/23p40 mAb (25 mg/kg, ip, ref 39) IL-18 inhibitor (rhIL-18BPa: 8 mg/kg, ip, ref 48) anti-IL-16 mAb (1 mg, ip, ref 49) MAdCAM-1 antisense oligonucleotide (1.5 mg/kg, sc, ref 51) CB2 agonist (JWH133: 20 mg/kg, qd or bid, ip; AM1241: 10 or 20 mg/kg, bid, ip, ref 52) steroid derivative (NCX-1015: 0.5 or 5 mg, sc, ref 53) curcumin (0.5, 2.0 or 5.0%, diet, ref 54) Na-H exchanger-1 inhibitor (amiloride: 3 mg/kg, po, ref 55) activin inhibitor (follistatin: 5 mcg, ip, ref 56) NF-kappaB inhibitor (decoy nucleotide: 75 mcg, ip or ir[8], ref 57) STAT3 inhibitor (antisense oligonucleotide: 15 nmol, ic, ref 58) calcitriol (0.2 mcg/kg, ip, ref 59)	
	Therapeutic effect in chronic model	anti-IL-12 mAb (1 mg, ip, ref 38) anti-IL-16 mAb (1 mg, ip, ref 49) steroid derivative (NCX-1015: 0.5, 5 mg, sc, ref 53) activin inhibitor (follistatin: 5 mcg, ip, ref 56) NF-kappaB inhibitor (decoy nucleotide: 75 mcg, ip or ir, ref 57) calcitriol (0.2 mcg/kg, ip, ref 59)	
Oxazolone	Prophylactic effect in acute model	5-ASA (25 mg/kg, ir, ref 63) steroid (prednisolone: 1 mg/kg, ir, ref 63) anti-IL-4 mAb (3 mg/mouse, ip, ref 60) $S1P_1$ agonist (FTY720: 1 or 3 mg/kg/day, ip, ref 64) IL-25 (10 mcg, ip, ref 65) sterol (guggulsterone: 30 mg/kg, ip, ref 66)	HMG-CoA reductase inhibitor (simvastatin: up to 40 mg/kg, qd, ip, ref 67) anti-TGF-beta (1 mg/mouse, ip, ref 60) anti-IL-12 (2 mg/mouse, ip, ref 60)
	Therapeutic effect in chronic model	$S1P_1$ agonist (FTY720: 1 or 3 mg/kg/day, ip, ref 64) IL-25 (10 mcg, ip, ref 65) sterol (guggulsterone: 30 mg/kg, ip, ref 66)	
SAMP1/Yit	Prophylactic effect	antibiotics (combination of 50 mg/kg/day of ciprofloxacin and 100 mg/kg/day of metronidazole in drinking water, ref 73) anti-MAdCAM-1 mAb (2 mg/kg, ip, ref 75) anti-PSGL mAb (2 mg/kg, ip, ref 76)	
	Therapeutic effect	antibiotics (combination of 50 mg/kg/day of ciprofloxacin and 100 mg/kg/day of metronidazole in drinking water, ref 73) anti-TNF-alpha mAb (0.1 or 1.0 mg, ip, ref 74) anti-MAdCAM-1 mAb (2 mg/kg, ip, ref 75) PDE-3 inhibitor (cilostazole: 0.02% in diet, ref 77)	
$CD4^+$ $CD45RB^{high}CD25^-$-transferred model	Prophylactic effect	anti-IFN-gamma mAb (2 mg at day 1 and day 14 or 2 mg at day 1 and 1 mg per week thereafter, up to 8 weeks, ip, ref 82) anti-TNF-alpha mAb (2 mg at day 1 and 1 mg per week thereafter, up to 8 weeks, ip, ref 82; 1 mg, ip, ref 93) anti-IL-12 mAb (1 mg/mouse, ip, ref 94) anti-beta$_7$ integrin mAb (100 mcg, ip, ref 95) anti-MAdCAM-1 mAb (100 mcg, ip, ref 95) anti-CD40L mAb (250 mcg, ip, ref 84) anti-OX40L mAb (250 mcg, ip, ref 96) anti-Fas ligand mAb (MFL1: 250 mcg, ip, ref 97) anti-ICOS mAb (250 mcg, ip, ref 98) anti-NKG2D mAb (250 mcg, ip, ref 99) anti-TLR4 mAb (20 mg/kg, ip, ref 100) CTLA4-Ig (100 mg, ip, ref 101)	cyclosporine A (5 mg/kg, ip, ref 101)

	Therapeutic effect	anti-TNF-alpha mAb (1 mg, ip, ref 93) anti-IL-12 mAb (1 mg/mouse, ip, ref 94) anti-OX40L mAb (250 mcg, ip, ref 96) Fas ligand inhibitor (MFL1: 250 mcg, ip, ref 97) anti-ICOS mAb (250 mcg, ip, ref 98)	
IL-10 KO	Prophylactic effect	anti-IL-12 mAb (0.5 mg, 1 mg the following week, 2 mg/week for 6 week, ip, ref 110) anti-TNF-alpha mAb (500 mcg, ip, ref 112) CXCR4 antagonist (TF14016: 100 mcg, ip, ref 119)	
	Therapeutic effect	anti-IL-12 mAb (2 mg/week, ip, ref 110) anti-TNF-alpha mAb (10 mcg, ip, ref 112) IkappaB kinase inhibitor (NEMO binding domain peptide: 10 mg/kg, ip, ref 115) anti-IP-10 mAb (200 mcL, ip, ref 116) calcineurin inhibitor (tacrolimus: 10 mg/kg, ir, ref 117) $S1P_1$ agonist (FTY720: 0.3 mg/kg/day, po, ref 89; KRP-203: 0.3 mg/kg/day, po, ref 120)	anti-IFN-gamma mAb (2 mg/week, ip, ref 110)
IL-2 KO	Prophylactic effect	cyclosporine A (5 mg/kg, ip, ref 122) anti-$alpha^E beta_7$ mAb (0.5 mg, ip, ref 131)	
	Therapeutic effect	anti-$alpha^E beta_7$ mAb (0.5 mg, ip, ref 131) OX40-IgG fusion protein (100 mcg, ip, ref 132)	
TCRalpha KO		steroid (dexamethasone, 3 mg/kg, po, ref 135)	
dnKO		anti-IFN-gamma mAb (1 mg, ip, ref 138) anti-IFN-gamma mAb + anti-TNF-alpha mAb (1 mg each, ip, ref 138) antibiotics (0.66 mg/mL of ciprofloxacin and 2.5 mg/mL of mertonidazole in drinking water, ref 138)	anti-TNF-alpha mAb (1 mg, ip, ref 138)

