

## REVIEW

# Uncovering Pathogenic Mechanisms of Inflammatory Bowel Disease Using Mouse Models of Crohn's Disease–Like Ileitis: What is the Right Model?



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## SUMMARY

This review highlights the use of animal models of intestinal inflammation in inflammatory bowel disease research, with a specific focus on 2 highly relevant models of Crohn's-like ileitis.

Crohn's disease and ulcerative colitis, together known as inflammatory bowel disease, are debilitating chronic disorders of unknown cause and cure. Our evolving understanding of these pathologies is enhanced greatly by the use of animal models of intestinal inflammation that allow in vivo mechanistic studies, preclinical evaluation of new therapies, and investigation into the causative factors that underlie disease pathogenesis. Several animal models, most commonly generated in mice, exist for the study of colitis. The appropriateness of their use often can be determined by their mode of generation (ie, chemical induction, T-cell transfer, targeted genetic manipulation, spontaneously occurring, and so forth), the type of investigation (mechanistic studies, pathogenic experiments, preclinical evaluations, and so forth), and the type of inflammation that occurs in the model (acute vs chronic colitis, tissue injury/repair, and so forth). Although most murine models of inflammatory bowel disease develop inflammation in the colon, Crohn's disease most commonly occurs in the terminal ileum, where a very limited number of mouse models manifest disease. This review discusses appropriate experimental applications for different mouse models of colitis, and highlights the particular utility of 2 highly relevant models of Crohn's-like ileitis—the spontaneous SAMP1/YitFc inbred mouse strain and the genetically engineered *Tnf*<sup>ΔARE-rich element/+</sup> mouse model of tumor necrosis factor overexpression, both of which bear strong resemblance to the human condition. Similar to patients with Crohn's disease, SAMP1/YitFc ileitis develops spontaneously, without chemical, genetic, or immunologic manipulation, making this model particularly relevant for studies aimed at identifying the primary defect underlying the occurrence of Crohn's ileitis, as well as preclinical testing of novel treatment modalities. (*Cell Mol Gastroenterol Hepatol* 2017;4:19–32; <http://dx.doi.org/10.1016/j.jcmgh.2017.02.010>)

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Inflammatory bowel disease (IBD) is a chronic, relapsing, and remitting inflammatory disease of the gastrointestinal tract that encompasses both Crohn's disease (CD) and ulcerative colitis (UC). IBD affects more than 3.5 million people in the United States and Europe, with a steep increase in incidence over the past 50 years.<sup>1,2</sup> There is no cure, and the cause of disease remains unclear, limiting our ability to prevent its occurrence, and focusing therapy toward mitigation of symptoms, rather than cure of disease. Traditionally, standard therapy for IBD has been centered on the use of corticosteroids to broadly combat intestinal inflammation through a systemic approach of immunosuppression. However, advances in monoclonal antibody–based therapeutics and other pharmacologic agents have spurred the development of new classes of biological drugs that can target very specific components of the gut mucosal immune system, facilitating the direct translation of mechanistic laboratory findings of disease pathogenesis to clinical development of novel therapies to treat intestinal inflammation in patients with CD and UC.

IBD pathogenesis involves multiple contributing genetic, environmental, and immunologic factors. Genome-wide association studies have shown IBD to be a polygenic disease with 163 identified susceptibility loci, most of which represent variants within DNA noncoding regions.<sup>3</sup> The largest genetic association study to date (29,838 patients) found that only 3 loci were associated with clinical subphenotypes in IBD patients (ie, nucleotide-binding oligomerization domain-containing protein 2 [*NOD2*], *MHC*, and *MST1 3p21*), mostly correlating to disease location.<sup>4</sup> Interestingly, this study also found genetic evidence supporting the concept that IBD actually may represent a spectrum of genetically distinct diseases that are classified more accurately as UC, Crohn's colitis, and Crohn's ileitis.

Although clearly a complex trait, genetic susceptibility alone is not sufficient to confer disease, as shown by family and monozygotic twin studies.<sup>5,6</sup> Genetically susceptible individuals also must be exposed to environmental triggers, most likely related to the gut microbiome. In fact, a reported

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genetic association with CD is *CARD15/NOD2*, which encodes the NOD2 protein, a receptor that detects the presence of bacterial lipopolysaccharide in the gut and activates nuclear factor- $\kappa$ B (NF- $\kappa$ B)-dependent inflammatory responses.<sup>7,8</sup> However, mutations in *CARD15/NOD2* are found in only a small subset of CD patients (~15%). Mounting evidence supports the concept that IBD may result from abnormal host-microbial responses of the intestinal mucosal immune system in a genetically susceptible host, within the context of host immunomodulatory dysfunction. Under normal conditions, the intestinal mucosal immune system maintains a delicate balance between self-tolerance toward the trillions of commensal bacteria present in the gut lumen and the ability to mount immune responses against invasive microorganisms or foreign antigens. Intestinal regulatory and effector T lymphocytes, and their respective cytokine mediators, play an important role in this process. During IBD, these homeostatic immunomodulatory mechanisms are thrown out of balance, leading to increased macrophage and T-cell activation and proliferation, as well as up-regulation of proinflammatory cytokines, which ultimately results in intestinal inflammation and subsequent tissue damage.

Animal models of intestinal inflammation have contributed greatly to our current understanding of IBD pathogenesis. Early studies in rabbits with formalin-induced colitis have shown the potential for in vivo therapeutic blockade of intestinal inflammation. By performing time course experiments in colitic rabbits, the proinflammatory cytokine, interleukin (IL)1, was identified as an early key mediator of intestinal inflammation, and that pretreatment of animals with IL1-receptor antagonist, a natural inhibitor of IL1 $\beta$ , could reduce the severity of intestinal inflammation significantly.<sup>9</sup> By using a similar approach, blocking studies against the proinflammatory cytokine, tumor necrosis factor (TNF), in the CD45Rb<sup>hi</sup> T-cell transfer model of murine colitis, showed significant anti-inflammatory activity.<sup>10</sup> Altogether, these studies provided proof of concept for the clinical development of infliximab, a monoclonal antibody therapy approved for the targeted treatment of IBD.

## Animal Models of IBD

A large number of animal models, primarily in mice, currently are available for the study of experimental IBD (Table 1). However, the majority of these models are generated by either chemical or immunologic manipulation, or gene targeting, and therefore do not fully resemble the multifactorial nature of the human condition.<sup>10</sup> In 1985, Warren Strober<sup>11</sup> proposed the characteristics of an ideal animal model for IBD, stating that the model should develop disease that is identical to human IBD, with the same casual factors, pathology, and clinical spectrum. It should occur in an animal that is accessible and inexpensive, with a defined genetic background, and a similar immune system to that in human beings. In addition, the disease should be able to be manipulated by diet, immunologic status, infection, and various forms of treatment. Although not all available models meet these standards, each has specific utility for different types of investigations and research questions. In this context, however, the SAMP1/YitFc mouse model

of CD-like ileitis fulfills most of the desired criteria and represents an ideal model to study IBD.

