CASE 37
CROHN'S DISEASE

A defect in mucosal innate immunity results in inflammation of the gut.

The intestinal epithelium is the innermost layer of the gut mucosa and provides the surface that is essential for nutrient and vitamin absorption, water handling, and secretion of waste. The gut mucosa is also a crucial immune organ playing key roles in pathogen surveillance, mucosal barrier function, and regulation of the composition of the intestinal microbial flora (the microbiota). The mucosa comprises the innermost layers of the intestine (adjacent to the gut lumen) and is composed of the glandular surface epithelium overlying the lamina propria, which is separated from the submucosa by the muscularis mucosae, one of three muscular layers (**Fig. 37.1**). Epithelial cells (enterocytes) are primarily involved in absorption (with some secretory functions), whereas goblet cells and Paneth cells are secretory cells. The absorptive surface area of the mucosa of the small intestine is increased by invaginations along the lumenal surface of enterocytes that form villi projecting into the gut lumen. Goblet cells secrete mucus into the lumen that protects the mucosa from the action of digestive enzymes and creates a biofilm in which microorganisms reside to form a microbial ecosystem in the gut. Paneth cells located in the base of the intestinal crypts between the villi are specialized epithelial cells that secrete enzymes and antimicrobial peptides (defensins) that prevent the translocation of potentially pathogenic bacteria and toxins across the bowel wall. The submucosa is the second layer of the gut and includes autonomic nerves, which not only control peristaltic contraction of the smooth muscle of the muscularis mucosae but also regulate secretion and immune function. The loose connective tissue of the serosa makes up the outer layer of the bowel wall.

Cells of the gut mucosal innate and adaptive immune system are interspersed between the cells of the mucosal epithelium and throughout the lamina propria,

TOPICS BEARING ON THIS CASE:

- NOD proteins
- Inflammatory reactions
- Gut mucosal immune system
- Host relations with the commensal microbiota

This case was prepared by Raif Geha, MD, in collaboration with Andrew Shulman, MD, PhD, and Harland Winter, MD.

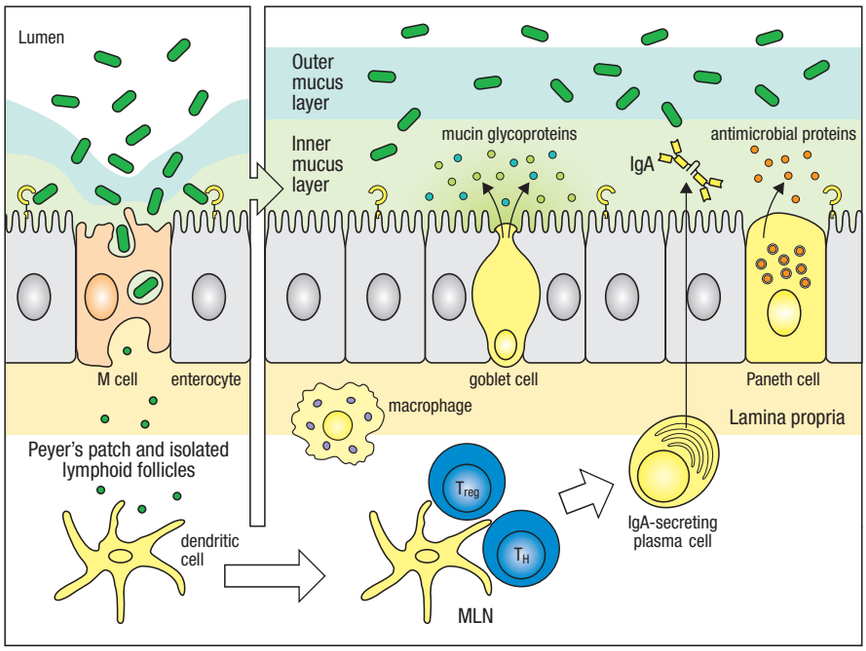


Fig. 37.1 A variety of mucosal responses keep the gut microbiota under control. The surface of the gut epithelium is protected by both innate and adaptive immune systems. A layer of mucus produced by goblet cells and antimicrobial proteins produced by Paneth cells help protect against pathogens and also keep commensal microorganisms under control, preventing them from colonizing the epithelial surface. Antigens from food, commensal microorganisms, or pathogens that enter the mucosal lymphoid tissues through M cells can initiate adaptive immune responses, including the production of IgA antibodies. In the absence of infection, dendritic cells presenting antigens (such as those derived from food and commensal microorganisms) to naive T cells in the mucosa tend to stimulate the production of regulatory T cells (T_{reg} cells), thus avoiding a damaging inflammatory response to the commensal microbiota. MLN, mesenteric lymph node.

and are also present as organized lymphoid organs (Peyer's patches) and isolated lymphoid follicles. The cells and organs of the gut mucosal immune system are collectively known as the gut-associated lymphoid tissue (GALT). The GALT is the largest lymphoid tissue in the human body, containing a significant portion of the body's 2×10^{12} lymphocytes.