[1]ip: intraperitoneally, [2]ref: reference, [3]po: orally, [4]ic: intracolonically, [5]bid: twice a day, [6]qd: once a day, [7]sc: subcutaneous, [8]ir: intrarectally

acute and chronic colitis models. Intraperitoneal administration of anti-MAdCAM-1 mAb (1 mg/kg, daily) fails to attenuate acute colitis in terms of disease activity index, colon length, ratio of colon weight to length and myeloperoxidase activity (27). Insufficient efficacy of anti-MAdCAM-1 mAb in acute colitis is also evidenced in the experimental system in which anti-MAdCAM-1 mAb (intraperitoneal, 200 mg/mouse, daily) is administered in the former half of 14-day DSS treatment period (28). However, when administered at the same dose in the latter half of 14-day DSS treatment period because the expression of MAdCAM-1 is significantly increased on day 7, anti-MAdCAM-1 mAb treatment provides beneficial effects. To the contrary, in chronic colitis established after four cycles of DSS application, anti-MAdCAM-1 treatment (intraperitoneal, 40 mcg/mouse) leads to a marked reduction (more than 60%) of leukocyte sticking and extravasation *in vivo*, compared to the controls (29).

Molecules in intracellular signal transduction pathways induced by cytokines binding to their receptors such as mitogen-activated protein kinase (MAPK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappaB) are evaluated in DSS model as a target for IBD treatment. NF-kappaB blocking by tetrandrine (oral, 40 mg/kg/day, the final day of DSS treatment and the following 7 days) or decoy oligonucleotide (intracolonical, 25 nmol, the final day of DSS treatment and the following 2 days) is evidently beneficial in recovering from acute colitis in this model (30,31). To the contrary, a p38 MAPK inhibitor, FR167653, aggravates DSS-induced colitis by intraperitoneal administration at a dose of 30 mg/kg/day once a day from the initial day of DSS administration, although it reduced mucosal IL-1beta and TNF-alpha production, suggesting a role of p38 MAPK-mediated proinflammatory cytokine induction in host defense mechanisms (32).

Phosphodiesterase (PDE)-4 promoted hydrolysis of cAMP which is closely associated with intracellular cytokine synthesis, suggesting that PDE4 inhibitors show anti-inflammatory effects. PDE4 inhibitors, like rolipram (intraperitoneal, 5 mg/kg/day, twice a day) and mesopram (oral or intraperitoneal, 10 mg/kg/day, once a day) ameliorated DSS colitis in prophylactic (rolipram: 11 days concomitant with DSS treatment; mesopram: 10 days concomitant with DSS treatment) and/or therapeutic (rolipram: for 8 days after completion of DSS treatment, mesopram: for 7 days after completion of DSS treatment) systems for efficacy evaluation (33-35).

Other compounds such as peroxisome proferator-activated receptor gamma (PPARgamma) agonists and 3-hydroxy-3methylglutaryl-CoA (HMG-CoA) reductase inhibitors were demonstrated to be efficacious in acute DSS colitis (36,37). Emerging role for Janus kinase (JAK) inhibitors in treating UC is currently being evaluated in the DSS UC model. In fact, a JAK inhibitor, CP-690,550, has demonstrated significant and remarkable efficacy in patients with UC (http://clinicaltrials.gov/show/NCT00787202).

3.2. Trinitrobenzene-sulfonic acid (TNBS) model

TNBS model was originally reported by Neurath *et al.* in 1995, which is an experimental colitis induced in mice by rectal instillation of TNBS (38). TNBS reacts as a hapten with autologous colonic proteins through its trinitrophenyl moiety and induces IL-12-mediated Th1-type colitis. Endpoints employed for efficacy evaluation in this colitis model are body weight, colon length, and/or histopathological examination of the intestine (Figure 2). The colitis mimics human CD at the immunologic and histopathologic levels (39). Similar to the DSS-induced colitis described above, the TNBS colitis is also easily induced and highly reproducible, and therefore widely utilized to evaluate the efficacy of drug candidates as well as to study the immunological mechanisms that play a role on the pathogenesis of IBD. There are two types of TNBS model, namely, acute and chronic models. Acute colitis is

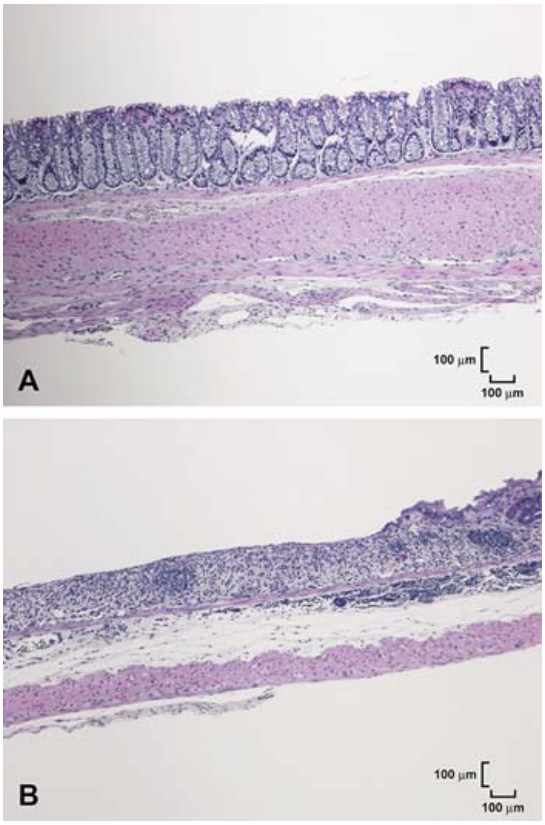

Figure 1. Histopathology of the colon from normal (A) and chemically-induced (DSS-induced) colitis model in BALB/c female mice (B). Note diffuse goblet cells depletion, mixed cells mucosal inflammation, mucosal erosion and submucosal edema.

induced by single administration of TNBS, while chronic colitis is developed by instillation of TNBS once a week for multiple weeks (8).