Animal models that use noxious chemicals to induce colitis (ie, dextran sodium sulfate [DSS], trinitrobenzene sulfate [TNBS], acetic acid, oxazolone, and so forth) are used most commonly because they are accessible, inexpensive, and relatively easy to induce. However, the resulting inflammation is generally less representative of the specific immunohistopathology present in the inflamed colons of patients with UC or Crohn's colitis. Chemically induced animal models of colitis can be quite useful, however, for the study of acute colonic tissue injury and repair mechanisms, or when paired with genetically engineered mice, to study the role of a targeted gene, or gene product, in mediating colitis.

IBD is a T-cell-mediated disease that involves the recruitment of lymphocytes to the inflamed gut mucosa, evidenced by the clinical effectiveness of vedolizumab, a recently approved drug for the treatment of IBD that specifically targets gut-homing lymphocytes by blocking their migration to the intestine.<sup>12,13</sup> Animal models that involve adoptive transfer of immune cells to induce colitis in recipient immunodeficient mice (eg, severe combined immunodeficient or Recombination-Activating Gene<sup>-/-</sup>), which lack B and T cells, are powerful tools for understanding the role of specific lymphocyte subpopulations in either promoting or preventing colitis. Bone marrow chimeric models similarly pair irradiated mice with adoptive transfer experiments to localize the cellular compartments (hematopoietic vs nonhematopoietic) responsible for conferring disease.

By their nature, genetically engineered animal models (the majority of which artificially alter only a single gene or locus) do not likely fully represent the underlying biological conditions that lead to IBD pathogenesis in human beings. However, such models can be extremely useful for understanding the functional role of a specific gene product in contributing to, or protecting against, experimental IBD. For example, introduction of a deletion for the gene encoding fibroblast growth factor inducible-14, the receptor for TNF-like weak inducer of apoptosis, a TNF superfamily member involved in intestinal tissue repair and innate and adaptive immunity, into C57BL/6 mice enhances their susceptibility to DSS-induced colitis, suggesting that the TNF-like weak inducer of apoptosis/fibroblast growth factor inducible-14 signaling pathway may play a protective role in mitigating acute colonic tissue injury and repair.<sup>14</sup>

## Mouse Models of CD-Like Ileitis

The vast majority of animal models of IBD only develop inflammation within the colon, however, active CD most commonly occurs in the terminal ileum and genome-wide association studies now provide evidence that Crohn's ileitis may, in fact, represent a genetically distinct form of IBD.<sup>4</sup> Two mouse models have been reported to develop Crohn's-like ileitis: the inbred SAMP1/YitFc strain and genetically engineered *Tnf* <sup>$\Delta$ AU-rich element(ARE)/+</sup> mice. Both strains show striking similarities to active Crohn's ileitis, making them particularly relevant to the human condition and valuable resources for furthering CD research.

**Table 1.** Mouse Models for the Study of Experimental IBD

Mode of generation and model	Inflammation phenotype/relevance to human IBD
<p><b>Chemical induction</b>  Advantages: easy to induce, inexpensive  Disadvantages: lack of reproducibility owing to different protocols, diminished pathogenic relevance to the human condition  DSS<sup>78,79</sup>  DNBS<sup>80</sup> and TNBS<sup>81,82</sup>  Acetic acid<sup>83</sup>  Oxazolone<sup>84</sup>  PG-PS<sup>85</sup></p>	<p>Tissue/epithelial injury and repair  Acute and chronic colitis (delayed hypersensitivity)  Acute colitis/motility  Acute Th2-mediated colitis  Acute and chronic colitis</p>
<p><b>Cell transfer</b>  Advantages: chronic features of intestinal inflammation, ability to investigate the role of specific T-cell subsets  Disadvantages: use of immunodeficient mice  CD45RB<sup>hi</sup> → SCID<sup>10</sup> CD8<sup>+</sup> → SCID,<sup>86</sup> SAMP1/YitFc CD4<sup>+</sup> → SCID<sup>18</sup>  ECOVA → SCID<sup>87</sup>  Bone marrow chimera/RAG<sup>-/-20</sup></p>	<p>Chronic ileitis and colitis/T-cell homing  Subacute colitis  Isolation of inflammatory defects to hematopoietic/nonhematopoietic compartments</p>
<p><b>Genetically engineered</b>  Advantages: ability to determine the role of specific genetic mutations, ability to target specific cell types  Disadvantages: diminished pathogenic relevance owing to lack of single-gene deletion in human disease  Conventional knock-out  IL10<sup>-/-88</sup>, IL2<sup>-/-89</sup>, JAK3<sup>-/-90</sup>, WASP<sup>-/-91</sup>, A20<sup>-/-92</sup>, TLR5<sup>-/-93</sup>, TCR<math>\alpha</math><sup>-/-94</sup>  Mdr1a<sup>-/-95</sup> and IL2R<math>\alpha</math><sup>-/-96</sup>  SHIP-1<sup>-/-97</sup>    TGF<math>\beta</math><sup>-/-98</sup>  RUNX3<sup>-/-99</sup>  Keratin 8<sup>-/-100</sup>  Muc2<sup>-/-101</sup>  Conditional knock-out  Myeloid/STAT-3<sup>-/-102</sup> and CD4/PDK1<sup>-/-103</sup>  Epi/C1 Galt1<sup>-/-104</sup>  Epi/XBP1<sup>-/-105</sup>  Epi/FADD<sup>-/-38</sup>  Epi/Casp8<sup>-/-39</sup>  Epi/N-cadherin<sup>-/-106</sup>  Conventional transgenic  IL7 transgenic<sup>107</sup>  T-bet<sup>+/+</sup>/RAG2<sup>-/-108</sup>  Anti-CD40/RAG<sup>-/-109</sup>  <i>Tnf</i><sup><math>\Delta</math>ARE/+37</sup></p>	<p>Chronic colitis  UC-like colitis  CD-like granulocyte and monocyte drive ileitis, lung inflammation  Chronic colitis and multi-organ failure  Chronic colitis, gastric lesions  Th2-driven colitis  Epithelial-driven colitis    Chronic colitis  Epithelial-driven UC-like colitis  Epithelial-driven acute ileitis  Epithelial-driven colitis  Terminal ileitis  CD-like ileitis    Chronic colitis  UC-like colitis, dendritic cell, and TNF-<math>\alpha</math> driven  Acute innate immune-mediated colitis  Acute and chronic CD-like ileitis, extra-intestinal manifestations (skin rashes, arthralgia)</p>
<p><b>Congenetic</b>  Advantages: spontaneous multifactorial disease with increased pathogenic relevance to the human condition  Disadvantages: poor breeding ability, increased cost of colony maintenance, and specific etiology unknown  SAMP1/Yit<sup>16</sup>    SAMP1/YitFc<sup>18</sup>    C3H/HeJBir<sup>110</sup></p>	<p>Spontaneous acute and chronic CD-like ileitis, extra-intestinal manifestations (skin lesions)  Spontaneous acute and chronic CD-like ileitis, extra-intestinal manifestations (skin lesions), early onset, perianal disease (5%), stricturing  Spontaneous colitis</p>

