



Inflammatory Diseases of the Gastrointestinal Tract and Pharmacological Treatments

Clinton B. Mathias, Jeremy P. McAleer, and Doreen E. Szollosi

Learning Objectives

1. Describe the components of the gut-associated lymphoid tissue and explain the various effector mechanisms involved in mucosal immune responses
2. Explain the factors involved in the balance between tolerance and immunity in the mucosal immune system in the gastrointestinal tract
3. Explain mechanisms by which commensal bacteria modulate the outcome of mucosal immune responses
4. Describe and explain the effector immune mechanisms involved in anti-parasitic responses in the gastrointestinal tract
5. Describe and explain the differences between ulcerative colitis and Crohn's Disease
6. Explain the various effector mechanisms involved in the pathogenesis of inflammatory bowel disease (IBD)
7. Explain the mechanism of action of the drugs used for the treatment of IBD
8. Describe and explain the effector mechanisms involved in the pathogenesis of celiac disease
9. Describe and explain the effector mechanisms involved in the development of food allergy
10. Review the available and developing therapeutic options for the treatment of celiac disease and food allergy

C. B. Mathias (✉)
Department of Pharmaceutical and Administrative Sciences, College of Pharmacy and Health Sciences, Western New England University, Springfield, MA, USA
e-mail: clinton.mathias@wne.edu

J. P. McAleer
Pharmaceutical Science and Research, Marshall University School of Pharmacy, Huntington, WV, USA
e-mail: mcaleer@marshall.edu

D. E. Szollosi
Department of Pharmaceutical Sciences, School of Pharmacy and Physician Assistant Studies, University of Saint Joseph, Hartford, CT, USA
e-mail: dszollosi@usj.edu

Drugs discussed in this chapter

Drugs	Classification
6-mercaptopurine (6-MP, Purinethol®)	Anti-proliferative, purine analog
Adalimumab (Humira®)	Anti-TNF monoclonal antibody
Azathioprine (Imuran®)	Anti-proliferative, purine analog
Balsalazide (Colazal®)	5-aminosalicylate
Budesonide (Entocort®)	Glucocorticoid
Certolizumab pegol (Cimzia®)	Anti-TNF, antibody fragment
Glucocorticoids	Global immunosuppressive agents
Infliximab (Remicade®)	Anti-TNF monoclonal antibody
Mesalamine (Asacol®, Canasa®, Lialda®, Pentasa®, Rowasa®)	5-aminosalicylate
Methotrexate (Rheumatrex®)	Anti-proliferative, DNA replication inhibitor
Natalizumab (Tysabri®)	Anti- α_4 integrin monoclonal antibody

Drugs	Classification
Olsalazine (Dipentum®)	5-aminosalicylate
Sulfasalazine (Azulfidine®)	5-aminosalicylate
Thalidomide (Thalomid®)	Anti-inflammatory
Ustekinumab (Stelara®)	Anti-IL-12/23p40 monoclonal antibody
Vedolizumab (Entyvio®)	Anti- $\alpha_4\beta_7$ integrin monoclonal antibody
Metronidazole	Antibiotic
Ciprofloxacin	Antibiotic

Introduction

Our gastrointestinal (GI) tract, including the oral cavity, stomach, small intestine (duodenum, jejunum, ileum), cecum, large intestine (colon) and rectum, constitutes one of the largest surface areas in our body. In an adult human being, the GI tract is approximately 9 m in length, equivalent to the size of roughly two tennis courts. The primary function of the GI tract is the processing of ingested food into nutrients that are absorbed by the body and waste that is eliminated from the body. The absorption of dietary nutrients into the body is facilitated through a physical epithelial barrier consisting of numerous finger-like projections called villi. This same epithelial barrier also prevents bacterial invasion, a remarkable accomplishment, considering that trillions of bacteria normally live within the lumen of the gut, reaching densities of up to 10^{12} organisms per gram in the colon. These normal intestinal flora are termed microbiota, the composition of which varies from individual to individual, and play a pivotal role in the regulation of mucosal immune responses within the GI tract. The coordinated efforts of multiple cell types help to limit opportunistic infections caused by commensal bacteria and protect the body's GI surfaces from colonization by pathogens. Perturbations of normal immune homeostasis within the GI tract, either due to infection or injury, defects in barrier function, induction of autoimmune reactions, and disruption of commensal bacterial composition may lead to chronic inflammatory diseases such as inflammatory bowel disease, celiac disease, food allergy and other conditions. In this chapter, we will examine the inflammatory processes and immunotherapeutic drug targets involved in the development of these conditions.

Immune Responses in the Gastrointestinal Tract

Role of Mucus in Preventing Microbial Attachment to the GI Epithelial Layer

The organs of the GI tract, along with parallel organs in the respiratory and urogenital tracts, are collectively referred to as **mucosal** organ systems. A primary characteristic of these organ systems is the continuous bathing of surfaces by a thick, viscous fluid called mucus. This fluid is secreted by specialized mucosal epithelial cells throughout the GI tract. The viscosity of mucus is caused by numerous gigantic glycoproteins called mucins, which are a major constituent of the mucosal fluid.

The production and secretion of mucus plays an important role in the protection of our surfaces from infection and injury. In addition to mucin, the mucus consists of numerous proteoglycans, antimicrobial peptides (e.g. defensins), and other enzymes that protect the epithelial cells from damage and limit the development of infection. Bacteria within the mucus are quickly trapped by mucins, lysed through the activity of antibacterial peptides such as defensins, and neutralized with antibodies such as dimeric or secretory IgA. The activity of dimeric IgA in the lumen of the GI tract is facilitated by mucins. By attaching to IgA, mucins poise the antibodies to perform the neutralization or opsonization of pathogens and their products. The secretion of mucus is a dynamic process and consists of several polypeptides that are encoded by different genes. Hence, the viscoelastic properties of mucosal tissues may vary depending on the particular organ, the state of the organ with respect to health or disease, and specific functions associated with the particular organ. The replenishment of mucus occurs continuously throughout the body; as epithelial cells turnover every few days, old mucus containing microorganisms is expelled from the body.

Roles for Commensal Bacteria in the Digestion of Food and Protection Against GI Pathogens

The absorption of nutrients from food is a dynamic process that depends in part on numerous enzymes and commensal bacteria. Species

residing in the oral cavity such as the mouth aid in the degradation of food particles by producing digestive enzymes. Food is then shuttled to the stomach followed by various compartments of the small and large intestines, where further interactions with commensal bacteria occurs. The major site for nutrient absorption is within the small intestine, whereas the large intestine is primarily for the storage, compaction, and elimination of waste. As food travels throughout the intestines, it comes into contact with increasing numbers of commensal bacteria, from approximately $10^8/\text{mL}$ in the small intestine to $10^{12}/\text{mL}$ in the large intestine. These bacteria aid in the digestion of food, produce metabolites such as vitamin K, inactivate toxins and other harmful enzymes, and limit the growth of opportunistic pathogens. The growth of the intestinal flora itself is a dynamic process, depending on inter-species competition for nutrients and resources as well as the production of antimicrobial

peptides from epithelial cells. Disruption of the microbial composition as a result of antibiotic treatment or other processes can increase susceptibility to infections or mucosal inflammation. The population of commensal microorganisms is stabilized by expelling vast numbers of organisms in the feces every day.