The expression pattern of cytokines, chemokines and their receptors in this model has been investigated (8) (Table 1). Regarding the serum cytokine profiles, acute colitis of this model displayed a cytotoxic and chemotactic profile with significantly elevated levels of IL-12, IL-17, IFN-gamma and macrophage inflammatory protein (MIP)-1alpha. This profile is consistent with an acute inflammatory response in that it is characterized by a macrophage-derived cytokine profile, strong chemotactic pattern, and a polarized Th1-Th17 panel, and also supported by the evidence that IL-17 receptor signaling plays a critical role in the development of this acute colitis (40). As the colitis becomes chronic in this model, Th1-Th17 response become notable, namely, elevation of IL-12 and IL-17 level is observed. However, there is one report showing that IFN-gamma is dispensable for the development of this colitis (41). In this model, macrophage-derived IL-18 is shown to have a prominent role in the establishment of colitis (42). In addition, TNF-alpha mRNA levels were highly increased in the colonic tissue and NF-kappaB activities were enhanced (43). Moreover, it is demonstrated that the TNF/TNFR-1 signaling system mediates mucosal damage through the enhancement of NF-kappaB activity, and that continuous infiltration of TNF-producing cells, probably a key for pathogenesis of colitis, may be closely associated with defective apoptosis of lamina propria mononuclear cells, which is possibly independent of the TNF/TNFR signaling system in this model.

Concerning the role of regulatory T cells in TNBS-induced colitis, TGF-beta production is a primary mechanism for counter-regulating Th1-type mucosal inflammation, and IL-10 is essential as a secondary factor that facilitates TGF-beta production (44).

As described above, TNBS colitis is an IL-12 driven Th1-mediated response and has elevation of TNF mRNA in colon, suggesting that IL-12 and TNF blocking is efficacious for the treatment of this colitis (Table 2). Treatment of the acute TNBS model with intraperitoneal dosing of an anti-TNF mAb (100 mcg) once a day from the whole study period (11 days) effectively recovered body weight, reduced intestinal mucosal inflammation and down-regulated proinflammatory cytokines such as IFN-gamma, TNF-alpha, IL-12 at the mRNA level in the colonic tissue. Apoptosis was also induced in lamina propria macrophages after treatment with anti-TNF (45). Considering the evidence of the IL-12 elevation in this model, IL-12 could be other therapeutic target cytokine to treat TNBS-induced colitis. Intraperitoneal administration of anti-IL-12 mAb (1 mg) to the TNBS-treated mice both early (day 5) and late phase (day 20) after induction of colitis leads to a striking improvement in both the clinical and histopathological aspects of the disease, and frequency abrogates the established colitis completely (38). In addition, treatment of TNBS mice with anti-IL-12 mAb brings recovery of tolerance toward resident intestinal flora and apoptosis of Th1 cells (46,47). Furthermore, anti-IL-12/23p40 mAb has been shown to be significantly efficacious in the acute TNBS colitis model, when intraperitoneally administered at the dose of 25 mg/kg on study day 0 (39). Another pivotal cytokine for the development of Th1 response is IL-18. Hove *et al.* reported that blockade of IL-18 by intraperitoneal daily dosing (8 mg/kg) of recombinant human IL-18 binding protein isoform a (rhIL-18BPa) ameliorates TNBS colitis by decreasing local TNF-alpha production (48). In addition to the cytokine blocking described above, anti-IL-16 mAb (intraperitoneal,1 mg, 24 hours before and 24 hours after TNBS treatment) is known to reduce colonic injury and inflammation induced by TNBS in mice (49).

MAdCAM-1 expression in inflamed colon of mice with acute TNBS colitis is reported to increase at transcriptional and translational levels (50). Actually, MAdCAM-1 antisense oligonucleotides (subcutaneous, 1.5 mg/kg/day, 7 days starting from the first day of the TNBS enema) significantly suppress the development of TNBS colitis clinically and histopathologically compared with controls, suggesting that antisense suppression of MAdCAM-1 can attenuate recruitment of lymphocytes bearing alpha4beta7 integrin to the inflamed colon (51)

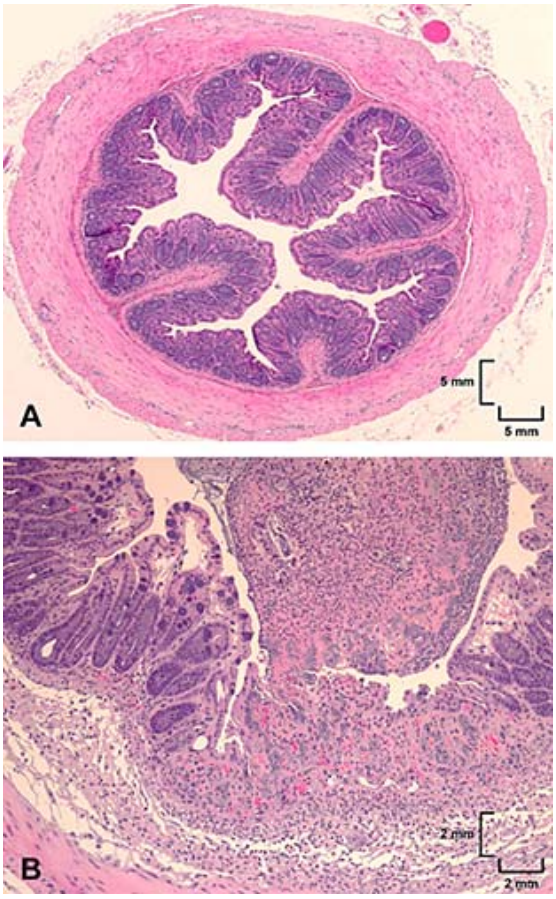

Figure 2. Histopathology of the colon from normal (A) and hapten-induced (TNBS-induced) colitis model (B). Note mucosal ulceration and mixed cell inflammation.

Regarding another novel therapeutic target for IBD treatment, the effect of intraperitoneal injection of cannabinoid (CB) 2 receptor agonists on TNBS-induced acute colitis has been evaluated (52). As a result, activation of CB2 receptor may also have a possibility to be a novel therapeutic target for IBD treatment. Moreover, other therapeutic targets and candidate compounds for IBD treatment are evaluated in this model (53-59).

3.3. Oxazolone model

Oxazolone model is another IBD model induced by haptenating agents along with TNBS model. This model is prepared by intrarectal administration of oxazolone and shows rapid onset of colitis marked by weight loss and diarrhea (60). Severe hemorrhagic colitis is observed in the distal half of the colon. Histopathological characteristics of the oxazolone colitis is epithelial cell loss, patchy ulceration, pronounced depletion of mucin producing-goblet cell, reduction of the density of the tubular glands, lymphocytes and granulocytes infiltration into the bowel lumen, and edema of submucosal layer. Taken together, these macroscopic and microscopic histopathological features of this model clearly demonstrate that this model has characteristics similar to that of human UC.

