

Chapter 21

Assessing Inflammatory Disease at Mucosal Surfaces in Murine Genetic Models

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Abstract

Inflammatory diseases of the mucosal surfaces are rising worldwide and particularly in the Western world that is witnessing unprecedented increases in the number and incidence of both asthma and inflammatory bowel disease. The laboratory mouse allows the application of the full panoply of modern genetic, immunological and biochemical tools to increase our understanding of how inflammation arises and how it might be controlled at mucosal surfaces. Here we provide a detailed description of how to systematically assess inflammatory disease in the lung and intestines of the laboratory mouse. We provide histopathology examples from SHIP mutant mice that are the only known genetic mutant to suffer from pulmonary consolidation, asthma, and Crohn's disease. The intent of this chapter is to facilitate increased surveillance of mucosal inflammation in studies where the laboratory mouse is utilized so that we can better understand the cell types, genes, and microorganisms that contribute to mucosal inflammatory disease and thereby develop more effective therapies and preventive strategies.

Key words: Pneumonia, Asthma, IBD, Mouse models, T cells

Crohn's disease (CD) and ulcerative colitis (UC) are chronic inflammatory diseases of the alimentary tract with multiple etiologies, distinguishable by unique histopathological phenotypes and pathogenic mechanisms (1–5). CD classically occurs in the ileum, begins with the formation of multiple aphthoid ulcers infiltrated by neutrophils, which progress and coalesce into a segmental transmural ileitis with granuloma, strictures, fissures, and fistulas. In contrast, UC is classically confined to the colon and comprises a diffuse, superficial, mucosal–submucosal colitis. Histopathological heterogeneity exists within each disorder.

Etiologically, this spectrum of enteric autoimmune inflammatory diseases results from complex polygenic and environmental interactions. Although genome-wide association studies have identified more than 30 susceptibility loci for IBD that disturb either intestinal epithelial cell (IEC) barrier homeostasis or immune effector or regulatory cell functions and interactions with luminal commensal

microflora, many susceptibility genes possess differing functions in IEC and hematopoietic cells, and the specific IEC or immunologic defects in a majority of genetically susceptible individuals remain enigmatic (6–9).

Mouse models representative of the major IBD phenotypes have contributed to an understanding of the pathogenic mechanisms of IBD, and more are needed to identify potential opportunities for therapeutic approaches. Models include those mice that spontaneously develop IBD-like enteritis/colitis as a result of a naturally occurring mutation or via gene targeting or transgene introduction, or mice that are induced to develop IBD-like enteritis/colitis by exposure to an exogenous (e.g., haptening) agent. Mouse models have contributed to identifying many of the interacting factors that contribute to IBD pathogenesis, including IEC integrity and function, alterations in the mucus–antimicrobial secreted barrier, and the presence of defective regulatory or excessive effector T-cell function (10–22).

Mouse models have revealed an etiologic interplay between immunologic dysregulation and commensal microbiota. Model strain-specific immunoregulatory defects that result in an IBD-spectrum phenotype include targeted deletion of receptors for either transforming growth factor (TGF)- β or interleukin (IL)-10, defects in regulatory T cell (Treg) modulation of effector functions via TGF- β - or IL-10-dependent mechanisms, aberrant B-cell specificity, serum autoreactivity, or myeloid cell dysfunction. Pro-inflammatory cytokines increase in IBD, including tumor necrosis factor (TNF)- α , the Th1-polarizing cytokines IL-12 and IFN- γ , and IL-23, the latter of which sustains a Th17-associated immune response (10–16). That primary defects in immune cell function contribute to CD in some human patients is supported by reports of disease remission following allogeneic bone marrow transplantation (17).

Mouse models have also revealed that epithelial barrier functions are critical to enteric homeostasis. Model strain-specific defects in epithelia proposed to result in enteritis include altered IEC cytokine expressions, increased IEC paracellular permeability due to inadequate IEC tight junctions, mucosal leakiness caused by a dominant negative epithelial N-cadherin, the absence of an intestinal mucus component in *Muc2*^{-/-} mice which contributes to an endoplasmic reticulum (ER) stress response in goblet cells causing reduced mucus secretion and an increase in pro-inflammatory cytokines, an enterocyte-specific ablation of nuclear factor kappa B (NF- κ B) activation which permits microflora to invade beyond the epithelial barrier and results in an exuberant inflammation, the inability of a Paneth cell-derived NOD2 variant to expel luminal bacteria, diminished IEC autophagy and antimicrobial granule exocytosis, and altered IEC toll-like receptor (TLR) expression with potentially dysregulated responses to common bacterial motifs (18–24).