Gut-Associated Lymphoid Tissue Mediates Host Defense in the GI Tract

Host defense in the GI tract depends on immune cells and the secondary lymphoid tissues present throughout the various organ systems. These are collectively referred to as the **gut-associated lymphoid tissue (GALT)**. These tissues include secondary lymphoid structures such as the Peyer's patches, specialized epithelial cells including M cells, and leukocytes that are present within the **lamina propria**, or loose connective tissue underlying the mucosal epithelium (Fig. 6.1). Furthermore, antigens from pathogens

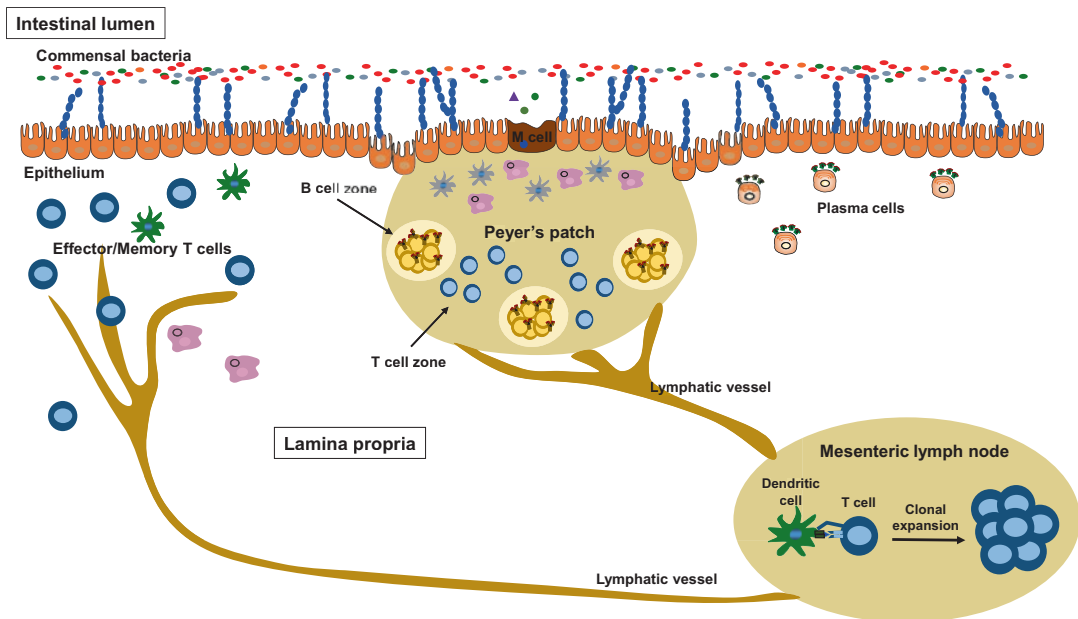


Fig. 6.1 Mucosal immune responses in the GI tract. Compartmentalization of the GI tract helps to facilitate immune responses while maintaining a physical barrier from microbial entry. Intestinal epithelial cells form a boundary between luminal contents and the underlying gut associated lymphoid tissue and lamina propria. Peyer's patches associated with the basolateral membrane of the epithelium are sites where immune responses are initiated. M cells interspersed between ciliated cells collect luminal antigens via phagocytosis. Dendritic cells and B cells residing in Peyer's patches then present antigens to T cells. Mesenteric lymph nodes are also sites where immune responses are initiated against intestinal antigens. Activated T cells undergo clonal expansion and migrate to the lamina propria where they provide effector functions. Antibody-producing plasma cells also reside in lamina propria, with IgA being the most predominant isotype produced in the GI tract

that invade the intestine are processed by antigen presenting cells and presented to naïve T cells within mesenteric lymph nodes, a large chain of lymph nodes within the mesentery, or connective tissue holding the gut in place.

Lymphoid tissues present in the mouth, tonsils and adenoids guard the entrance to the GI tract. They produce dimeric IgA that recognizes numerous oral microorganisms that individuals are exposed to, especially during the first few years of life. The presence of lymphoid tissue is most concentrated in the small intestine, which is also the major site of nutrient absorption. In the small intestine, finger-like projections called villi increase the surface area available for absorption. Numerous patches of discrete lymphoid tissue, named **Peyer's patches** after the scientist who discovered them, are present throughout the small intestine. These lymphoid organs vary in size, forming a dome-like structure containing numerous B cell follicles with germinal centers, interspersed with T cell areas and dendritic cells. In addition, numerous isolated lymphoid follicles consisting of B cells also line the intestinal wall. Within the follicle-associated epithelium, a specialized cell type called the microfold cell or **M cell** resides, which facilitates the transport of microbial and food antigens from the lumen of the gut to the secondary lymphoid tissue (Peyer's patches) and underlying lamina propria. The **lamina propria** directly underneath the basolateral side of the epithelium contains immune cells including macrophages, dendritic cells (DCs), memory T cells and B cells. Secretory IgA produced by B cells is transported through the epithelium into the lumen where it helps to prevent microbial attachment and neutralizes toxins.

A primary characteristic of the immune system in the GI tract is the prevention of inflammation under normal homeostatic conditions. The growth of commensal microorganisms is held in check by dimeric IgA and numerous effector cells within the lamina propria, which remain poised to defend against microbial invasion and quickly terminate pathogens. At the same time, tolerogenic mechanisms ensure that inflammation is primarily directed against pathogens rather than symbiotic microbes and does not contribute

to unnecessary damage to the GI tract. For instance, unactivated DCs induce oral tolerance to food antigens and FoxP3-positive T_{reg} cells prevent and resolve inflammation. The mechanisms through which T_{regs} function include competing with naïve T cells for costimulation, and producing anti-inflammatory cytokines (TGF- β , IL-10) which suppress the activation of effector T_H1 , T_H2 and T_H17 cells.

Role of Individual Cell Types in Gastrointestinal Host Defense

The induction of immune responses in the gut is coordinated by a number of cell types. While their functions help to prevent and terminate colonization with pathogens, their activity must be tightly regulated in order to limit tissue damage caused by inflammation. Intestinal epithelial cells play a vital role by facilitating the uptake of nutrients and potential antigens from the lumen and sensing various microbial products with pattern recognition receptors. As described in Chap. 2, toll-like receptors (TLRs) are expressed on the cell surface and intracellularly, while NOD-like receptors (NOD1, NOD2) that recognize bacterial-derived muramyl dipeptide are localized to the cytoplasm. Signaling cascades initiated *via* TLR or NOD receptor binding results in the activation of transcription factors including NF- κ B, leading to the production of pro-inflammatory cytokines (IL-1, IL-6, TNF- α), antimicrobial enzymes (defensins), and chemokines (CXCL8/IL-8) that attract other cells including neutrophils to sites of infection. As described earlier, M cells residing within the epithelium overlay Peyer's patches and isolated lymphoid follicles. They have numerous folds or ruffles on their surface, enabling the transcytosis of microbial antigens from the luminal side of the cell to the basal region. In secondary lymphoid tissues, these antigens are captured by DCs and B cells, and surveyed by T cells, leading to the initiation of adaptive immune responses.