The nature of the immune response of oxazolone colitis is an IL-4-driven Th2 T cell response which is marked by elevated IL-4/IL-5 production and normal IFN-gamma production of lamina propria cells in colon and which is prevented by the systemic coadministration of anti-IL-4 mAb. Interestingly, this proinflammatory cytokine response is counterbalanced by a massive TGF-beta response, which limits both the extent and duration of disease. In addition, IL-13 production is a significant pathogenic factor which is produced by natural killer T cells in oxazolone colitis (61). This T-cell dependent experimental colitis is crucially regulated by one of the nuclear factor of activated T cells (NFAT) family of transcription factors, NFATc2, as the expression of NFATc2 is up-regulated in the oxazolone colitis (62).

There are several reports regarding utility of this model for pharmacologic evaluation of medicines currently prescribed for IBD patients and candidate compounds. Kojima *et al.* demonstrated that intrarectal administration of 5-ASA (25 mg/kg, once a day) or prednisolone (1 mg/kg, once a day) for 3 days reduces myeloperoxidase activity in the colonic tissue of this model (63). With regard to neutralization of cytokines, prophylactic effects of anti-IL-4, anti-TGF-beta and anti-IL12 mAbs have been evaluated (60). As described above, intraperitoneal administration of anti-IL-4 mAb (3 mg/mouse) leads to a striking amelioration of disease, because an IL-4-driven Th2 T cell response is the nature of this colitis. In contrast, anti-TGF-beta administration (1 mg/mouse, intraperitoneal) leads to more severe inflammation which involves the entire colon, and anti-IL-12 administration (2 mg/mouse, intraperitoneal) either has no effect or exacerbates disease, suggesting an important counterregulatory role of TGF-beta and Th2-mediated colitis in the oxazolone model.

FTY720, a sphingosine-1-phosphate (S1P) analogue known to alter migration and homing of lymphocytes via sphingosine-1-phospahte receptors, prominently reduced the clinical and histopathological severity of this colitis, abrogating body weight loss, diarrhea, and macroscopic and microscopic intestinal inflammation by intraperitoneal dosing at 1 or 3 mg/kg in both prevention and treatment of colitis (64). In this case, decreased expression of IL-4, IL-5 and IL-13 in lamina propria cells was also observed in the mice by administration of FTY720. Moreover, intraperitoneal administration of recombinant IL-25 (10 mcg/mouse) and cis-guggulsterone (30 mg/kg) showed therapeutic and prophylactic effects on the oxazolone colitis, respectively (65,66). On the other hand, intraperitoneal administration of an HMG-CoA reductase inhibitor, simvastatin (up to 40 mg/kg, once a day) to the oxazolone colitis model failed to attenuate colitis, whereas it could attenuate the colitis in the acute TNBS model (67).

4. SPONTANEOUSLY DEVELOPED MODELS

4.1. Senescence accelerated mouse prone (SAMP) 1/Yit model

SAMP1/Yit mouse is a unique model of spontaneous and chronic ileitis established by Matsumoto *et al.* in 1998 (68). Histopathologically, thickening of the intestinal wall of the terminal ileum is detected, where the

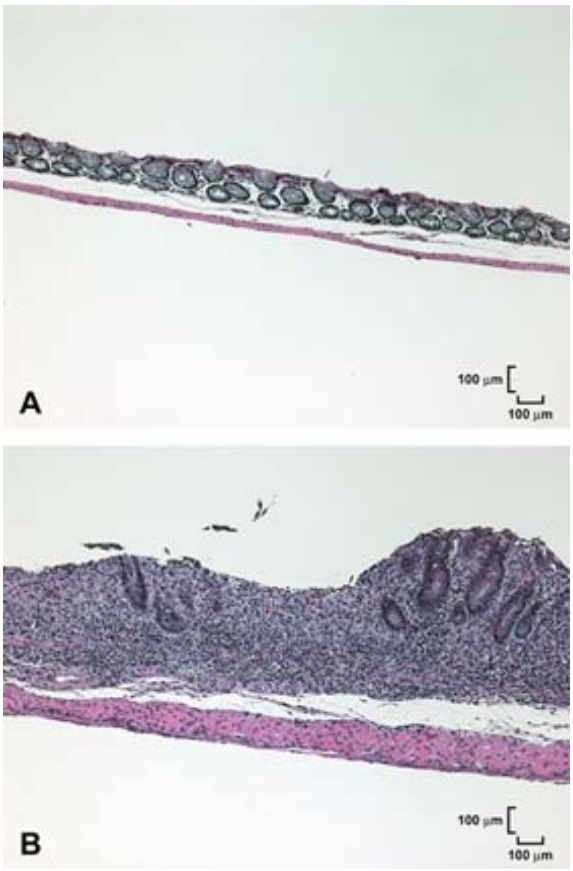

Figure 3. Histopathology of the colon from normal (A) and $CD4^+CD45RB^{high}CD25^-$-transferred colitis female C.B.-17 SCID mouse model (B). Note mucus depletion with mucosal thickening and expansion due to epithelial cell hyperplasia and lymphoplasmocytic inflammation.

discontinuous lesion is maximal and gradually decreased as it reaches the upper part of the small intestine (Table 1). The intestinal inflammation is restricted to the distal part of the jejunum, ileum and cecum. The duodenum, proximal part of the jejunum, colon and rectum have normal features. Infiltration of inflammatory cells into the lamina propria and submucosal is observed. Inflammatory infiltrates in the lamina propria consist of macrophages, neutrophils and lymphocytes, and transluminal neutrophils are seen in the necrotic crypts. The overall features of this ileitis are closely similar to that of human CD (69). The results of adoptive transfer experiments suggest that $CD4^+$ T cells that produce a Th1-like profile of cytokines like IFN-gamma and TNF-alpha mediate the intestinal inflammation, and TNF-alpha plays an important role in the pathogenesis of intestinal inflammation (63). In addition, IL-5 is remarkably increased in Peyer's patches, mesenteric lymph nodes and mucosa involved in ileitis and this ileitis is attenuated by the administration of anti-IL-5 antibodies, suggesting that IL-5 also participates in the pathogenesis of the ileitis in this model (70). Moreover, the signal transducer and activator of transcription (STAT)-3 activation via IL-6 trans-signaling contributes to this ileitis (71). As well as T cells and the cytokines secreted from macrophages or T cells, the disorders of regulatory B-cell function under innate immune activation is reported to have possibility to be associated with pathogenesis in this model (72).