Luminal commensal microbiota is normally host protective, but in the presence of mucosal immunologic dysregulation, contribute to IBD progression. Normally, TLR signaling activated by commensal microbiota provides cytoprotective signals to epithelia. In the absence of microflora in germ-free mice, immune dysregulation does not result in IBD. Indeed, dysregulated, exaggerated immune responses in IBD may be directed against microflora initially and later evolve into an autoimmune disease, to include autoantibody formation. CD and UC phenotypes appear to have unique pathogenic mechanisms with respect to host interactions with commensal microflora. *NOD2* (*CARD15*), the intracellular sensor of bacterial peptidoglycan, and autophagy factors *ATG16L1* and *IRGM*, are genetic factors for CD but not for UC. Understanding how host–microbiota interactions are disrupted in IBD phenotypes also requires an understanding of the composition of normal luminal microflora.

Mouse models of IBD are often induced rather than spontaneously developing models and frequently involve the colon (5, 7, 25). Three CD mouse models that spontaneously develop chronic inflammation of the ileum are the SAMP1/Yit mouse, the TNFΔARE mouse, and the Src homology 2 (SH2)-containing inositol-5-phosphatase (SHIP)^{-/-} mouse (26–28). SAMP1/Yit mice spontaneously develop terminal ileitis by 10 weeks of age driven by Th1- and Th2-type immune responses, the severity of which can be decreased by anti-TNF antibodies (25, 29, 30). Although SAMP1/Yit mesenteric lymph node CD4⁺ T cells are capable of adoptively transferring ileitis to SCID mice, increased IEC paracellular permeability may be a principle susceptibility factor in this model (22). The TNFΔARE mouse model was generated by deletion of 69 bp within the AU-rich region (ARE) of the gene that encodes TNF, resulting in elevated systemic TNF levels associated with expansion of CD8⁺ effector cell subpopulations that mediate chronic ileitis and arthritis in both heterozygous and homozygous mice (27, 31). Hematopoietic cells of neither the SAMP1/Yit nor the TNFΔARE mouse are capable of adoptively transferring CD-like disease to an immunocompetent host. SHIP^{-/-} mice develop segmental, transmural pyogranulomatous ileitis that recapitulates many features of CD enteric pathology by 8 weeks of age (28). Reconstitution of SHIP^{-/-} mice with SHIP-competent hematopoietic cells prevents CD-like ileitis, while transfer of SHIP^{-/-} spleen cells to an immunocompetent host elicits CD-like ileitis. SHIP signaling limits the number and function of immunoregulatory cells either by limiting the survival of FoxP3⁺ Treg cells or by preventing the inappropriate acquisition of FoxP3 expression by naïve CD4 T cells (32–41). FoxP3⁺ Treg cells play an important anti-inflammatory role. SHIP-deficiency also promotes the inappropriate expansion of myeloid immunoregulatory (MIR) cells, and limits PI3K activation of key downstream immune effector

pathways such as Akt and NF- κ B (35, 36). SHIP^{-/-} granulocytes are less susceptible to apoptotic signals, and granulocyte–monocyte infiltrations are found in multiple organs in SHIP^{-/-} mice. A role of SHIP1 in human IBD is suggested by the demonstration that a chromosome 2 polymorphism located at 2q37 is associated with a significantly increased and early onset of IBD, and that the SHIP1 locus is found in the 2q37 region (42).

Mucosal homeostasis appears to result from various cooperative host contributions that reinforce the IEC structural barrier including its tight junctions, relies on an appropriately orchestrated mucosal immune response, all interacting with commensal microbiota at the luminal surface. These host contributions include goblet cell secretion of mucus, Paneth cell secretion of antimicrobial peptides, IEC NF- κ B activation by bacterial products, IEC transport of dimeric immunoglobulin A (IgA) produced by plasma cells in the lamina propria, and the IEC release of intra-luminal secretory IgA. Diminution of this structural and functional mucosal barrier may permit commensal microbiota to aberrantly or excessively stimulate the mucosal-based immune system and elicit a pro-inflammatory T cell-mediated immune response, acutely manifest as a granulocyte–monocyte infiltration. Primary defects in immunologic regulation may also initiate IBD in the absence of an IEC breach, as commensal microflora may routinely trigger mucosal-based antigen-presenting cells (APC) to produce pro-inflammatory cytokines, and encourage a mixed Th1/Th17-driven inflammation, which in the absence of adequate immunologic regulation manifests as an IBD spectrum disease.

Evaluations of future IBD mouse models require a systematic and comprehensive approach. Since the spectrum of enteric autoimmune inflammatory diseases that may model IBD are histopathologically heterogeneous, the entire alimentary tract, including the esophagus, stomach, duodenum, jejunum, ileum, cecum, and colon and associated mesentery and mesenteric lymph nodes may need to be evaluated morphologically. A comprehensive evaluation of all organs will assist with determining whether other inflammatory lesions in organs outside the alimentary tract develop concordant with the IBD phenotype. For example, TNF Δ ARE mice develop both ileitis and arthritis and SHIP^{-/-} mice develop both ileitis and eosinophilic crystalline pneumonia (43–45).