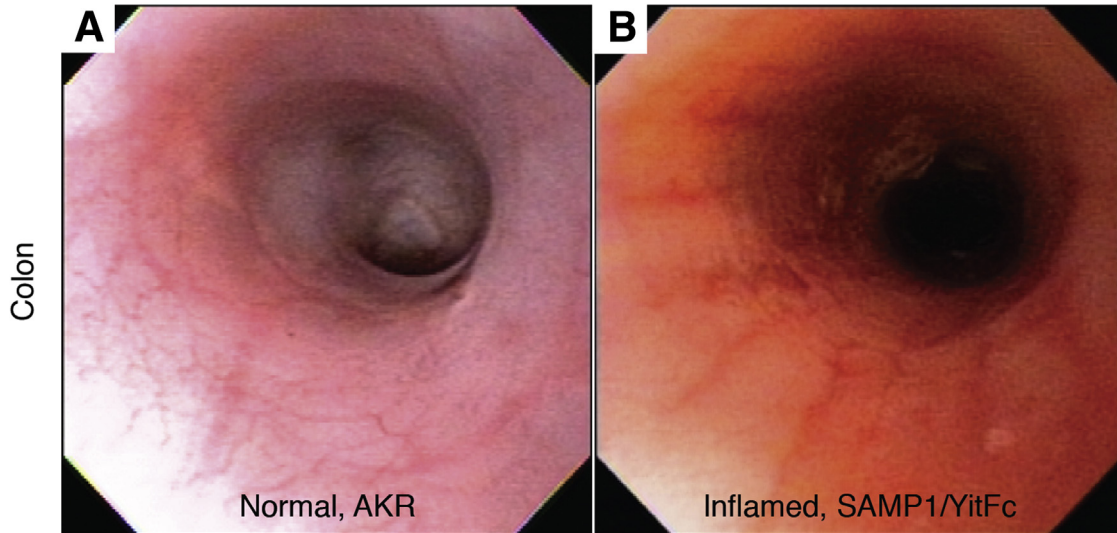
Casp8, caspase 8; C1 Galt1, Core 1 synthase glycoprotein-N-acetylgalactosyltransferase 1; DNBS, dinitrobenzene sulfonic acid; ECOVA, *E. Coli*-expressing OVA peptide; Epi, epithelial cell; FADD, FAS-Associated via Death Domain; IEC, intestinal epithelial cell; JAK3, Janus kinase 3; Mdr1a, multi-drug resistance protein 1a; Muc2, mucin 2; PDK1, phosphoinositide dependent protein kinase-1; PG-PS, peptidoglycan-polysaccharide; RAG, Recombination-Activating Gene; RUNX3, runt related transcription factor 3; SCID, severe combined immunodeficient; SHIP-1, SH2-containing inositol phosphatase-1; STAT3, signal transducer and activator of transcription 3; TCR, T-cell receptor; TGF, transforming growth factor; TLR, Toll-like receptor; WASP, Wiskott-Aldrich syndrome protein; XBP1, X-box binding protein 1.

**SAMP1/YitFc Mouse Model**

The spontaneous, inbred SAMP1/YitFc mouse strain originally was derived from selective brother-sister mating of parental AKR/J mice (The Jackson Laboratory, Bar Harbor, ME).<sup>15</sup> After 24 generations, the mice developed a

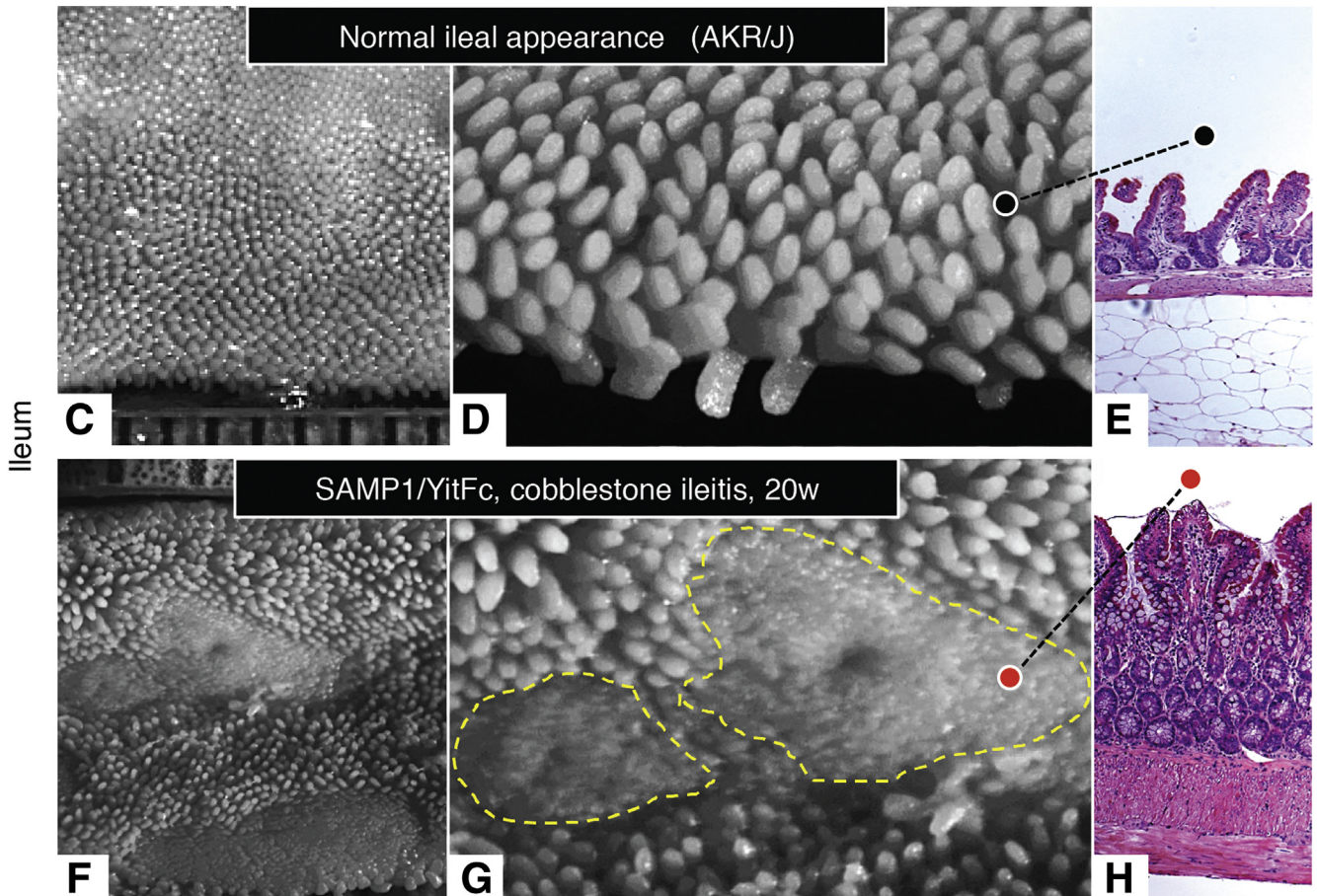
senescence-accelerated phenotype, yielding 10 lines of senescence-prone mice (ie, SAMP 1-10) that showed accelerated aging, skin lesions, and autoimmune dysfunctions. The SAMP1/Yit substrain subsequently was generated through continued brother-sister mating based on the