Other important cell types in the GI tract include intestinal macrophages and dendritic cells. While macrophages are efficient in the capture and phagocytosis of microorganisms, they typically do so in a manner that elicits few proinflammatory cytokines and do not promote

inflammation under steady-state conditions. The activation status of intestinal macrophages is regulated by cytokines such as TGF- β . Intestinal DCs play an important role in the activation of T cell-mediated immune responses. Under normal conditions, DCs induce tolerance by promoting T_{reg} differentiation. Infections lead to DC activation, including the upregulation of costimulatory molecules and production of cytokines. Thus, intestinal T cells recognizing pathogen-derived antigens presented by activated DCs will undergo differentiation into pro-inflammatory Th subsets.

T follicular helper cells (T_{FH}) are one of these subsets (Fig. 3.12). Their primary function is to promote isotype switching and somatic hypermutation in B cells recognizing antigens from the same pathogen. In the GI tract, this interaction usually leads to the production of dimeric IgA. Lymphocytes that are activated in mucosal lymphoid tissues preferentially reside within these tissues. This is due to their expression of certain adhesion molecules and chemokine receptors that are preferentially expressed in mucosal tissues. Thus, T and B cells activated in mesenteric lymph nodes or Peyer's patches will migrate to the epithelial and lamina propria compartments following their differentiation into effector cells. Specifically, activated lymphocytes lose the expression of CCR7 and gain the expression of CCR9. Ligands for these chemokine receptors are expressed in systemic secondary lymphoid organs and blood vessels supplying the GI tract, respectively. This process, termed **imprinting**, results in lymphocytes trafficking to the mucosal surfaces where the antigen was originally encountered. Cells in the lamina propria produce CCL25, serving as a chemoattractant for T and B cells expressing CCR9. DCs contribute to the imprinting of T cells and induce their expression of integrin $\alpha_4\beta_7$ in the GI tract. By interacting with **mucosal vascular addressin cellular adhesion molecule (MAdCAM-1)** expressed on intestinal endothelial cells, $\alpha_4\beta_7$ promotes the diapedesis of T cells and B cells into the intestinal parenchyma.

A variety of other immune cells also participate in GI host defense, including mast cells, $\gamma\delta$ T cells, CD8 T cells, and a distinctive type of

CD8 T cell, referred to as the **intraepithelial lymphocyte (IEL)**. Effector IELs integrate into the epithelial layer of the small intestine and help to confer immunity against a limited range of antigens. Unlike other T cell subsets, IELs possess both innate and adaptive immune characteristics and are thought to have been pre-activated against antigens. Upon their encounter with antigens, they immediately release various cytokines and induce the killing of target cells.

Anti-parasitic Immune Responses

A good illustration of how different cell types cooperate to provide host defense occurs during infections with parasites (Fig. 6.2). Helminth worms such as nematodes, trematodes, and cestodes are a major cause of debilitating diseases throughout the developing world. These infections are controlled by T_{H2} responses, resulting in anti-parasitic IgE antibodies from B cells and the destruction of helminths coated with these antibodies by mast cells, basophils, and neutrophils. Infections with helminths results in the production of cytokines such as IL-33 and TSLP by epithelial cells, leading to DC activation and the differentiation of naive T cells into T_{H2} and T_{FH} subsets in draining lymph nodes. B cells that recognize helminth-derived antigens then undergo isotype switching to IgE under the direction of T cell-derived signals. When helminths are coated by IgE antibodies, innate immune cells are recruited to destroy the infectious organisms. Specifically, mast cells, basophils and eosinophils express a high affinity receptor for the constant region of IgE, named Fc ϵ RI. The activation of Fc ϵ RI on mast cells, basophils and eosinophils leads to their expulsion of granular contents such as histamine and various degradative enzymes including major basic protein. Degranulation also results in smooth muscle contraction, intestinal spasms, and diarrhea. This powerful response ensures the killing of helminth worms and their expulsion in the mucus and the feces. Overall, cytokines produced by T_{H2} cells drive this process. IL-3, IL-9 and IL-10 drive mast cell expansion and function, IL-5 promotes eosinophil activation and survival, IL-4 promotes B cell activation and propagates the T_{H2} response,

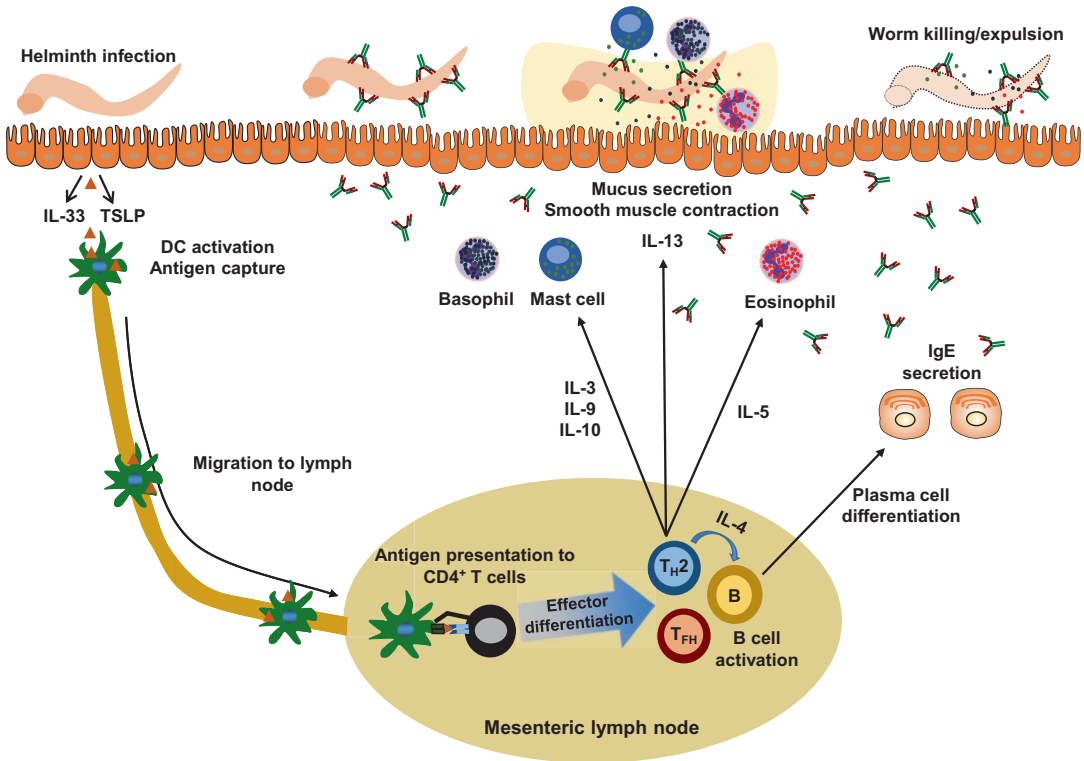


Fig. 6.2 T cell immune responses against parasites. Infections with helminth parasites stimulate epithelial cells to produce IL-33 and TSLP, resulting in DC activation and migration to lymph nodes. Antigen presentation to CD4 T cells results in their differentiation into T_H2 and T_{FH} cells. Cytokines produced by T_H2 cells promote the activation of B cells, granulocytes and mast cells. T_{FH} cells also help to induce the secretion of parasite-specific antibodies from B cells, namely IgE. This results in antibody coating the surface of parasites, marking them for attack by granulocytes and mast cells. Mucus produced in response to IL-13 also helps to prevent parasite attachment to epithelium

and IL-13 induces mucus hypersecretion, smooth muscle reactivity, and epithelial cell turnover. Collectively, these actions ensure the pathogen is driven out of the body by the combined activity of IgE-triggered mast cells and eosinophils.