Neutrophils, macrophages, and dendritic cells of the innate immune system express pattern recognition receptors and act as sensors for potentially pathogenic microorganisms in the gut. Pattern recognition receptors recognize lipids, carbohydrates, nucleic acids, and peptides that are common features of bacteria, viruses, fungi, and parasites. One important class of these receptors is the Toll-like receptors (TLRs), which are located at the cell surface and in the membranes of endosomes. Activation of TLRs by microorganisms and their products triggers innate immune pathways that mediate inflammation and host defense (see Fig. 29.1). NOD proteins (nucleotide-binding oligomerization domain-containing proteins) comprise another class of pattern recognition receptors; these are located in the cytoplasm and bind to bacterial peptidoglycans released during infection (**Fig. 37.2**).

The professional antigen-presenting cells of the gut mucosa—the macrophages and dendritic cells—take up and kill bacteria via phagocytosis, and present bacterial antigens to, and activate, the resident naive T cells and B cells within the GALT. B cells within the bowel wall are activated to produce antigen-specific secretory dimeric IgA antibodies, which are transported into the gut lumen. Between 3 and 5 g of IgA is secreted into the intestinal lumen daily, where it functions to neutralize pathogenic bacterial proteins required for mucosal colonization and translocation and also helps prevent invasion by the gut's commensal microbiota. The GALT includes a full complement of T lymphocytes: CD4 helper T cells (T_H1 and T_H2) and CD8 cytotoxic T cells, and the more recently characterized lineages of CD4 effector T cells, regulatory T cells (T_{reg} cells) and T_H17 cells. The role of T_H17 cells in autoimmunity and inflammation has generated intense research interest. In some contexts, T_H17 cells may have a protective role in preventing gut inflammation and maintaining homeostasis with the bacterial flora.

Given the high concentration of bacteria in the distal ileum and the colon (more than 10^{12} organisms per gram), the mucosal immune system performs a remarkable

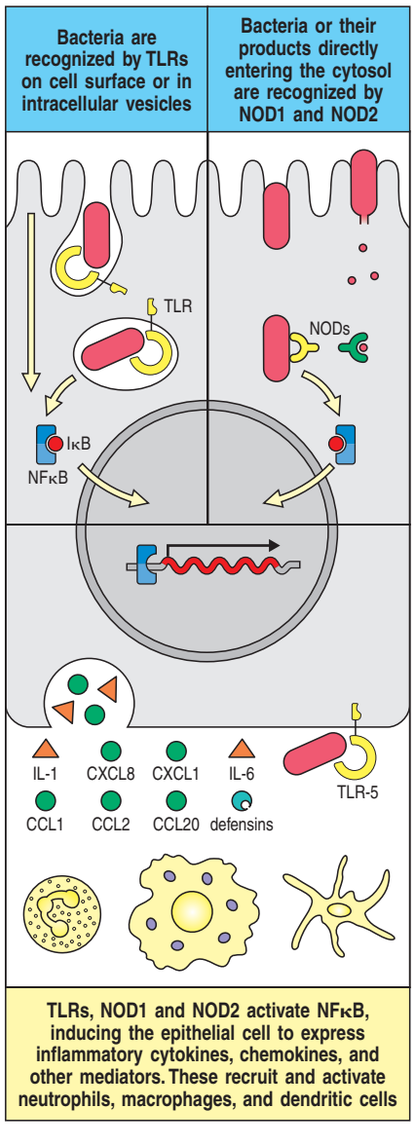


Fig. 37.2 Epithelial cells have a crucial role in innate defense against pathogens. TLRs are present in intracellular vesicles or on the basolateral or apical surfaces of epithelial cells, where they recognize different components of invading bacteria. NOD1 and NOD2 pattern-recognition receptors are found in the cytoplasm and recognize cell-wall peptides from bacteria. Both TLRs and NODs activate the NFκB pathway (see Fig. 23.1), leading to the generation of pro-inflammatory responses by epithelial cells. These include the production of chemokines such as CXCL8, CXCL1 (GROα), CCL1, and CCL2, which attract neutrophils and macrophages, and CCL20 and β-defensin, which attract immature dendritic cells in addition to possessing antimicrobial properties. The cytokines IL-1 and IL-6 are also produced and activate macrophages and other components of the acute inflammatory response. The epithelial cells also express MIC-A and MIC-B and other stress-related nonclassical MHC molecules, which can be recognized by cells of the innate immune system. IκB, inhibitor of NFκB.