**ENDOSCOPY**



**STEREOMICROSCOPY**

**HISTOLOGY**



presence of skin lesions and ileitis.<sup>16</sup> By the 20th generation, the strain had lost its senescence-accelerated phenotype, and consistently showed both acute and chronic transmural small intestinal inflammation with nearly 100% penetrance by 30–40 weeks of age, and persisting up to 80 weeks of age. The disease was concentrated within the terminal ileum and cecum and manifested in a discontinuous pattern, similar to that seen in human CD. Continuous inbreeding of a SAMP1/Yit colony led to the development of a unique SAMP1/YitFc substrain, with a novel phenotype that included an earlier emergence of ileitis by 10 weeks of age, preceded by increased levels of intestinal interferon (IFN) $\gamma$  production by 4 weeks of age, and the development of perianal fistulae in 5%–10% of the colony, as well as intestinal strictures in nearly 50% of mice older than 40 weeks of age.<sup>17,18</sup> Of note, perianal disease and fibrostenotic/stricturing are both hallmark features of human CD that are not observed commonly in animal models of IBD.

SAMP1/YitFc ileitis is characterized by areas of discontinuous cobblestoning with transmural inflammation dispersed between areas of normal gut mucosa within the terminal ileum and cecum, similar to the “patchy” nature of CD (Figure 1).<sup>19</sup> Early alterations in epithelial architecture and defective barrier function are present by 3 weeks of age, preceding the onset of overt ileitis, and include increased numbers of secretory Paneth, goblet, and intermediate cells, with a simultaneous reduction in absorptive enterocytes. This trend increases at sites of active inflammation as the animals age and as the disease becomes more severe.<sup>20,21</sup> Ileal epithelial barrier dysfunction leads to infiltration of active and chronic immune cells that populate the intestinal lamina propria, and include both polymorphonuclear and mononuclear cells.<sup>17,18</sup> In fact, chronic SAMP1/YitFc ileitis shows large numbers of activated Th1-type polarized CD4<sup>+</sup> cells within the lamina propria that produce increased levels of intestinal TNF and IFN $\gamma$ , as well as increased numbers of CD8<sup>+</sup> $\alpha^+$  T-Cell Receptor (TCR) $\alpha\beta^+$  T cells. Likewise, the intraepithelial lymphocytic compartment also is shifted toward CD8<sup>+</sup> $\alpha^+$ TCR $\alpha\beta^+$  cells and away from CD8<sup>+</sup> $\alpha^+$ TCR $\gamma\delta^+$  cells.<sup>17</sup> The majority of SAMP1/YitFc mice with advanced disease (age, >40 wk) show thickening of the bowel wall that often leads to collagen deposition and terminal ileal stricture formation (Figure 1), as well as focal granulomatous inflammation, basal plasmacytosis, and neural hyperplasia. Interestingly, SAMP1/YitFc mice also show extraintestinal manifestations of CD (eg, skin rashes and inflammation of the eye), as well as pathologies similar to human perianal CD, including anal fissures, rectal prolapse, and perianal fistulae.<sup>18</sup> SAMP1/YitFc mice also have been reported to develop Crohn's-like gastritis (in the absence of *Helicobacter* infection),<sup>22</sup> autoimmune hepatitis,<sup>23</sup> and periodontitis.<sup>24</sup>