In summary, the GI tract is a dynamic environment due to constant changes in food and nutrient availability, bacterial species growth, and activation of the immune system. Homeostasis refers to the remarkable ability of the GI tract to promote nutrient absorption while limiting pathogen colonization, repair injuries caused by inflammation, and maintain the epithelial lining. The role of the immune system in maintaining intestinal homeostasis has been demonstrated in studies showing that deficiencies in anti-inflammatory genes, notably IL-10, leads to spontaneous inflammatory

bowel diseases. On the other hand, pro-inflammatory cytokines including IL-6, IL-23 and TNF- α are therapeutic targets for IBD. Thus, maintaining intestinal homeostasis is a complex process that depends on a multitude of environmental (diet, microorganisms, allergens) and genetic factors. Injuries or disruptions to the epithelial barrier integrity can lead to acute or chronic inflammation, which is treated by various biologics that target the immune system, as described below.

Inflammatory Bowel Diseases

Inflammatory bowel disease (IBD) is an idiopathic disease thought to be caused by abnormal immune responses to host intestinal microbiota.

Two types of IBD have been described. They include **Ulcerative Colitis (UC)** which is typically confined to the colonic mucosal surface, and **Crohn's Disease (CD)** which may involve transmural inflammation along the length of the GI tract from the mouth through the anus (Table 6.1). Both diseases involve chronic inflammation that is relapsing-remitting in nature and can cause various complications including stenoses, abscesses, fistulas, extraintestinal manifestations, colitis-associated neoplasias, and cancer. The presence of blood in the stool, along with other symptoms, is a defining characteristic of IBD, aiding in distinguishing it from similar symptoms associated with irritable bowel syndrome. It is estimated that approximately 2.5 million people in the Western world suffer from IBD including UC and CD, with developing countries seeing more and more cases.

Ulcerative Colitis

In UC, the immune response and tissue damage is confined to the colon. The rectum is predominantly affected in 95% of patients, with variable degrees of proximal extension. Inflammation can be acute or chronic and is limited to the mucosa. Several immune cell types are involved, and the disease is characterized by ulceration, edema, hemorrhage along the length of the colon, crypt abscesses and goblet cell depletion (Fig. 6.3). Patients with UC typically present with bloody diarrhea accompanied by intense lower abdominal cramps, the severity of which increases during bowel movements. The disease can involve various parts of the colon resulting in fulminant colitis, leading to the development of peritonitis. The risk of colon cancer development in patients

Table 6.1 Differences observed between ulcerative colitis and Crohn's disease

Characteristic	Ulcerative colitis (UC)	Crohn's disease (CD)
Location of inflammation	Colonic mucosa, with the rectum affected in 95% of patients	Transmural inflammation can occur throughout the GI tract, from mouth to anus
Presence of skip areas	No	Yes
Symptoms	Bloody diarrhea; intense lower abdominal cramps	Determined by location, extent, and severity of inflammation Ileocecal: Abdominal pain Small intestine: Non-bloody, intermittent diarrhea, abdominal pain, weight loss Colonic: Bloody diarrhea, similar to UC Gastroduodenal: Nausea, vomiting, anorexia Perianal: Perirectal pain and rectal fistulas
Extra-intestinal manifestations observed	Yes	Yes

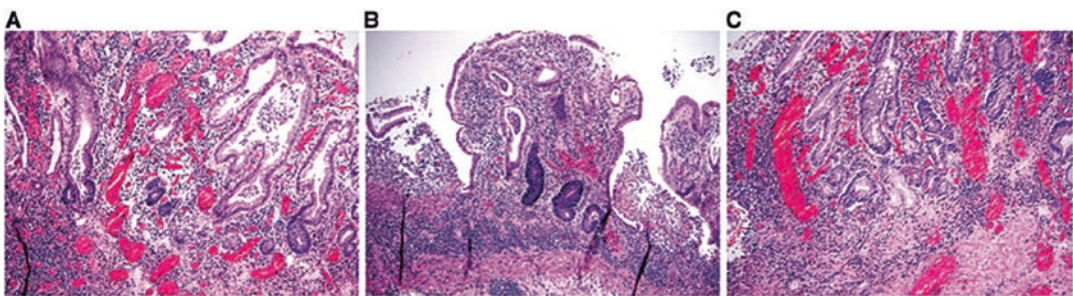


Fig. 6.3 Histologic evaluation of ulcerative colitis. Microscopic features of ulcerative colitis. (a and b): architectural distortion, including shortening of crypts, variation in the sizes and shapes of crypts, and basal lymphoplasmacytosis (a and b): H&E stain; $\times 100$. (c) Paneth cell metaplasia and pyloric gland metaplasia in the left colon (H&E stain; $\times 100$). Credit: Tom C. DeRoche, Shu-Yuan Xiao, and Xiuli Liu. *Histological evaluation in ulcerative colitis*. Gastroenterol Rep (Oxf). 2014 Aug; 2(3): 178–192. Reprinted under Creative Commons License Agreement (Attribution)

with UC increases from 2% in the first 10 years after diagnosis to 18% for those who have had UC for 30 years.

Crohn's Disease

In contrast to UC, CD can involve any part of the GI tract, and is characterized by the frequent separation of diseased segments of the GI tract from normal bowel areas, referred to as “skip” areas. The inflammation in CD is transmural and extends to the serosa, resulting in the formation of abscesses and fistulas. The disease often begins with inflammation and abscesses in the crypts, which then progresses to the formation of aphthoid ulcers (superficial ulcers over a Peyer's patch) and transverse, chronic inflammation extending into the submucosa, sometimes accompanied by non-caseating granulomas. The most common region for CD development is the ileocecal region, followed by the terminal ileum alone, diffuse small bowel, and isolated colonic regions. Transmural inflammation results in the development of lymphedema and thickening of the bowel wall and mesentery. The mesenteric lymph nodes are often enlarged, and the development of abscesses, fistulas, and strictures

often leads to obstruction of the bowel. Non-caseating granulomas, which may be observed in up to 50% of patients, can occur in the lymph nodes, peritoneum, and throughout the intestinal walls. In contrast to UC, the diagnosis of CD is subtle and often delayed since GI symptoms depend on the location, extent, and severity of the inflammation. In patients with ileocecal CD, the development of abdominal pain is usually post-prandial (occurring after consumption of a meal), and in children, may extend to the periumbilical area. CD of the small intestine often presents with non-bloody, intermittent diarrhea, abdominal pain, weight loss, and anorexia. In contrast, colonic CD presents with bloody diarrhea, making it indistinguishable from UC. Gastroduodenal CD presents with nausea, vomiting, and anorexia, and perianal CD can present with severe peri-rectal pain and discharge from rectal fistulas. Similar to UC, CD is associated with an increased risk of development of colon cancer, increasing from 3% at 10 years following diagnosis to 8% at 30 years. In Fig. 6.4 below, a biopsy specimen depicting pathologic changes consistent with Crohn's colitis is shown. Severe crypt atrophy and destruction can be observed, along with the accumulation of lymphoid cells and granulomas.