During necropsy, collection and preparation of the gastrointestinal tract should be accomplished first to minimize autolysis. The small intestine should be separated from the cecum at the rotund sac and gently lifted from the abdominal cavity, removing the attached mesenteric tissue, and the pancreas at the duodenojejunal flexure, and then separated from the stomach at the pars pylorica. After removal of the reproductive organs, the cecum is lifted and the colon removed by freeing it from attached mesentery. The intestines must then be insufflated with a 10 ml syringe of fixative

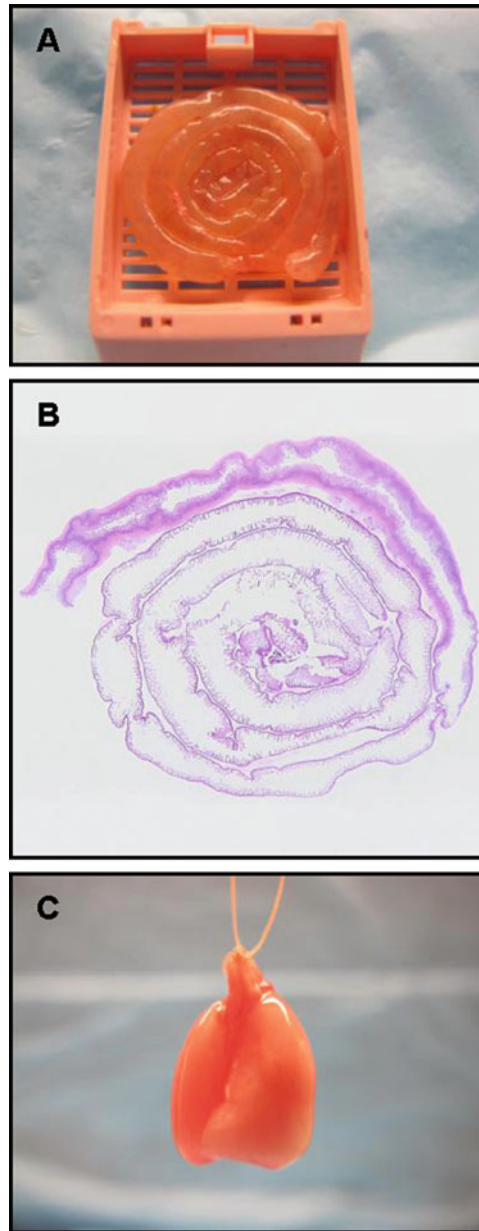


Fig. 1. (a) Insufflated small intestine rolled to fit as a “Swiss roll” into a cassette for histological processing. (b) Full-length small intestine thin section preparation for microscopic assessment from an SHIP^{-/-} mouse with regional thickening of the ileum. (c) Insufflated lungs ready for cassette placement and histological processing.

by introducing a 17–22 gauge sharp needle along the length and at the ends of the bowel, and holding the intestine and penetrating needle firmly with forceps, or by using a 20 gauge ball-tip gavage needle introduced at the ends of the bowel. Long intestinal segments (e.g., small intestine, large intestine) should be rolled to fit as “Swiss rolls” into cassettes for histological processing (Fig. 1a).

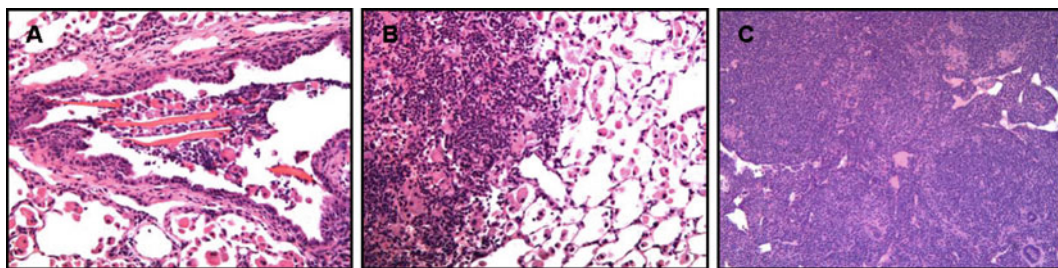


Fig. 2. Eosinophilic crystalline pneumonia of SHIP^{-/-} mice with (a) large crystals in bronchiolar airways, focal mixed leukocyte infiltration, bronchiolar subepithelial fibrosis, hypertrophy, and mucous metaplasia of bronchiolar epithelium, numerous alveolar macrophages, and multinucleate cells, which result in patchy (b) to lobar (c) pulmonary consolidation.