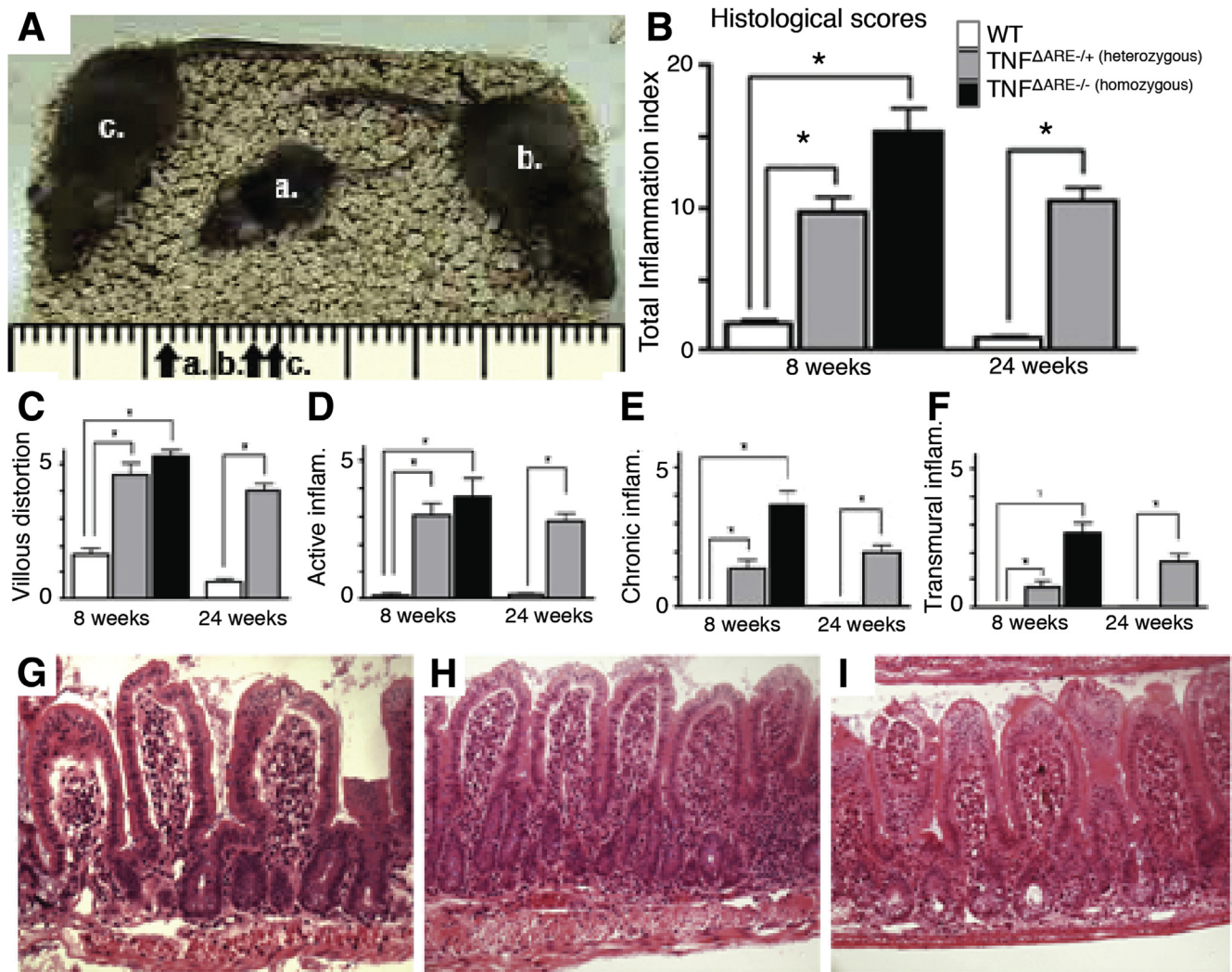
SAMP1/YitFc ileitis can be considered a truly spontaneous model of Crohn's ileitis because the disease presents independent of any need for genetic, chemical, or immunologic manipulation, and closely resembles the human condition with regard to location, histologic features, and extra-intestinal manifestations. The disease occurs in virtually 100% of mice by 10 weeks of age and persists for up to 80 weeks. Unknown host-microbial interactions amplify the severity of disease because SAMP1/YitFc mice reared under germ-free conditions still develop an attenuated ileitis, but exposure to the natural flora is necessary for its full manifestation.<sup>25</sup> Adoptive transfer experiments and cytokine blockade studies have shown that the disease also requires the presence of T cells and proinflammatory cytokines.<sup>17,26</sup> Finally, the disease is responsive to standard CD therapies, such as anti-TNF drugs and steroids,<sup>27</sup> and also responds to probiotics for disease prevention,<sup>28</sup> further supporting its use as a highly relevant model for understanding the pathogenic mechanisms that underlie the development and perpetuation of CD. Because of the spontaneous and multifactorial nature of this model, SAMP1/YitFc mice can be used to characterize specific new biomarkers, including microbiome and metabolomic markers, which may be important for monitoring drug response and management. One disadvantage of this model is the poor breeding ability of this strain, requiring a large breeding colony for yielding experimental mice and associated costs compared with other IBD models. Specific advantages and disadvantages of individual models are listed in Table 1.

### The *Tnf* <sup>$\Delta$ ARE/+</sup> Model

TNF is a central mediator of intestinal inflammation and IBD, most notably evidenced by the clinical effectiveness of anti-TNF monoclonal antibodies in treating both CD and UC.<sup>29–36</sup> The *Tnf* <sup>$\Delta$ ARE/+</sup> mouse model of Crohn's-like ileitis originally was developed to understand the mechanisms of TNF-driven Crohn's-like IBD and chronic inflammatory arthritis. Mice were generated by introducing a 69-bp targeted deletion of the ARE of *Tnf* into Sv/129-C57BL/6 mice.<sup>37</sup> The AU-rich element is an area of adenosine-uracil nucleotide repeats (AUUUA) located in the 3' untranslated region of a gene, and is responsible for messenger RNA destabilization and translational silencing. Interestingly, mice with intestinal-specific deletion of FAS-Associated via Death Domain and caspase-8, which are important regulators of TNF, develop small intestinal inflammation and terminal ileitis,<sup>38,39</sup> highlighting the critical role of TNF in mediating chronic small intestinal inflammation.

Mice homozygous for the deleted AU-rich element (*Tnf* <sup>$\Delta$ ARE/ $\Delta$ ARE</sup>) overexpress TNF messenger RNA and

**Figure 1.** (See previous page). **Characteristics of chronic intestinal inflammation in SAMP1/YitFc mice.** (A) Normal endoscopic appearance of colonic mucosa in AKR control mice showing a normal vascular pattern. (B) Endoscopic appearance of severe colitis in SAMP1/YitFc mice showing friable mucosa with hemorrhage and ulcerations. (C) Normal stereomicroscopic ileal appearance of a 20-week-old AKR control mouse with intact mucosa. (D) Higher magnification shows normal structure of intestinal villi with no visible lesions. (E) Histologic image of D (black dot) shows normal histologic morphology of intestinal villi and mucosa. (F) Abnormal stereomicroscopic ileal appearance of age-matched SAMP1/YitFc mouse showing the presence of cobblestone lesions. (G) Higher magnification shows a large cobblestone surrounded by distorted intestinal villi. (H) Histologic image of panel G (red dot) shows distorted and blunted villi with severe inflammatory cell infiltrate in mucosa.