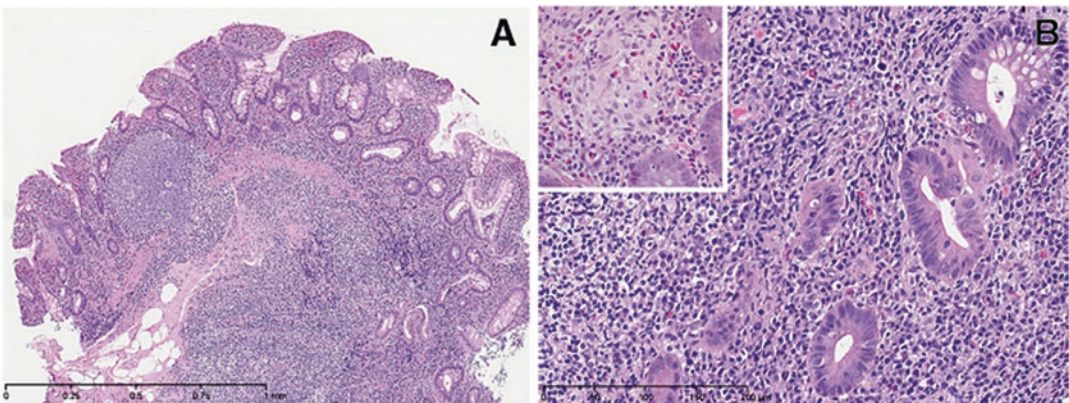


Fig. 6.4 Crohn colitis in a 10-year old child. Endoscopic biopsy specimen from right colon showing changes compatible with Crohn disease. (a) Colon mucosa with crypt atrophy and irregularity, and lymphoid hyperplasia (H&E, bar = 1 mm). (b) Higher magnification revealing crypt destruction and in the lamina propria severe accumulation of lymphocytes and plasma cells as well as a single small epithelioid cell granuloma (inset) (H&E, bar = 200 μ m). Credit: Erling Peter Larsen, Allan Bayat and Mogens Vyberg. *Small duct autoimmune sclerosing cholangitis and Crohn colitis in a 10-year-old child. A case report and review of the literature.* Diagnostic Pathology 2012, 7:100. Reprinted under Creative Commons License Agreement (Attribution)

Extra-Intestinal Manifestations (EIM)

Both diseases are associated with various EIMs. Growth abnormalities and delayed sexual maturation are the most common in CD. Other systems affected include the skin (red scaly patches), joints (arthritis, ankylosing spondylitis), eyes (uveitis), and mouth (aphthous ulcers). The release of cytokines from immune cells, including TNF- α and IL-6, contributes to maintenance of the pro-inflammatory state and symptoms.

Pathogenesis of IBD

While the exact etiology of IBD is undetermined, several factors are thought to play a role in the development of the disease. This involves a complex interplay of distinct mechanisms including genetic, environmental, microbial, and immunologic influences (Fig. 6.5).

Genetics

Genome-wide association studies have identified more than 160 single nucleotide polymorphisms that may be associated with the development of IBD. Of these, the gene for *NOD2* (also called

CARD15) was the one first identified and holds one of the strongest associations. **Nucleotide binding oligomerization domain containing 2 (NOD2)** is a pattern recognition receptor (PRR) in the cytosol which helps the immune system recognize intracellular bacteria. It is expressed in Paneth cells of the intestinal epithelium, lamina propria immune cells, and other cell types. By detecting muramyl dipeptides in the peptidoglycan of bacterial cell walls, NOD2 stimulates innate immune functions including antigen presentation, antimicrobial peptides, bactericidal activity, and cytokines. Its role as a PRR helps to modulate the composition of normal gut flora. The immunological effects of NOD2 in the GI tract are complex, and a regulatory function has been identified due to its suppression of T_H17 responses that are driven by IL-23. T_H17 cells have roles in the development of both UC and CD, and IL-23 contributes to T_H17 cell differentiation and proliferation. Polymorphisms in the gene encoding for a subunit of the IL-23 receptor, *IL-23R*, have been implicated in IBD. Additional genes include *ATG16L1* and *IRGM* which are involved in autophagy, a cellular process that facilitates the lysosomal degradation and recycling of cellular components including organelles. In addition, autophagy can promote the lysosomal degradation of intracellular bacteria, limiting colonization with pathogens. Epithelial cells and dendritic cells with polymorphisms in these genes display defective antimicrobial responses, possibly leading to intestinal dysbiosis and susceptibility to IBD.

Environment

Several environmental factors are linked to the development of IBD, including cigarette smoking, the use of non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics, frequent infections, and diet. Of these, smoking has been the most studied, and found to be inversely correlated with the development of UC, but positively correlated with CD. The use of NSAIDs such as aspirin at high doses or for prolonged or frequent use contributes to IBD symptoms. Antibiotics contribute to IBD by disrupting the composition of the normal microbial flora. While having a diverse

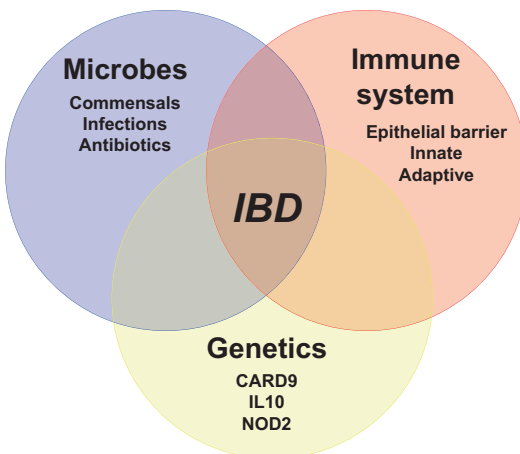


Fig. 6.5 Several factors contribute to the development of Inflammatory Bowel Diseases (IBDs) in susceptible individuals. IBDs are complex diseases that depend on interactions between the immune system, microorganisms and genetic susceptibility, giving rise to the manifestation of either ulcerative colitis or Crohn's Disease

microbiota is beneficial for GI homeostasis, antibiotics may kill protective species of bacteria and increase the space available for colonization with pathogens, promoting inflammation. Likewise, a lack of anti-inflammatory components in the diet as well as vitamin D deficiency have been implicated in development of the disease.

Microbial Factors

The human gut microbiome is established within the first 2 weeks of life, remaining relatively stable after that. While the intestines of healthy individuals are colonized by diverse species of bacteria, the intestines of patients with IBD exhibit remarkably reduced microbial biodiversity, with over-representation of enterobacteria in CD and increased numbers of *Escherichia coli* in UC. Residential intestinal bacteria are referred to as commensal microbiota and provide a number of important functions. Commensals aid in food digestion including dietary fiber, regulation of glucose homeostasis, and protection against infection from pathogens. Towards this, commensal bacteria compete with pathogens for nutrients or space in the gut, induce the secretion of antimicrobial peptides from intestinal Paneth cells, and stimulate adaptive immune responses including IgA production from B cells. Thus, interactions between commensal microbiota and our immune system help to maintain intestinal homeostasis in healthy individuals. Beneficial metabolites produced by the *Bacteroidetes* and *Firmicutes* phyla include short chain fatty acids, which have anti-inflammatory effects on immune cells. Several factors determine the composition of gut microbial species, including maternal transfer during birth, diet, antibiotic use, and potential drug/alcohol use. Further, the immune system shapes the composition of microbial species by producing antimicrobial peptides, IgA, and mucus which limits bacterial adhesion to the epithelial lining. The detection of microbes by pattern recognition receptors expressed on innate immune cells (see Chap. 2) may precipitate the inflammatory cascade in patients with a genetic susceptibility to IBD. As described earlier, genetic variants of NOD2 increase susceptibility to CD. By activating NF- κ B and MAP kinases in

monocytes, NOD2 induces the production of inflammatory cytokines. CARD9 contributes to innate immunity against fungi and intracellular pathogens by activating proinflammatory MAP kinases. These findings suggest that detection of commensal microbiota by the immune system is essential for maintaining intestinal homeostasis.