TLRs, NOD1 and NOD2 activate NFκB, inducing the epithelial cell to express inflammatory cytokines, chemokines, and other mediators. These recruit and activate neutrophils, macrophages, and dendritic cells

function in promoting the colonization of beneficial bacteria, which contribute to nutrient digestion, while mediating the destruction, clearance, and development of immunologic memory to pathogens and toxins. Dysregulation of the complex mechanisms responsible for mucosal immune function can result in inflammatory bowel disease (IBD)—most commonly, Crohn's disease and ulcerative colitis.

The case of Dorian Gray: a boy with fever, chronic abdominal pain, and weight loss.

Dorian was a healthy 8-year-old when he developed painful swelling and redness of his right elbow. The following day, he developed mouth ulcers (aphthous ulcers) that persisted for several days. He was taken to the pediatrician, who thought that the ulcers could be due to a Coxsackie virus infection. X-rays of the elbow were done and showed no fracture or injury. Over the next 2 months, Dorian developed frequent poorly localized abdominal pain. Passing stools was particularly difficult, and his parents would often find Dorian crying in the bathroom. He had difficulties with constipation, passing three hard stools per week, but had no bloody stool or sensation of incomplete bowel evacuation.

The severity of Dorian's abdominal pain, elbow swelling, and oral ulcers seemed to wax and wane over the next 2 months. Dorian developed daily low-grade fevers, at times as high as 39ºC. His abdominal pain became more severe and he passed stools more frequently. Dorian was fatigued and listless and was unable to attend school. His appetite was very poor and his parents noticed that he had lost about 3.5 kg over the previous 2 months. He was seen again by his pediatrician, who set up outpatient appointments to investigate Dorian's illness. But when Dorian developed painful red lesions on his right shin and a pain in his jaw soon after this visit, his parents became concerned and brought him to the Children's Hospital Emergency Department.

The Grays were asked about the health of their family and told the staff that there was no history of inflammatory bowel disease or autoimmune illness. When examined, Dorian looked ill, tired, and pale. Numerous aphthous ulcers were present in his mouth. His abdominal examination showed no focal tenderness or masses. Two inflamed anal skin tags were found and the rectal exam showed no tenderness, fissures, or evidence of occult blood. Several raised, red skin lesions were present on Dorian's shins and his right elbow was swollen and warm. The skin lesions were recognized as erythema nodosum—acute nodular erythematous eruptions that typically occur on the lower extremities.

Mouth sores and a swollen elbow.

Fever, abdominal pain, weight loss.

Arthritis and nodular erythematous eruption on the legs.

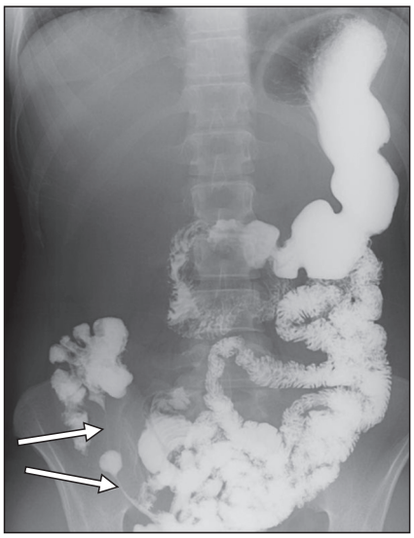


Fig. 37.3 Barium study showing two areas of small intestinal narrowing due to Crohn's disease.

High leukocyte count, elevated sedimentation rate and high CRP.

Active ileitis and colitis with rare crypt abscesses.

Fig. 37.4 Histology of the small intestine in Crohn's disease compared with the colon in ulcerative colitis. Left panel: Crohn's disease—biopsy from a terminal ileum with active disease. The figure illustrates a discrete granuloma composed of compact macrophages, giant cells, and epithelioid cells. Surrounding the nodule there is marked infiltration of lymphoid cells, plasma cells, and other inflammatory cells, but there is no necrosis. Right panel: ulcerative colitis—colonic mucosal biopsy taken from a patient with active disease. The crypt abscess is composed of transmigrated neutrophils and the surrounding epithelium exhibits features of acute mucosal injury. Photographs from *Nature*, 2007, 448:427–434, with permission from Macmillan Publishers Ltd.