**Figure 2. Characteristics of chronic intestinal inflammation in  $Tnf^{\Delta ARE/+}$  mice.** (A) Phenotypic appearance of 8-week-old  $Tnf^{\Delta ARE/+}$  littermates.  $Tnf^{\Delta ARE/\Delta ARE}$  mice (a) have severe wasting syndrome, ruffled coat, alopecia-like lesions, impairment in mobility, and increased mortality as compared with  $Tnf^{\Delta ARE/+}$  (b) or  $Tnf^{+/+}$  mice (c). (B) Total inflammatory scores (calculated as the sum of panels C–F) show severe ileitis in  $Tnf^{\Delta ARE/\Delta ARE}$  mice and moderate ileitis in  $Tnf^{\Delta ARE/+}$  compared with  $Tnf^{+/+}$  littermates. (C) Villous distortion index, (D) active inflammation index, (E) chronic inflammation index, and (F) transmural inflammation index are increased significantly in  $Tnf^{\Delta ARE/\Delta ARE}$  and  $Tnf^{\Delta ARE/+}$  vs  $Tnf^{+/+}$  littermates ( $*P < .001$ );  $n = 12$ /group. H&E-stained sections of the terminal ileum from 8-week-old mice show increased intestinal inflammation in (I)  $Tnf^{\Delta ARE/\Delta ARE}$  and (H)  $Tnf^{\Delta ARE/+}$  as compared with (G)  $Tnf^{+/+}$  mice. inflam, inflammation; WT, wild type.

protein, and develop early severe inflammatory disease, limiting their lifespan to only 5–12 weeks (Figure 1). Heterozygous  $Tnf^{\Delta ARE/+}$  mice develop chronic arthritis and Crohn's-like ileitis, with a normal lifespan and sporadic evidence of mild liver and lung inflammation, as well as occasional proximal colitis. The intestinal disease in  $Tnf^{\Delta ARE/+}$  heterozygotes includes early villous blunting, with severe patchy terminal ileitis by 8 weeks of age and acute and chronic transmural inflammation by 16 weeks of age; older mice (age, 5–7 mo) show a loss of villous architecture and the development of granulomas, similar to that seen in patients with severe active CD (Figure 2). Unlike the associated arthritis,  $Tnf^{\Delta ARE/+}$  ileitis is dependent on the presence of mature B and T cells, suggesting differential mechanisms of disease within the 2 organ systems.

Moreover, the intestinal disease is driven by T-helper (Th)1 cytokines (IL12, IFN $\gamma$ ) and cytotoxic CD8 $^{+}$  effector T cells.<sup>40</sup>

### Pathogenic Mechanisms of Crohn's-Like Ileitis

The SAMP1/YitFc and  $Tnf^{\Delta ARE/+}$  mouse models are particularly useful for understanding mechanisms of IBD pathogenesis that may uniquely lead to the development of Crohn's ileitis. We summarize some of the important findings from studies performed using these 2 models, and how they have contributed to our general understanding of experimental Crohn's ileitis and its application to the human condition.

### The Th1/Th2 Paradigm Revisited

CD traditionally was regarded as an adaptive immune disease, primarily driven by Th1-type immune responses. By using SAMP1/YitFc mice, Bamias et al<sup>26</sup> tested the hypothesis that the natural course of ileitis may be characterized instead by multiple immunologically distinct phases, and that the Th2 pathway also may be important during the maintenance phase of ileitis. Histologic analysis showed that a distinct natural course of SAMP1/YitFc ileitis exists with an early inductive phase of acute ileitis present from 4 to 8 weeks of age that correlates with increased production of the Th1-type cytokines, TNF and IFN $\gamma$ , followed by a chronic phase that begins after 8 weeks of age that is associated with continued increased production of Th1 cytokines, but also increased levels of the Th2 cytokines IL4 and IL5 by 23 weeks of age.

Recent studies have investigated the role of IL12/IL23 and IL17 blockade as a novel therapy for IBD, leading to the recent approval of a monoclonal antibody against p40 (ustekinumab) for the treatment of CD.<sup>41</sup> Interestingly, blockade of IL17 had no effects and a higher rate of adverse events in a randomized controlled trial of patients with CD.<sup>42</sup> Of note, the role of IL12/IL23 and IL17 in SAMP1/YitFc and *Tnf* <sup>$\Delta$ ARE/+</sup> mice have not been studied fully, and it would be interesting to see whether blockade of either IL23 or IL17 in these models correlates with the reported results in patients with CD.

### Epithelial Alterations and Barrier Dysfunction

The precise regulation of the intestinal barrier allows the maintenance of mucosal immune homeostasis and prevents the onset of uncontrolled inflammation. In support of this concept, several lines of evidence point to defects in components of the epithelium as etiologic factors in the pathogenesis of IBD.<sup>43</sup> For example, early SAMP1/YitFc ileitis results from an underlying defect in epithelial barrier function. By using bone marrow chimera experiments in SAMP1/YitFc and AKR/J control mice, Olson et al<sup>20</sup> discovered a primary defect in SAMP1/YitFc mice attributed to nonhematopoietic cellular sources, and found decreased epithelial barrier resistance and increased epithelial permeability before the onset of ileitis, suggesting early epithelial barrier dysfunction that precedes the development of disease. Moreover, additional bone marrow chimera experiments showed that hematopoietic cells isolated from AKR/J control mice were capable of inducing ileitis in the presence of abnormal SAMP1/YitFc epithelium. The effect occurred in the absence of a commensal flora and was supported by differential expression of tight junction proteins, claudin-2 and occludin, in ileal epithelial cells from SAMP1/YitFc compared with AKR/J mice. Lopetuso et al<sup>44</sup> recently showed that SAMP1/YitFc mice express increased ileal epithelial Toll-like receptor 5 and serum anti-flagellin IgG antibodies, which both were augmented further in the presence of the gut microbiome. Toll-like receptor 5 activation in response to bacterial flagellin, in fact, altered expression of tight junction proteins and resulted in an inability to maintain appropriate epithelial barrier integrity of ex vivo-stimulated ileal tissues from

SAMP1/YitFc mice. In *Tnf* <sup>$\Delta$ ARE/+</sup> mice, 2 separate reports clearly show the ability of TNF, specifically overexpressed in the intestinal epithelium, to sufficiently generate chronic ileitis in the absence of extra-intestinal manifestations, and point out the central role of the gut epithelium in this model.<sup>45,46</sup> This concept is supported further by Marini et al,<sup>27</sup> who identified that decreased intestinal epithelial apoptosis was a key mechanism of action of anti-TNF therapy in SAMP1/YitFc mice.

The role of mucosal healing and resolution of inflammation is an active area of investigation in the field of IBD pathogenesis. SAMP1/YitFc and *Tnf* <sup>$\Delta$ ARE/+</sup> mice develop Crohn's-like ileitis in the absence of spontaneous colonic inflammation, making them very useful models to study the contribution of mucosal healing in the presence of immunologic and genetic dysfunction, independent of active colonic inflammation. In fact, Corridoni et al<sup>47</sup> recently showed that induction of acute colitis by DSS administration in SAMP1/YitFc mice generated more severe and prolonged colitis compared with AKR control mice, suggesting that mucosal healing and impaired resolution of inflammation may be important in this model. Further studies are warranted to determine the precise potential dysfunction of mucosal healing in both models and its contribution to the pathogenesis of IBD.