Immunologic Mechanisms

The immune system plays a critical role in the pathogenesis of IBD, and a number of factors have been proposed. These include chronic inflammatory responses against self-antigens or the intestinal commensal bacteria. A widely-held hypothesis is that environmental triggers including infections can lead to the loss of intestinal epithelial barrier integrity and oral tolerance to intestinal and microbial antigens. This results in the activation of DCs, which subsequently migrate to the mesenteric lymph nodes and induce the activation and differentiation of T_H1, T_H2, and T_H17 effector subsets which may recognize self or microbial antigens (Fig. 6.6). Potential target antigens in IBD may be derived from mucin, goblet cells or colonocytes. In addition, anti-neutrophil cytoplasmic antibodies (ANCA) and antibodies targeting neutrophil myeloperoxidase are associated with both CD and UC development. Changes in microbial composition, or dysbiosis, can further propagate the immune response to individual microbial species, particularly in UC development. Thus, the pathogenesis of IBD involves a complex crosstalk between innate and adaptive immune cell types, intestinal epithelial cells and the microbiome.

The loss of immune tolerance to intestinal antigens can impair the epithelial barrier function and facilitate the proliferation of commensal bacteria, resulting in the activation of immune cells. Moreover, patients with IBD have decreased microbial diversity in their GI tract, or **dysbiosis**, including decreased colonization by *Firmicutes* species, which can further impact food metabolism, vitamin synthesis, and nutrient absorption, and favor the development of microbial-specific immune responses. Several microbial species are thought to induce immune responses during IBD including viruses, protozoans, and specific bacte-

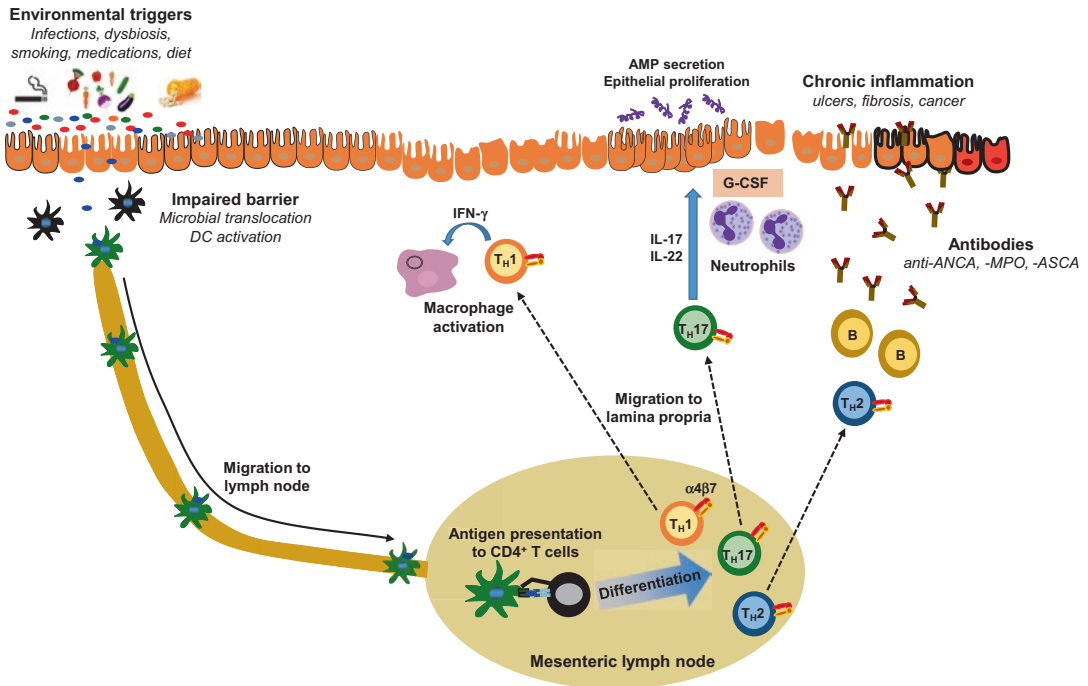


Fig. 6.6 Effector mechanisms of immune cells in IBD. In susceptible individuals, certain environmental triggers impair the intestinal barrier function, leading to microbial translocation across the epithelial layer and DC activation. This is followed by DC migration to lymph nodes, where they present antigens to T cells to induce differentiation into T_H1 , T_H2 and T_H17 subsets. Effector differentiation is accompanied by their upregulation of the intestinal-specific integrin $\alpha 4\beta 7$, resulting in the migration of effector T cells to intestinal lamina propria. T_H1 cells mediate inflammation by secreting IFN- γ , resulting in macrophage activation and villus atrophy. Cytokines produced by T_H17 cells induce epithelial proliferation, antimicrobial peptide secretion and neutrophil recruitment. T_H2 cells induce B cell activation and antibody production against antigens commonly found in the GI tract. Chronic inflammation mediated by T cells results in cycles of epithelial cell injury followed by repair, impairing the epithelial barrier function, and leading to intestinal ulcers, fibrosis and cancer

rial species such as *Mycobacterium paratuberculosis*, *Mycobacterium avium*, *Listeria monocytogenes*, *Chlamydia trachomatis*, and *E. coli*. Antibodies to several commensal organisms have been detected in the sera of patients with IBD. In addition, circulating antibodies against *Saccharomyces cerevisiae* have also been found to be present. Due to the compromised mucosal barrier, increased numbers of bacteria adhere to the intestinal wall, increasing intestinal permeability and inflammation. This pro-inflammatory microenvironment can perpetuate IBD and lead to the development of intestinal ulcers, fibrosis, and increased susceptibility to the development of colon cancer. While the role for microbiota in the development of IBD in experimental rodent models is well-established, its significance in

human IBD development is variable. However, a subgroup of patients respond to fecal microbiota transplantation or antibiotic use, suggesting that the microbiota can regulate the severity of disease but may not be sufficient to cause IBD in otherwise healthy people. Lastly, studies in animal models demonstrate that the immune responses during IBD are associated with increased production of the pro-inflammatory cytokine TNF- α and decreased production of the anti-inflammatory cytokine IL-10. Interestingly, the development of colitis does not occur in germ-free animals, whereas IL-10-deficient mice can spontaneously develop colitis when housed in environments colonized by certain types of bacteria. Furthermore, the production of IL-10 by CD4 T_{reg} cells is particularly important in pre-

venting the development of inflammation, suggesting that the induction of inflammatory and protective mechanisms is regulated *via* a delicate balance of immune processes that control the activation of inflammatory T_{H1} , T_{H2} , and T_{H17} subsets and regulatory T_{reg} cell subsets.