Efficacy of several medicines currently prescribed for IBD patients has been evaluated in this model (Table 2). Combination of antibiotics (50 mg/kg/day of ciprofloxacin and 100 mg/kg/day of metronidazole in drinking water) significantly decreases the severity of this ileitis both in the prevention (for 6 weeks before the development of ileitis) and the treatment (for 4 weeks after the establishment of ileitis) protocols by a mechanism involving down-regulation of activated gut lymphocytes and inhibition of intestinal IFN-gamma and TNF-alpha production (73). Direct neutralization of TNF-alpha by intraperitoneal administration of anti-TNF-alpha antibody (0.1 or 1.0 mg/mouse) also ameliorates the severity of the established ileitis by abrogation of intestinal epithelial cell apoptosis (74). In addition to the drugs targeting cytokines, inhibitors of adhesion molecules are expected to be efficacious based on the fact that inflammatory infiltrates are observed at ileitis of this model. Actually, periodical intraperitoneal administration of anti-MAdCAM-1 antibody every other day for 7 weeks significantly is effective for both prevention of the developing ileitis and attenuation of established ileitis at 2 mg/kg (75). Anti-P-selectin glycoprotein ligand (PSGL) antibody (2 mg/kg) also prevents the development of ileitis in this model by intraperitoneal administration every other day for 7 weeks (76). Furthermore, decrease in monocyte recruitment to intestinal mucosa through suppression of platelet-monocyte interactions by a specific phosphodiesterase-3 (PDE-3) inhibitor of platelets (0.02% cilostazole in diet for 3 weeks) provides the beneficial effects on the established ileitis of this model (77).

5. T-CELL TRANSFER MODELS

5.1. $CD4^+CD45RB^{high}CD25^-$-transferred model

In 1993, it was reported that adoptive transfer of $CD4^+CD45RB^{high}$ T cells to C.B-17 SCID mice leads to the development of a lethal wasting disease with chronic intestinal inflammation (78,79). Following these reports, it was also clarified that similar colitis can be induced when the aforementioned T cell population is transferred into recombinase activating gene-1-deficient ($RAG^{-/-}$) mice. Mice in this chronic model begin to show progressive weight loss 3-4 weeks after the cell transfer followed by colonic inflammation (80). Histopathologically, the inflammation extends diffusely from the cecum to the rectum and becomes transmural in severe cases. Inflammatory infiltrate contains numerous macrophages accompanied by moderate numbers of activated $CD4^+$ lymphocytes. Histopathologically, mucin depletion and epithelial hyperplasia resulting in glandular elongation and mucosal thickening are also consistently observed (Figure 3). These changes of this model are similar to these seen in patients with CD (Table 1).

In the inflamed colon in this colitis model, the gene expression of CC chemokine receptor (CCR) 1,

CCR2, CCR5, CXC chemokine receptor 3, their ligands, TNF-alpha, IFN-gamma and IL-6 was progressively augmented as colitis developed (81). $CD4^+$ T cells from colonic lesion of this colitis model display high level of IFN-gamma synthesis, suggesting that this colitis is induced by the disregulated Th1 response (82). Actually, IFN-gamma is absolutely required in the pathogenesis of this colitis and has critical role in this model (83). From the evidence obtained from the experiment using IL-6 deficient mice, T cell-derived IL-6 may induce this augmentation of gene expression of proinflammatory molecule, thereby causing transmural inflammation (81). In addition, $CD40^+$ and $CD40L^+$ cells as well as their mRNA levels are significantly increased in inflamed mucosa as observed in IBD in humans, and CD40-CD40L interactions are suggested to be essential for the inflammatory response in this colitis (84). Moreover, IL-7 is also essential for the development and the persistence of chronic colitis of this model (85). On the other hand, the colitis of this model is prevented when $CD4^+CD45RB^{low}$ T cells are co-transferred along with $CD4^+CD45RB^{high}$ T cells (86). The key components associated with this prevention do not seem IL-4, but TGF-beta and $CD4^+CD45RB^{low}$-derived IL-10 (87,88). After these reports, it was clarified that the true T cells responsible for this prevention are $CD4^+CD25^+$ regulatory T cells (Treg) (89,90). These Treg prevent colitis by inhibiting the accumulation of tissue-seeking effector cells and that Treg accumulation in the intestine is dispensable for colitis suppression (91). Comparative analysis clearly demonstrates that the pattern of gene expression in this model most closely reflects altered gene expression in IBD (92).

This model is not so often used for pharmacologic evaluation of efficacy of candidate compounds by pharmaceutical companies due to its lower throughput resulting from the complex experimental procedure and long study duration (Table 2). However, prophylactic efficacy of mAbs for neutralizing TNF-alpha and IFN-gamma has been evaluated in this model, because their levels would be augmented in the inflamed colon of this model as described above. The intraperitoneal administration of anti-IFN-gamma mAb to this model soon after T cell transfer prevents development of colitis for up to 12 weeks regardless of the following number and timing of dosing (82). With regard to TNF-alpha, continual neutralization with intraperitoneal weekly dosing of anti-TNF mAb reduces the incidence of severe disease; however, neutralization during only the first 3-4 weeks has no effect (82). In addition, studies of another group show that immediate and delayed treatment of this model after T cell transfer by anti-TNF mAb (1 mg/mouse, intraperitoneal, once a week) demonstrates prophylactic and therapeutic effects on intestinal mucosal inflammation, respectively, with concomitant suppression of leukocyte infiltration in the inflamed colon, down-regulation of IFN-gamma, IL-2 and TNF-alpha secretion by lamina propria $CD4^+$ T cells, and decrease in mRNA levels of IL-23p19 and IL-17 in inflamed colon (93). Considering the elevated IFN-gamma levels in the colonic lesion of this model, IL-12 which is the main inducer of IFN-gamma is likely to be another promising target to prevent and ameliorate this colitis. In fact, administration of anti-IL-12 mAb (1 mg/mouse, intraperitoneal, every other week) provides beneficial effects in both developing and established colitis with accompanying abrogated mucosal inflammation and down-regulation of IFN-gamma and IL-2 (94). Other mAbs whose efficacy has been evaluated in this model are ones for beta7 integrin, MAdCAM-1, CD40L, OX40L, Fas ligand, ICOS, B7RP-1, NKG2D and TLR4 (84,95-100). Among them, prophylactic effects to prevent development of colitis after cell transfer are investigated for anti-beta7 integrin, anti-MAdCAM-1, anti-CD40L, anti-NKG2D and anti-TLR4 mAbs and they evidently showed such effects. On the other hand, regarding anti-OX40L, anti-Fas ligand and anti-ICOS mAbs, therapeutic effect to improve developed colitis are analyzed in addition to prophylactic effect. These mAbs successfully demonstrate both prophylactic and therapeutic effects in this model. Furthermore, efficacy of a fusion protein between the extracellular portion of cytotoxic T lymphocyte associated antigen-4 (CTLA-4) and the Fc portion of human IgG1 (CTLA4-Ig), and cyclosporine A have been evaluated in this colitis (101). When administered intraperitoneally twice weekly, CTLA4-Ig prevents developing colitis at 100 mg/kg with lower inflammation score and pro-inflammatory cytokine levels in lamina propria lymphocytes as compared to control. On the other hand, cyclosporine A (2.5 mg/kg, intracolonic, daily) cannot prevent developing colitis effectively.