### Mechanisms of Leukocyte Trafficking

One of the major recent advances in the treatment of IBD has been the approval of agents that specifically block leukocyte trafficking to the gut, such as vedolizumab.<sup>48</sup> Seminal work using both SAMP1/YitFc and *Tnf* <sup>$\Delta$ ARE/+</sup> mice have shown the importance of blocking leukocyte trafficking to the inflamed intestinal mucosa, and the critical role of adhesion molecules, such as endothelial PSGL1,<sup>49</sup> CCL25/CCR9,<sup>50</sup>  $\alpha_4\beta_7$ ,<sup>51</sup> CCL21,<sup>52</sup> and S1P1<sup>53</sup> in the pathogenesis of IBD.

### The Role of Innate Immunity and NOD2

*CARD15/NOD2* (also known as *IBD1*) is a gene associated with CD in human beings.<sup>7,8</sup> The gene product, NOD2, is an intracellular pattern recognition receptor and key mediator of intestinal innate immunity through sensing of its ligand, bacterial muramyl dipeptide (MDP), and initiation of NF- $\kappa$ B-dependent immune responses within the gut. The identification of *NOD2* generated a fundamental shift in understanding CD pathogenesis, with a new focus on mechanisms of innate immunity and host-microbial interactions within the gut. Interestingly, *NOD2* genetic deletion in SAMP1/YitFc mice significantly suppresses chronic ileitis by approximately 50%,<sup>54</sup> supporting the concept of a dichotomous role of *NOD2* in early vs late (chronic) phases of intestinal inflammation.<sup>47</sup>

Of note, only 10%–15% of Crohn's patients actually possess mutations within the *NOD2* gene,<sup>7</sup> raising the question of whether the remaining 85% of patients actually experience a functional defect in their MDP/*NOD2* pathway despite having a normal *NOD2* gene. SAMP1/YitFc mice represent a suitable model system to address this question

because these mice also lack *Nod2* mutations.<sup>55,56</sup> MDP administration is known to prevent the development of DSS colitis in normal mice.<sup>57</sup> However, treatment of SAMP1/YitFc mice with MDP before DSS colitis induction has no mitigating effects, and deletion of *Nod2* in SAMP1/YitFc mice causes a decrease in the severity of ileitis, supporting the concept that a functional defect related to the NOD2/MDP pathway contributes to Crohn's-like ileitis in SAMP1/YitFc, despite their wild-type *Nod2* status.<sup>55</sup>

### Defining New Predisposing Genes

Because SAMP1/YitFc mice are one of the only animal models of IBD that develops intestinal inflammation spontaneously without genetic, chemical, or immunologic manipulation, these mice can provide insights into the possible genetic determinants of CD in human beings. A genome-wide scan of SAMP1/YitFc mice identified 4 susceptibility loci on chromosomes 9, 6, 8, and X.<sup>58</sup> The loci on chromosome 9 (*Ibdq1*) was linked strongly to epithelial changes in SAMP1/YitFc mice and contains several potential candidate genes that may play a role in promoting epithelial abnormalities during Crohn's-like ileitis. Interestingly, the *Ibdq1* region overlaps with 2 chemically induced colitis susceptibility loci (*Dss* and *Tnbs1*), as well as the genes that encode IL10-receptor antagonist and IL18, an epithelial-derived Th1-polarizing cytokine that has protective effects in TNBS-induced murine colitis.<sup>59</sup> The loci on chromosome 6 (*Ibdq2*) also was linked to ileitis and includes a homolog for the human Chr3 (p21-p26) region in which several genome-wide association studies have identified CD susceptibility loci.<sup>60-64</sup> The remaining 2 loci on chromosomes 8 and X (*Ibdq3* and *Ibdq4*, respectively) appear to associate with epithelial changes in SAMP1/YitFc ileitis.<sup>58</sup> Linkage with *Ibdq3* only occurs in young mice (age, 10–12 wk), suggesting that this region may include susceptibility loci involved in the early/acute phase of disease.

The *Ibdq2* region also contains the gene that encodes peroxisome proliferation-activated receptor (PPAR) $\gamma$ , a nuclear receptor that is highly expressed by intestinal epithelial cells and inhibits innate immune responses through suppression of NF- $\kappa$ B-dependent signaling. Sugawara et al<sup>56</sup> reported that PPAR $\gamma$  expression levels were reduced in the ilea of SAMP1/YitFc mice compared with AKR/J controls, and the magnitude of this difference correlated with age and the severity of ileitis. A mutation in the gene encoding PPAR $\gamma$  could provide an explanatory mechanism for the observed functional abnormality in the NOD2 pathway in SAMP1/YitFc mice despite the fact that these mice lack any *Nod2* mutations because PPAR $\gamma$  and NOD2 may cooperatively regulate NF- $\kappa$ B-dependent signaling and innate intestinal immune responses.<sup>65</sup> After the advent of new methodologies, genome-wide sequencing of SAMP1/YitFc mice has been performed by our group and the results will be available in the near future.

### New Imaging Modalities

The development of advanced murine imaging technologies and validated scoring systems for quantifying the

extent of intestinal inflammation has the potential to greatly enhance the quality and generalizability of data obtained from animal studies in IBD. Kodani et al<sup>66</sup> used SAMP1/YitFc mice to develop and validate murine endoscopy as a useful technology for assessing intestinal inflammation without killing the animal. This nonlethal procedure allows comparative assessments of endoscopic inflammation within a single animal (Figure 1). An accompanying validated scoring system allows investigators to systematically quantify the severity of inflammation and the presence of tumors within the murine intestine in a manner that is generalizable across laboratories and studies, as well as within a particular experiment.

Another newly developed technology for assessing intestinal inflammation in mice uses stereomicroscopic analysis of the 3-dimensional intestinal architecture in conjunction with localized myeloperoxidase activity levels to characterize the microscopic features and quantify levels of intestinal inflammation. By using this method, Rodriguez-Palacios et al<sup>19</sup> found SAMP1/YitFc mice showed unique 3-dimensional stereomicroscopic features (cobblestoning) within their inflamed intestines that was not seen in *Tnf<sup>ΔARE/+</sup>* mice; cobblestone lesions are also a signature pathologic feature reported in the inflamed intestinal tissues of patients with CD. This technology has the potential to be paired with molecular imaging. In fact, prior studies of molecular imaging using anti-mucosal addressin cellular adhesion molecule-1 antibodies conjugated to encapsulated gaseous microbubbles have been used successfully to detect and quantify ileal inflammation in both the SAMP1/YitFc and *Tnf<sup>ΔARE/+</sup>* mice.<sup>67</sup> In addition, our laboratories also are involved in developing novel noninvasive magnetic resonance imaging techniques to quantify intestinal inflammation in both SAMP1/YitFc and *Tnf<sup>ΔARE/+</sup>* mice.