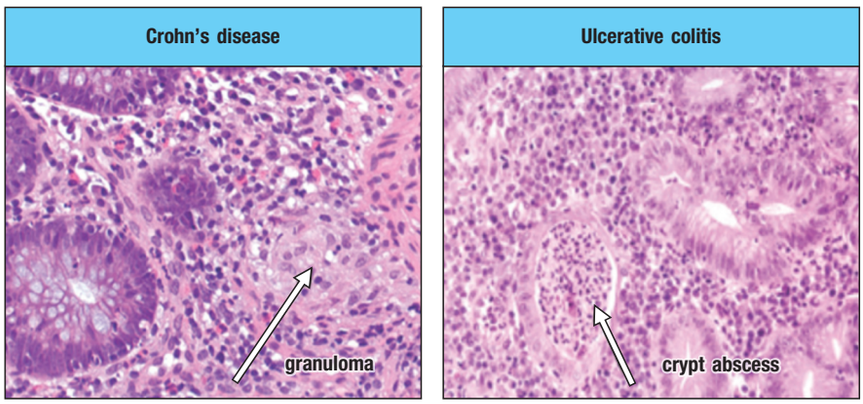
Blood tests were sent to check for evidence of infection or inflammation. The white blood cell count was elevated at 14,700 μl^{-1} (normal 5,700–9,900 μl^{-1}), and the platelet count was high at 759,000 μl^{-1} (normal 198,000–371,000 μl^{-1}). The erythrocyte sedimentation rate (ESR), an index of systemic inflammation, was elevated at 80 mm h^{-1} (normal 0–20 mm h^{-1}). The level of another acute-phase reactant, C-reactive protein (CRP), was also high at 2.2 mg dl^{-1} (normal less than 0.5 mg dl^{-1}). Dorian was told to return for an upper and lower endoscopy the following week.

On endoscopy, Dorian's pediatric gastroenterologist found ulceration of the esophagus and small intestine, and a perianal fistula (Fig. 37.3). The pathologist found neutrophilic infiltration of the esophageal and ileal lesions, active colitis with rare crypt abscesses, but no granulomatous inflammation (Fig. 37.4). The arthritis, oral aphthous ulcers, erythema nodosum lesions on the shins, and the endoscopic findings were all consistent with a diagnosis of Crohn's disease, and Dorian was started on a proton pump inhibitor to treat the esophageal lesions and oral corticosteroids as an initial anti-inflammatory treatment.

Over subsequent months, additional immunosuppressive agents in combination with oral steroids were tried, including 6-mercaptopurine and infusions of antibodies (infliximab and adalimumab) that inhibit the proinflammatory cytokine tumor necrosis factor-α (TNF-α). Unfortunately, Dorian's symptoms were not completely controlled. A year after receiving his diagnosis, Dorian developed worsening fever and abdominal pain, and an abdominal mass in the right lower quadrant could be felt. He was admitted to the Children's Hospital; abdominal ultrasound and CT scans revealed a large abscess of the distal small intestine (ileum and cecum). He was treated with intravenous antibiotics and was taken to the operating room, where the ileum and cecum were resected (ileocecectomy) and multiple smaller abscesses were drained. Dorian recovered well from surgery, continues to take weekly adalimumab injections, and has been able to discontinue steroid therapy. Although the weekly injections are difficult for Dorian and his family, the abdominal, skin, and joint symptoms of his Crohn's disease are all well controlled.

Crohn's disease.

Crohn's disease was first described in the medical literature by the Italian physician Giovanni Battista Morgagni, who presented the case of a chronically ill young man with diarrhea in 1769. American physician Burrill Crohn and colleagues published a case series of patients with "regional ileitis" in 1932. Crohn's disease is a disorder of mucosal immune dysregulation that is characterized by inflammatory lesions that can involve the entire gastrointestinal tract, from mouth to anus. In 40–50% of pediatric cases, Crohn's disease involves the ileum and colon.



By contrast, ulcerative colitis, typically involves only the colon and rectum. Biopsy evidence of inflammatory cell infiltrate is 'transmural' in Crohn's disease in that it may involve the epithelium, the lamina propria, and other layers of the bowel wall (see Fig. 37.4). As a result, fistulas and intra-abdominal abscesses, as in Dorian's case, are frequent complications. The presence of extra-intestinal inflammatory manifestations of IBDs, including skin (erythema nodosum, pyoderma gangrenosum), joints (IBD-related arthritis), and eyes (uveitis), underscores that IBDs are systemic inflammatory diseases. Colon cancer occurs with increased frequency in individuals with ulcerative colitis.