In addition, several pharmaceutical agents used for IBD treatment in humans are evaluated in a similar cell-transfer model in which whole spleen and mesenteric lymph node cells from IL-10 deficient mice are transferred to C.B-17 SCID mice (102). In this study, TNFR-Ig, prednisolone, sulfasalazine, azathioprine, tacrolimus and cyclosporine A were assessed. As a result, TNFR-Ig and prednisolone has markedly attenuated pathological clinical indices when administered one day after cell transfer, whereas other agents showed no significant efficacy.

6. TRANSGENIC (TG) MODELS

6.1. Interleukin-7 (IL-7) TG model

The IL-7 transgenic mice were established by Uehira *et al.* in 1993 (103). In these TG mice, IL-7 is constitutively expressed in several tissues, especially in lymphoid organs. The disorder of IL-7 expression was originally reported to provide the symptoms of severe dermatitis infiltrated by gamma delta T cells and an increased population of gamma delta T cells in the lymphoid organs (103). Subsequently, Watanabe *et al.* clarified that these TG mice develop acute and chronic colitis (104). Acute colitis with infiltrating neutrophils and lymphocytes is observed at 1 to 3 weeks of age (Table 1). In the inflamed colonic mucosa, IL-7 protein is significantly expressed. Infiltrating T cells in the colitis lesion are $CD4^+$ and T cell receptor gamma/delta T cells. In contrast, chronic colitis is developed at 4 to 12 weeks of age. In the chronic colitis, expression of IL-7 receptor (IL-7R) increases in mucosal lymphocyte, whereas IL-7 accumulation is decreased in the goblet cell-depleted colonic epithelium. Erosion and neutrophil infiltration are

observed in the anal ring, but no ulceration is demonstrated. The inflammatory cell infiltration and goblet cell depletion found throughout entire colon is most prominent in the rectum. Crypt abscess, paneth cell metaplasia and infiltration of eosinophils are also observed in the lesions. These features are similar to the histopathological characteristics of human UC. Lymphoid infiltrates in the lamina propria are dominated by Th1 $CD4^+$ T cells. Increases in production of IFN-gamma and IL-2, and decreases in production of IL-4 in isolated colonic mucosa cells are observed (104).

To date, there are few reports on pharmacologic evaluation of efficacy of candidate compounds. However, these TG mice would be helpful for understanding the mechanism of action of several candidate compounds like JAK1/3 inhibitors one of targets of which is the IL-7/IL-7R signaling pathway.

7. IMMUNOREGULATORY KNOCKOUT (KO) MODELS

7.1. Interleukin-10 (IL-10) KO model

IL-10 KO model was generated by Kuhn *et al.* in 1993 (105) and has been considered to have histological, physiological and biochemical features similar to those of human CD (106).

This model has the characteristics of lymphocyte development and antibody responses being normal, but most animals are growth retarded and anemic and suffer from chronic enterocolitis under conventional conditions at the age of 4-8 weeks. There is no colitis observed when maintained in a microbiologically clean facility, but colitis is developed when they are moved under conventional condition by about 12 weeks of age (107).

The intestinal pathology has been characterized by a regionally variable pattern of mucosal inflammation associated with either hyperregenerative or degenerative lesions of the intestinal epithelia. Gastrointestinal regions predominantly affected are the duodenum, proximal jeojunum and proximal colon. In the duodenum and adjoining jejunum, the chronic inflammatory process causes excessive regenerative hyperplasia of the mucosa leading to a marked thickening of the intestinal wall. The typical architecture of the mucosa is disturbed by the formation of abnormal crypt and villus structures consisting of branched and fused villi, enlarged and branched crypts, and labyrinthine sheets of enterocytes on the surface of the mucosa (Table 1).

Microbial milieu plays a major role in determining the characteristics of this model. This model derived under conventional conditions develops histopathological changes characterized by discontinuous transmural lesion affecting both the upper and lower gastrointestinal tract, epithelial hyperplasia, mucin depletion, crypt abscesses, ulcers, and thickening of the bowel wall. Particularly, *Helicobacter hapaticus* is known to induce chronic mucosal inflammation in immune-deficient mice and was identified as part of the complex intestinal flora present in IL-10 KO. In contrast, IL-10 KO derived under specific pathogen-free (SPF) conditions develops a mild-to-moderate inflammation restricted almost entirely to the colon. Moreover, germ-free IL-10KO mice repopulated with six defined bacterial strains, including *Bacteroides vulgatus*, developed a very mild form of colitis. (108). This is consistent with the emerging role of microbiota in IBD.

In addition to the major role of microbial milieu, endogenous prostaglandins are also shown to have an important role in this model, because the induction of this colitis can be accelerated by the treatment of non-steroidal anti-inflammatory drugs (NSAIDs) (109).

IL-10 is a potent suppressor of macrophage activation, inhibiting the production of IL-1, IL-6 and TNF-alpha. IL-10 also inhibits the ability of macrophage to promote the development of Th1-type T cells, which secrete IL-2 and IFN-gamma. Therefore, it was originally believed that IL-10 KO had Th1 pathogenesis, in which IL-12 and IFN-gamma play a critical role. Intraperitoneal administration of anti-IL-12 mAb once a week for 8 weeks (0.5 mg, 1 mg the following week, 2 mg/week for 6 week) has been shown to completely prevent colitis development in young IL-10 KO mice at ten days of age. Treatment of three-month-old adult mice by intraperitoneally injecting anti-IL-12 mAb once a week for 8 weeks (2 mg/week) resulted in significant amelioration of established colitis accompanied by reduced numbers of mesenteric lymph node and colonic $CD4^+$ T cells and of mesenteric lymph node T cells spontaneously producing IFN-gamma. In contrast, intraperitoneal administration of anti-IFN-gamma mAb once a week for 8 weeks (2 mg/week) has had minimal effect on disease reversal in adult mice, despite a significant preventative effect in young mice. From these findings, it is suggested that IL-12 sustains the colitis of IL-10 KO (110). However, a study comparing contribution of IL-12 to the pathogenesis of IL-10 KO with that of IL-23 clearly demonstrates that IL-23 is essential for spontaneous induction of colitis followed by production of IL-17 and IL-6, whereas IL-12 is not (111). Therefore, this model seems to have Th17 pathogenesis. Nevertheless, it is clear that cells component for expression of both IFN-gamma and IL-17 are detectable at all stages of disease in T cell-dependent mouse models, as well as in human CD. The fact that early, but not late, blockade of IFN-gamma prevents disease suggests that IFN-gamma might be more critical for disease onset than for its persistence.