Role of Macrophages and DCs in IBD Initiation

Macrophages and DCs in the lamina propria of the GI tract recognize microbes through pattern recognition receptors (TLRs, NLRs, C-type lectin receptors, and RIG-I-like receptors). These receptors activate transcription factors including NF- κ B, MAP kinases, and interferon regulatory factors (IRFs), resulting in the production of pro-inflammatory cytokines, chemokines and type I interferons (IFNs). Macrophage activation in the GI tract can lead to the direct killing of microbes as well as the recruitment of neutrophils. Furthermore, the production of pro-inflammatory cytokines may activate intestinal DCs, promoting their antigen presenting function to T cells. Thus, the balance of pro-inflammatory (IL-6, IL-12, IL-23, TNF- α) and anti-inflammatory (IL-10) cytokines produced by lamina propria macrophages has potential to impact the inflammatory milieu. IL-10 is required to prevent spontaneous intestinal inflammation in response to microbiota and promotes regulatory T cell (T_{reg}) maintenance in the gut. In addition, IL-10 suppresses the production of IL-12/23 p40 from intestinal macrophages, leading to diminished T_{H1} and T_{H17} responses. In healthy individuals, pro-inflammatory responses to intestinal bacteria are transient and resolve over time. Intestinal macrophages appear to be able to distinguish between commensal microbiota and pathogens such that disease-causing bacteria are most likely to trigger inflammation. However, dysregulation of these pathways can enhance susceptibility to infections or produce the chronic inflammation characteristic of IBD. Several single nucleotide polymorphisms (SNPs) associated with IBD susceptibility are expressed in macrophages, including the genes encoding NOD2, NF- κ B, IRF5, CARD9, IL-10, IL-12 and the IFN- γ receptor. The activation status of DCs can also modulate GI

inflammation by inducing effector CD4 T cell differentiation. Various subsets of DCs can induce the differentiation of pro-inflammatory T cells (such as T_{H1} , T_{H2} , and T_{H17}) or anti-inflammatory cells (e.g. T_{reg}). DCs also positively regulate IgA production in the gut, helping to limit bacteria growth and adhesion to the epithelium. Secretory IgA (sIgA) facilitates antigen delivery to DCs and is significantly decreased in IBD, especially UC, although the serum levels of monomeric IgA may be elevated. Thus, both macrophages and DCs can contribute to inflammatory responses during IBD.

T Cell Immunity and IBD

CD4 T cells are central for driving gut inflammation and IBD pathogenesis. Following their activation by antigen presenting cells, CD4 T cells differentiate into specialized subsets in response to the cytokine milieu (Chap. 3). The production of IL-12 by innate cells induces T_{H1} differentiation while the combination of IL-1 β , IL-6, IL-23 and TGF- β promotes T_{H17} differentiation. The differentiation of T_{H2} subsets is driven by IL-4. Once activated within the mesenteric lymph nodes, effector T cells express the intestinal homing integrin $\alpha_4\beta_7$ and migrate to the gut where they secrete inflammatory cytokines. While these subsets are required for adaptive immunity, they contribute to pathogenic inflammation when directed against innocuous- or self-antigens. IFN- γ produced by T_{H1} cells activates macrophages to augment their microbicidal properties, thus further amplifying the macrophage response. Similarly, IL-17 and IL-22 produced by T_{H17} cells will stimulate the intestinal epithelium to proliferate, secrete various antimicrobial peptides and neutrophil-recruiting chemokines. Increased numbers of IFN- γ -producing T_{H1} cells as well as IL-17-producing T_{H17} cells have been selectively identified in patients with CD, suggesting a pathogenic role for these T_{H} subsets. In contrast, IL-5 and IL-13-producing T_{H2} cells, as well as IL-13-producing NKT cells, are thought to be predominant in UC patients. However, the role of IL-13 in disease is complex, as it has been shown to have both pro- and anti-inflammatory effects in UC patients. The IL-23R

and its signaling pathway appears to impact susceptibility to both CD and UC. Lastly, regulatory T cells (T_{regs}) contribute to tolerance in part by producing IL-10. Their protective role in CD is corroborated by studies demonstrating the efficacy of autologous T_{reg} transfer.

IBD Treatment

Given the many possible genetic, immune, environmental, and microbial interactions leading to the development of IBD, therapeutic approaches are aimed at either resolution of the inflammatory response or an attempt to suppress immune cell activation. As described below, the most commonly used drugs for inducing a state of remission are corticosteroids, methotrexate, sulfasalazine, azathioprine, cyclosporine, or TNF inhibitors. Initially, antibiotics such as **metronidazole** (Flagyl) or **ciprofloxacin** (Cipro) may be given, as many CD patients have increased levels of colonization with *E. coli*, *Serratia marcescens*, and/or *Candida tropicalis* in the GI tract. These may form a biofilm on the intestinal walls, precipitating inflammation.

Aminosalicylates (ASAs) exert their therapeutic efficacy in the colon after being chemically reduced from their prodrug form by coliform bacteria. **Sulfasalazine** and other 5-aminosalicylate drugs are effective at reducing inflammation and achieving remission in UC, but sulfasalazine's effects are more controversial in CD. This may be due to the sulfapyridine component of the drug, which is responsible for many of its adverse effects. While this component contributes to its therapeutic effects in rheumatoid arthritis, only the 5-ASA component is necessary for treating IBD. Thus, the use of the 5-ASA only drugs (Asacol, Colazal, and Delzicol, among others) are preferred over sulfasalazine.

Corticosteroids such as methylprednisolone, prednisone, hydrocortisone, or budesonide may be used to help relieve symptoms and flareups, as well as inducing remission. While these medications tend to work quickly, they are not used long-term due to adrenal suppression, and the patient should be gradually tapered off the drug.

While corticosteroids are often taken orally, methylprednisolone and hydrocortisone can be given locally in a rectal formulation for severe forms of the disease in the lower colon or rectum. Budesonide is given orally and targets inflammation in the distal small intestine and beginning of the large intestine.

Patients who are refractory to steroid therapy, 5-ASAs, or antibiotics may require therapy with calcineurin inhibitors, anti-metabolites, or biologics. For instance, TNF inhibitors (adalimumab, infliximab, certolizumab) may be used in combination with purine antimetabolites (thiopurines; azathioprine, 6-mercaptopurine). The class of antimetabolites function by inhibiting purine synthesis, which is required for cellular replication. Thus, dividing cells are most susceptible to antimetabolites and undergo apoptosis in their presence. In addition, it is thought that azathioprine may target Rac1, a small GTPase involved in CD4 T cell activation. When azathioprine is metabolized to 6-mercaptopurine and subsequently converted to 6-thioguanine, one of its metabolites (6-thio-GTP) binds to Rac1, acting as a competitive inhibitor of endogenous GTP, promoting apoptosis of the T cell.

Integrin-neutralizing monoclonal antibodies are used to suppress lymphocyte migration to the GI tract. By targeting α_4 integrins, natalizumab blocks the function of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ heterodimers. While $\alpha_4\beta_7$ is required for T cell migration to the gut, $\alpha_4\beta_1$ is involved in trafficking to the brain. By inducing immunosuppression in the brain, natalizumab comes with a risk of progressive multifocal leukoencephalopathy (PML), and patients must be monitored and tested for JC virus. Since vedolizumab specifically blocks the interaction of $\alpha_4\beta_7$ heterodimers with MAdCAM-1 on intestinal vascular endothelium, the risk of PML is thought to be much lower. Both drugs have demonstrated efficacy as Crohn's therapies by inhibiting/slowing leukocyte trafficking to the gut, thus suppressing inflammation and tissue damage.