Breakthroughs in understanding the pathogenesis of IBDs as a result of new human genetics tools and animal disease models have focused attention on the dysregulation of mucosal immunity to the gut commensal microbiota and a resulting impaired mucosal barrier function (see Fig. 37.1). These recent discoveries have identified proteins that participate in innate immune recognition of bacteria, cellular stress-response pathways, and host–microbiota interactions as targets of new therapies for IBD patients. The finding that deficiencies in the NFκB signal pathway component NEMO (see Case 23), IL-10, the IL-10 receptor, or T_{reg} cells result in IBD provides evidence that the innate immune system and T_{reg} cells are essential in maintaining intestinal homeostasis. Mice with genetic disruption of genes for the cytokines interleukin (IL)-2, which is essential for T_{reg} cell function, or IL-10, an immunosuppressive cytokine, are protected from the development of intestinal inflammation only when raised in germ-free conditions, revealing an essential role for host–microbiota interactions in initiating IBD pathogenesis. Isoforms of the cytokine receptor IL-23R have been shown to confer both susceptibility to and protection from Crohn's disease, indicating complex regulatory roles of T_{reg} and T_H17 cells in intestinal inflammation. The cytokine IL-23 is required for T_H17-cell maintenance and survival. T_H17 and T_{reg} cells have a reciprocal relationship, because both compete for the cytokine TGF-β for their induction, which in the case of T_H17 cells, but not T_{reg} cells, also requires IL-23R.

Genome-wide association studies, which mine genetic data from large populations of patients and control individuals to identify variants of genes that confer increased disease risk, have led to new knowledge related to Crohn's disease (**Fig. 37.5**). Mutations in NOD2, an intracellular innate immune receptor, expressed in macrophages and epithelial cells, that binds to muramyl dipeptides from bacterial cell walls and activates the production of inflammatory cytokines (see Fig. 37.2) results in severe ileal Crohn's disease. Intestinal epithelial cells from individuals with a Crohn's disease-associated mutation in *NOD2* have impaired secretion of antimicrobial peptides called defensins when exposed to invasive bacteria. Blau syndrome, a rare autosomal dominant condition characterized by granulomatous inflammation of the eyes, skin, and joints, has also been found to be due to mutations in *NOD2*.

Autophagy is an evolutionarily conserved cellular stress-response pathway that delivers intracellular organelles and cytoplasmic contents to the lysosomes for degradation. Autophagy is important for walling off and eliminating bacteria that escape into the cytosol or enter it directly. *ATG16L1* and *IRGM* are two genes whose products are important for the formation of the autophagocytic vacuole. Identification of disease-related variants of the *ATG16L1* and *IRGM* genes implicate defects in autophagy in Crohn's disease, possibly because inefficient elimination of microbes from the cytosol leads to sustained cytokine secretion and Paneth cell dysfunction, resulting in intestinal inflammation. Cellular stress caused by misfolded proteins, metabolic factors, and microbes activate what is known as the 'unfolded protein response.' This response activates the transcription factor XBP1, which regulates the expression of genes important for the proper function of the immune system. Defects in XBP1 result in IBD in mouse models, and XBP1 variants have been described in patients with Crohn's disease, suggesting that abnormalities in the cellular stress response may result in intestinal inflammation and decreased survival of Paneth cells.

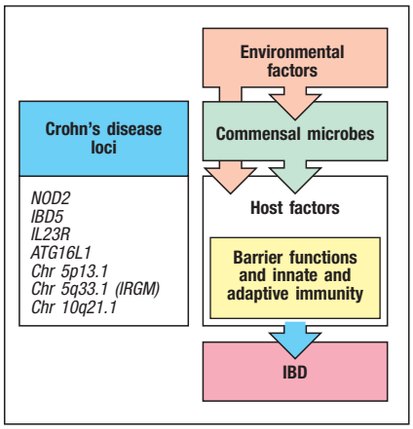


Fig. 37.5 Genome-wide association studies reveal patterns of Crohn's disease pathogenesis.

Questions.

1 *How can you account for Dorian's markedly elevated platelet count found on his initial visit to the Emergency Department?*

2 *6-Mercaptopurine was developed as a chemotherapeutic agent for the treatment of cancer. What could be the basis for its use in the treatment of inflammatory bowel disease?*

3 *Infliximab is a chimeric mouse–human monoclonal antibody generated against the inflammatory cytokine TNF-α. Adalimumab is a fully humanized anti-TNF-α antibody. How might some patients become resistant to infliximab therapy and yet still respond to adalimumab?*

4 *Natalizumab is a therapeutic monoclonal antibody that targets the cell-adhesion molecule α4-integrin and is used as a second-line therapy for severe Crohn's disease. What might be its mechanism of action?*