### Role of Novel Cytokines

Initial studies of IL1 blockade in rabbit formalin-induced colitis clearly showed the power of animal models to decipher the role of cytokines in mediating intestinal inflammation.<sup>9,68</sup> Given their spontaneous nature and marked similarities to the human condition, both the SAMP1/YitFc and *Tnf<sup>ΔARE/+</sup>* models have proven highly useful for evaluating the role of novel cytokines in experimental Crohn's-like ileitis. TNF-like ligand 1A (TL1A) is a member of the TNF superfamily of proteins that binds to death receptor 3 (DR3) and provides co-stimulatory signals to activated T lymphocytes. Inflamed intestinal tissues from patients with CD produce increased TL1A levels, specifically antigen-presenting cells within the intestinal lamina propria and CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes; in vitro studies have shown that TL1A induces IFN $\gamma$  production by activated lamina propria mononuclear cells isolated from CD patients.<sup>69</sup> To better understand the pathogenic mechanisms related to this novel cytokine and its receptor in CD, Bamias et al<sup>70</sup> used SAMP1/YitFc and *Tnf<sup>ΔARE/+</sup>* mice to study the role of the TL1A/DR3 signaling complex during spontaneous experimental Crohn's-like ileitis. Increased levels of both TL1A and the active transmembrane form of DR3 were



found in the inflamed mucosa of both strains. Specifically, TL1A was expressed by CD11c<sup>+</sup> dendritic cells within the lamina propria and induced proliferation of memory CD4<sup>+</sup>CD45RB<sup>lo</sup> T cells, but not CD4<sup>+</sup>CD45RB<sup>hi</sup> cells. The TL1A/DR3 signaling complex synergized with IL12/IL18 signaling to induce IFN $\gamma$  production by activated T lymphocytes, promoting Th1 immune responses and ileitis.

SAMP1/YitFc ileitis shows a dichotomous immune profile, with Th1 immune responses driving the early acute phase and Th2 immune responses present during chronic ileitis (after 8 weeks of age).<sup>26</sup> IL33 is a Th2-polarizing cytokine that is increased in the intestinal epithelium of patients with UC,<sup>71</sup> and recently was found to be increased in a subpopulation of pediatric patients with stricturing Crohn's ileitis.<sup>72</sup> To determine whether IL33 plays a protective or pathogenic role in IBD, De Salvo et al<sup>73</sup> used SAMP1Yit/Fc mice to study the role of IL33 within the context of chronic intestinal inflammation. SAMP1/YitFc ileitis was associated with a large infiltration of eosinophils by 12 weeks of age, which persisted through 20 weeks of age and correlated with disease severity and production of Th2 cytokines (ie, IL33 and IL5) and eotaxins. IL33 levels correlated positively with the level of inflammation and the number of invading eosinophils, and blockade of IL33 resulted in decreased inflammation and eosinophils, as well as reduced Th2 cytokine production, suggesting a proinflammatory role for IL33 within the context of chronic experimental IBD. Interestingly, induction of IL33 was dependent on interaction with the gut microbiome, and fecal transplantation from specific pathogen-free SAMP1/YitFc mice donors into germ-free-raised SAMP1/YitFc recipients restored increased IL33, as well as eosinophilia, in these mice.

### Role of the Gut Microbiome

The vast majority of animal models of IBD do not develop inflammation when reared under germ-free conditions, strongly implicating the gut microbiome as a contributor to IBD pathogenesis. However, Bamias et al<sup>25</sup> showed that germ-free SAMP1/YitFc mice develop ileitis with varying degrees of severity, the majority showing a milder form of the disease, confirming the importance of the gut microbiome as a modulating factor of chronic intestinal inflammation. The absence of a commensal flora appears preferentially to impact the Th2-driven chronic phase of SAMP1/YitFc ileitis, with significantly reduced levels of IL33, and downstream IL5 and IL13 at 13 weeks of age, and decreased chronic inflammatory scores through 30 weeks of age, providing further evidence that interactions with the gut microbiome drive production of Th2 cytokine production during the chronic phase of SAMP1/YitFc ileitis.<sup>26,73</sup>

The gut microbiome also appears to influence the function of regulatory T cells during SAMP1/YitFc ileitis. Mesenteric lymph nodes from germ-free SAMP1/YitFc mice have decreased numbers of CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> regulatory T cells. Moreover, CD4<sup>+</sup> lymphocytes from germ-free mice are able to confer disease to severe combined immunodeficient recipients, unlike CD4<sup>+</sup> cells derived from SPF SAMP1/YitFc mice, suggesting that absence of exposure to

the gut microbiome compromises normal development of the regulatory component of the CD4<sup>+</sup> T-cell population in SAMP1/YitFc mice.<sup>25</sup> This finding is supported further by studies in SAMP1/YitFc mice showing dysfunction within their mesenteric lymph node regulatory T-cell compartment.<sup>74</sup> Studies performed in *Tnf<sup>ΔARE/+</sup>* mice also confirm the importance of host-gut microbiome interactions and dysbiosis in the pathogenesis of CD-like ileitis.<sup>75,76</sup> Schaubek et al<sup>77</sup> recently showed that germ-free *Tnf<sup>ΔARE/+</sup>* mice were free of intestinal inflammation. In addition, 16S analysis and metaproteomics showed specific compositional and functional alterations of bacterial communities in inflamed mice. Finally, transplantation of disease-associated, but not healthy, microbiota transmitted CD-like ileitis to germ-free *Tnf<sup>ΔARE/+</sup>* recipients. In comparative studies, Roulis et al<sup>76</sup> showed that defective expression of antimicrobials and dysbiosis are characteristic of TNF-driven CD-like ileitis. In addition, they showed that indigenous microbiota is sufficient to drive TNF overexpression and CD-like ileitis in this model.

### Conclusions

Mouse models of IBD are extremely useful tools for exploring pathogenic mechanisms of chronic intestinal inflammation. The many available models vary by the type of inflammation that they produce (acute vs chronic), their mode of generation (chemical, genetic, immunologic, or spontaneous), and whether they show colitis or ileitis. These factors require careful consideration when choosing a model to use in scientific investigations. However, to understand pathogenic mechanisms that are relevant to the human condition and preclinical testing of novel treatments, spontaneous models, such as SAMP1/YitFc mice, offer a unique advantage and strong scientific premise. With continuing advances in targeted drug technologies, important mechanistic findings from animal studies now can be translated readily into clinical development of novel therapies to combat these devastating diseases.

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#### Conflicts of interest

The authors disclose no conflicts.

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