This model often has been used for evaluation of pharmacologic efficacy of several candidate compounds (Table 2). The serum level of TNF-alpha has been known to increase, so the efficacy of anti-TNF-alpha mAb has been evaluated in this model (112,113). Intraperitoneal injection of anti-TNF-alpha mAb once daily for 10 days (10 mcg) or three times a week for 16 weeks (500 mcg) improves inflammation histologically with correlation with a resolution of diarrhea and rectal bleeding. Clinical score similar to CD Activity Index in humans also shows the amelioration of colitis evidently, and analysis of cytokine levels in stools reveals a marked diminution of

inflammatory cytokines. Furthermore, oral administration of *L. lactis* secreting an anti-TNF nanobody has showed efficacy in this model (114). Taken together, these results clearly demonstrated that TNF-alpha neutralization is efficacious in this model, as is observed in clinical treatment of CD. Relating to TNF-alpha neutralization, blocking of intracellular signaling of TNF receptor by inhibiting NF-kappaB activation by peptides (intraperitoneal dosing at 10 mg/kg for 10 of 14 days) corresponding to the NF-kappaB essential modulator (NEMO) binding domain of the IkappaB kinase complex also improved colitis of this model (115).

Another study on the efficacy of mAb is inhibition of IFN-gamma-inducible protein-10 (IP-10) (116). Because of high expression of IP-10 and CXCR3 at sites of colitis, IP-10 inhibition in this model (intraperitoneal dosing of 200 mcL every 3 days) attenuates the associated increases in serum and/or local amyloid A, IL-2, IL-6, TNF-alpha, IFN-gamma, IL-1alpha and IL-1beta, and inflammation score with colitis as compared with IL-10 KO that develop colitis similar to human CD.

In addition to these mAbs, some compounds with low molecular weight have also been evaluated in this model. Tacrolimus which is a strong immuno-suppressing agent originally used for prevention of rejection of allograft has recently been approved as a novel drug for UC treatment. Rectal administration of tacrolimus at 10 mg/kg every other day 3 times a week for 2 weeks evokes apoptosis of colonic macrophages and ameliorates colitis in this model with significant reduction of gene expression of inflammatory cytokines in colonic mucosa (117). Other studies regarding efficacy of low-molecular weight compounds or peptides in this model also demonstrate that inhibition of CXCR4 (118) and stimulation of $S1P_1$ (119,120).

7.2. Interleukin-2 (IL-2) KO and IL-2 receptor alpha (IL-2Ralpha) KO models

In 1993, Sadlack *et al.* reported that approximately 50% of IL-2 KO mice die within the first 9 weeks of age due to splenomegaly, lymphoadenopathy and severe anemia, and that the remaining animals develop an IBD (121). Disease symptoms observed in IL-2 KO mice between 6 and 15 weeks of age are chronic diarrhea remittant intestinal bleeding and a frequent rectal prolapse (Table 1). The disease progresses and leads to death within 10-25 weeks. The lesions are observed in the mucosa and submucosal tissue of the large bowel with pronounced thickening of the bowel wall. Prominent ulcerations and frequent crypt abscesses are found, and the epithelial layer shows a loss of goblet cells. Typically, a continuous involvement of the large intestine with increasing intensity from the caecum to the rectum is seen. The mucosa is infiltrated with granulocytes, lymphocytes and plasma cells. In the lymphocytes from colon, drastic increase in both B and T cells is observed. The isolated lymphocytes show that T cell counts are up to 100 times higher as compared with control mice, and that IgA- and IgG1-secreting cells are elevated. Moreover, anti-colon antibodies are detected. In the caecum and colon, a large numbers of $CD4^+$, $CD8^+$, TCR-alpha $beta^+$ and TCR-gamma $delta^+$ T cells, and macrophage, dendritic cells and $MAdCAM\text{-}1^+$ endothelial cells are observed (122). This is associated with an increase in the number of IFN-gamma, IL-1 and TNF-alpha transcripts and a decrease in IL-4 and IL-10 transcripts. In terms of the cytokine level in colonic tissues, the level of IL-1beta drastically increases whereas that of IL-10 evidently decreases (123,124). However, the level of TNF-alpha remains unaltered (123). In the severely inflamed colonic sections, up to 19-fold increase in transcripts of inducible NO synthase (iNOS) is also shown (125). The severe, rapid and predictable development of colitis in IL-2 KO mice can be performed by intraperitoneal injection of 2,4,6-trinitrophenol (TNP)-substituted protein in complete Freund's adjuvant (CFA) (126). In contrast to other genetically engineered rodents, IL-2 KO mice develop mild focal gastrointestinal and active portal tract inflammation in the absence of viable bacteria (127). Currently, the chronic inflammatory responses observed in IL-2 KO mice is considered to be induced by dysregulation of activation induced cell death (AICD) and intrathymic differentiation of regulatory T cells due to the absence of IL-2 (128,129). The characteristics of IL-2Ralpha KO mice would not be mentioned in this review, because phenotypes and their pathogenesis including inflammatory bowel disease in IL-2Ralpha KO mice are similar to those of IL-2 KO (130).

In IL-2KO mice, efficacy of few IBD medicines has been evaluated (Table 2). Intraperitoneal injection of 5 mg/kg of cyclosporine A twice weekly provides survival of mice compared to the untreated mice (122). Moreover, blocking of $alpha^E beta_7$ by intraperitoneal administration of anti-$alpha^E beta_7$ mAb (0.5 mg, every other day) to TNP-ovalbumin-immunized IL-2 KO mice results in a significantly reduced colitis which is associated with a significant reduction in $CD4^+$ lamina propria lymphocyte subpopulation (131). The beneficial effects of anti-$alpha^E beta_7$ mAb are also observed in the established colitis of these immunized mice. In addition, administration of OX40-IgG fusion protein (intraperitoneal, 100 mcg daily) to IL-2 KO mice with established colitis ameliorates disease in this model accompanied with a reduction in tissue myeloperoxidase, transcripts for TNF-alpha, IL-1, IL-12 and IFN-gamma, and T cell infiltrates (132).