This will create a full-length intestinal preparation for microscopic assessment (Fig. 1b). The insufflated intestine is rolled in concentric circles, orienting the proximal end within the center of the roll. Similarly, the lungs should be insufflated with fixative to ensure adequate presentation for microscopic morphologic evaluation (Fig. 2). During necropsy, the soft tissues of the ventral neck as well as the ventral thoracic ribcage are removed exposing the epiglottis, larynx, trachea, esophagus, thymus, heart, and lungs. Using a similar needle and syringe as with the intestine, the needle is placed in the glottis and the lungs are insufflated to a size normal achieved during inhalation.

Histological sections of each segment of the alimentary tract should be masked, assessed, and assigned an inflammatory grade relevant to the IBD phenotype that presents. Grading schemes used may vary depending on the IBD phenotype that manifests, but should reflect disease progression and represent the most advanced lesion identified in each section. As an example, in the SHIP^{-/-} mouse model of CD, grades of 0–6 were assigned to sections of ileum, with grade 6 representing the most progressed, severe lesion (Fig. 3). Inflammatory grades assigned reflected progressively more severe disease with an inflammatory grade 0 representing no significant abnormalities; an inflammatory grade 1 representing mild predominantly polymorphonuclear (PMN) leukocyte infiltrations (<25 PMN/hpf) of the lamina propria and/or enteric lymph nodule; an inflammatory grade 2 representing moderate predominantly PMN leukocyte infiltrations (>25 cells/hpf) of the lamina propria and/or lymph nodule; an inflammatory grade 3 representing marked inflammatory cell infiltrations with extension below the muscularis mucosa causing architectural distortion of the mucosa and submucosa with attendant crypt hyperplasia; an inflammatory grade 4 representing marked infiltrations with extension into the tunica muscularis; an inflammatory grade 5 representing marked transmural leukocyte infiltrations; and an inflammatory grade 6 representing marked transmural leukocyte infiltrations

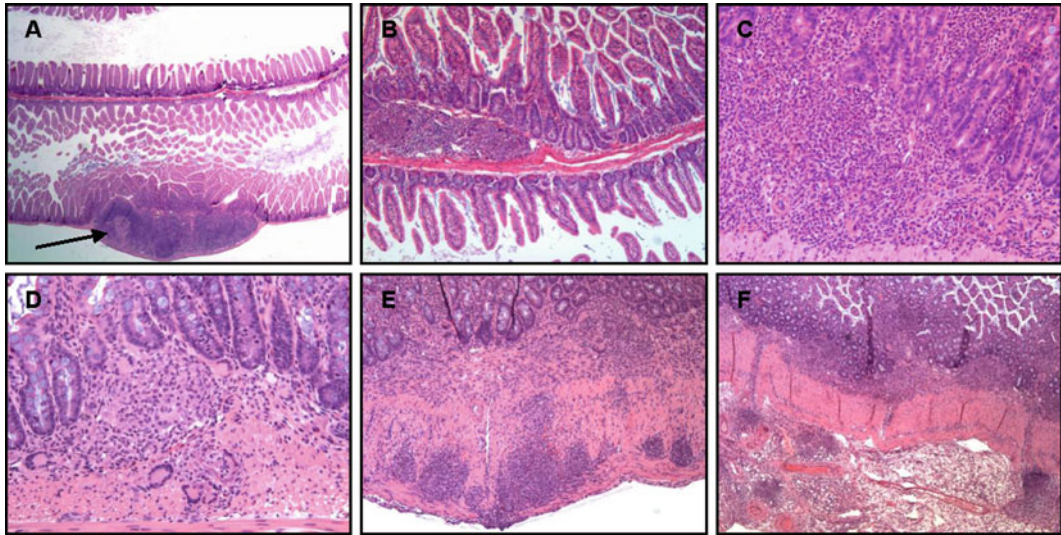


Fig. 3. Histopathological appearance of the small intestine of SHIP^{-/-} mice with CD-like disease assessed as inflammatory (a) grade 1 with mild polymorphonuclear (PMN) leukocyte infiltrations of the lamina propria and enteric lymph nodule (arrow); (b) grade 2 with moderate PMN leukocyte infiltrations; (c) grade 3 with marked PMN infiltrations extending below the muscularis mucosa; (d) grade 4 with marked infiltrations extending into the tunica muscularis; (e) grade 5 with marked transmural leukocyte infiltrations; (f) grade 6 with transmural leukocyte infiltrations extending into the mesentery.

with extension into the mesentery and/or other organs. In each section the presence of granuloma, crypt abscess, stricture, or fissure can be noted.

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