7.3. T-cell receptor alpha (TCRalpha) KO model

TCRalpha KO was originally created by Momberts *et al.* in 1992 and reported to spontaneously develop colitis with similar characteristics to human IBD in 1993 (133,134) (Table 1). The area where colitis is observed is restricted to large intestine and its histopathological feature including loss of goblet cells, elongation of crypts, crypt microabscesses, and inflammatory cell infiltrate in the lamina propria are reminiscent of UC. In this model, Th2-type $CD4^+$TCR-beta beta T cells produce increase in IL-4, suggesting that these cells are associated with the development of the colitis in this model. In addition, autoantibodies are produced as a result of the immunological disorder, one of which is the autoantibody to tropomyosin which is observed in a large percentage of UC patients (135). Generally, the colonic

inflammation of this model begins at 6-8 weeks of age and reaches to chronic colitis in about 60% of mice by 16-20 weeks of age (136), indicating that this model is not appropriate for an *in vivo* evaluation system of pharmacological effects of candidate compounds in the aspects of timing or rate of colitis induction. However, a recent study demonstrates that piroxicam which is one of typical non-steroidal anti-inflammatory drugs (NSAIDs) accelerates the development of uniform colitis of this model through its effect on mucosal barrier function (137). It is also shown that the observed colitis has characteristics similar to the ones of the colitis previously developed in this model without addition of piroxicam. Moreover, dexamethasone which is one of standard of cares for IBD in humans markedly suppressed the induction of colitis and colonic cytokine levels for IL-1beta, IL-17, TNF-alpha and IFN-gamma in this accelerated model, showing that this model is applicable to an *in vivo* evaluation system of pharmacological effects of candidate compounds (Table 2).

7.4. dnKO model

This novel mouse line was generated by Kang *et al.* in 2008 by breeding dominant negative TGFbetaRII mice with mice lacking IL-10R2, and called dnKO as an UC model (138). The unique characteristic of this model is that both IL-10R2 signaling in all compartment and TGFbetaRII signaling specifically in the T cell compartment are defective. Total body weights of this model and controls showed that dnKO mice failed to thrive by 3-4 weeks and demonstrated rapid weight loss culminating in death as compared to all controls by 4-6 weeks. At the entire cecum, descending colon and rectum in dnKO mice, striking disruption in the gross morphology were observed. Histopathological examination revealed that dnKO mice had severe and diffuse alterations in mucosal structure that correlated with the gross pathology findings and included marked extensive epithelial hyperplasia, diminished goblet cell and crypt number, erosion of surface epithelial cells, numerous crypt abscesses, and mixed leukocyte infiltrates localized in both the mucosa and submucosa of cecum, descending colon and rectum. In lamina propria and mucosally associated lymphoid tissue in the cecum, descending colon, and rectal regions, there were a 10-fold increase in cell numbers of T cells, immature myeloid/monocytes, neutrophiles, NK/NKT, and B cells observed. In addition, increase in serum levels of TNF-alpha, IFN-gamma and IL-6 were also seen (Table 1).

Intraperitoneal injection of anti-IFN-gamma mAb (1 mg) prior to disease assessment alone significantly diminished loss of crypts, goblet cells, and surface epithelial cell height, whereas intraperitoneal injection of anti-TNF-alpha mAb (1 mg) alone did not have any significant alterations in colitis induction. However, neutralization of both IFN-gamma and TNF-alpha by mAbs for them (1 mg/each) resulted in greater amelioration of pathology including significantly decreased hyperplasia. Moreover, antibiotics like ciprofloxacin and mertonidazole completely inhibits disease in this model from all aspects of survival, growth observations, gross and histologic examination of intestines, and the pro-inflammatory cytokine levels in serum, when administered 0.66 mg/mL of ciprofloxacin and 2.5 mg/mL of mertonidazole in drinking water (Table 2).

As described above, this is the first mouse model of fulminant UC by combining multiple genetic hits in immune regulation and that the resulting disease is sensitive to both anticytokine therapy and broad-spectrum antibiotics.

8. PERSPECTIVE

The future IBD research undoubtedly will deliver more innovative therapeutic targets of this chronic and often devastating illness especially in the geriatric population which is prone to more long term complications (139). In this review, we have focused on the pharmacologic efficacy of novel therapeutic targets and drug candidates for IBD treatment in the most commonly utilized IBD models in a pharmaceutical setting. There are several other IBD models applicable to the evaluation of pharmacologic efficacy of drug candidates. However, most of them evidently have limitation in reproducibility and/or not being high throughput. Chemically- and hapten-induced models are easy to execute and are of high throughput. This is especially accurate for the acute TNBS-induced colitis model which is often utilized in pharmacologic efficacy evaluation of drug candidates. With respect to the pharmacologic intervention related to pathogenesis of this colitis, the recent anti-cytokine and anti-adhesion molecule therapies are efficacious, demonstrating the suitability of this model to a pharmacological setting. Concerning the DSS colitis model, the chronic model is more suitable for the evaluation of pharmacologic efficacy than the acute model despite its low throughput, because its immunopathological profile is similar to that of human IBD in which both leukocyte migration and lymphocyte activation are major pathogenesis thereby the recent therapies such as anti-cytokine and/or anti-adhesion molecule antibodies as well as traditional standard of care for IBD are efficacious. Oxazolone model is one of the very few models showing definitive Th2 cytokine profile. More immunopathological information is expected to accumulate through the efficacy analyses of current standard of care for IBD. As is the case with the chronic DSS colitis, anti-cytokine and/or anti-adhesion molecule antibody therapies show beneficial effects in adoptive transfer models, suggesting that the leukocyte migration and the following lymphocyte activation seem to be major pathogenesis of this model. However, low throughput provides fewer opportunities of pharmacologic efficacy evaluation of drug candidates. On the other hand, some spontaneously developed, TG and immunoregulatory KO mouse models are attractive due to their high reproducibility and high throughput, whereas their genetic condition related to knocked-out genes is often clearly distinct from that of human IBD. Among them, SAMP1/Yit and dnKO models are the scarce CD and UC models, respectively, in which antibiotics are clearly shown to be efficacious. Especially, ileitis of SAMP1/Yit is also improved by blockings of TNF-alpha and MAdCAM which are current 1st and 2nd line therapies combined with standard of care for CD, respectively. Recently, role of

autophagy and microbiota is emerging as an essential component in the pathogenesis of IBD as well as susceptibility based on immune and/or genetic condition. Some of IBD models discussed here are known to have less severe colitis when bred under germ-free condition. There will continue to be a compelling need to develop and test novel medicines based on emerging data from basic immunologic studies. Therefore, it would be important that relation of autophagy and microbiota with colitis is better defined and understood in IBD models in order to investigate human relevance as well as to improve reproducibility and throughput as pharmacologic efficacy evaluation systems.

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Abbreviations: mcg: microgram, mcL: microliter, Da: dalton

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