Paying close attention to the diet and maintaining good nutrition may help decrease symptoms in CD patients and allow for the healing of tissues. Spicy or fibrous foods may lead to flareups, diarrhea, and impaired nutrient absorption.

Approximately 50–75% of patients with complications (fistulas, abscesses) whose condition is not well-controlled with medication require surgical resection of affected tissues within 10 years of their diagnosis. Even with surgery, up to 30% of patients may experience a recurrence within 3 years, with 60% experiencing a recurrence within 10 years.

Mechanism of Action of IBD Drugs

5-Aminosalicylates

Drug formulations containing 5-aminosalicylic acid (5-ASA) are often used as first line therapy for treating IBD and to maintain remission. Produced as inactive prodrugs, they must undergo chemical reactions in the gut to become active. This is essential because unformulated 5-ASA is mostly absorbed in the small intestine prior to reaching inflamed regions of the GI tract. The azo (sulfasalazine, olsalazine, balsalazide) and mesalamine formulations allow for high concentrations of the active drug to reach the terminal ileum and colon. **Olsalazine (Dipentum[®])** contains two 5-ASA moieties bound through an azo bond, reducing its absorption from the small intestine. In the terminal ileum and colon, bacteria cleave the azo bond, releasing two active 5-ASA molecules. In **sulfasalazine (Azulfidine[®])**, 5-ASA is bound to the inert compound sulfapyridine, which is metabolized by intestinal bacteria to yield free 5-ASA. In **balsalazide (Colazal[®])**, 5-ASA is bound to 4-aminobenzoyl-beta-alanine. **Mesalamine formulations** deliver 5-ASA to different sections of the small or large bowel. **Pentasa[®]** contains microgranules that target 5-ASA release to the small intestine. **Asacol[®]** and **Lialda[®]** are coated in resins that dissolve at pH 7, releasing 5-ASA into the distal ileum and proximal colon. For treating inflammation confined to the distal colon or rectum, enema formulations (**Rowasa[®]**) and suppositories (**Canasa[®]**) are preferred for delivering high concentrations of 5-ASA to these regions. The mechanism through which 5-ASA provides anti-inflammatory action is not well understood, although it has structural similarity

to the cyclooxygenase (COX) inhibitor acetylsalicylic acid (aspirin). Likewise, 5-ASA suppresses arachidonic acid metabolism into prostaglandins and leukotrienes, implicating a possible inhibitory effect on COX and lipoxygenase enzymes. Further, 5-ASA is a free radical scavenger and decreases oxidative stress. An animal model of colitis showed that 5-ASA inhibits NF- κ B activation, an important transcription factor for pro-inflammatory cytokine production.

Glucocorticoids

Therapeutic glucocorticoids (GCs) are structurally similar to the endogenous hormone cortisol, which is involved in the stress response and has anti-insulin effects, increasing blood glucose levels. Their mechanism of action is described in detail in Chaps. 1 and 4 and is briefly alluded to below. The potent immunosuppressive properties of GCs render them very useful for treating inflammation, including moderate to severe relapses of UC and CD. GCs are lipophilic and largely bound to serum albumin or steroid binding globulin, while the free hormone passively diffuses through cell membranes. Cortisol is an essential hormone and nearly every cell in the body expresses the GC receptor. The initial interaction between GCs and the GC receptor occurs in the cytoplasm, resulting in the nuclear translocation of this complex and binding to genetic sequences called glucocorticoid response elements (GREs) (Fig. 6.7). In addition to directly regulating gene expression, the GC receptor interacts with other transcription factors including NF- κ B and AP-1, antagonizing their activity. It is estimated that 10–20% of the human genome is positively or negatively regulated by GCs. The mechanism through which GCs suppress pro-inflammatory cytokines, prostaglandins, chemokines, and tissue damage involves the inhibition of NF- κ B, COX-2, phospholipase A2, and nitric oxide synthase. The adverse systemic effects of GCs include adrenal suppression, acne, edema, glucose intolerance, osteoporosis and susceptibility to infection. While endogenous cortisol can bind to and activate the mineralocorticoid receptor, therapeutic GCs can be selected for their anti-inflammatory

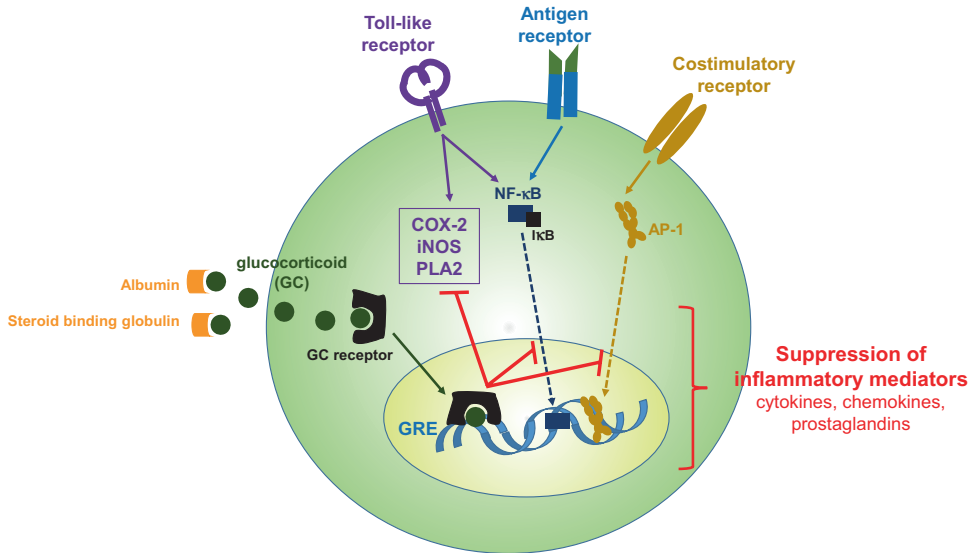


Fig. 6.7 Mechanism of action for therapeutic glucocorticoids. Immune cell activation is mediated by several types of receptors including those involved in antigen recognition, costimulation or pathogen detection (Toll-like receptor). Signaling through these receptors results in the activation of transcription factors that induce cell proliferation and the secretion of inflammatory cytokines and prostaglandins. Glucocorticoids are potent immunosuppressive agents that diffuse across cell membranes and activate cytoplasmic glucocorticoid receptors. This results in its nuclear translocation and binding to promoter regions in genes containing glucocorticoid response elements (GRE). Thus, glucocorticoids suppress immune cell functions by regulating gene expression

potency with minimal mineralocorticoid effects. Common IBD treatments are prednisone, prednisolone and budesonide. For active IBD in the rectum and distal colon, topical formulations of hydrocortisone or budesonide are used (enemas, foam, suppositories) since they produce fewer systemic side effects than oral or intravenous forms due to their poor absorption. **Budesonide (Entocort®)** binds to the GC receptor with 200-fold greater affinity than cortisol and 15-fold greater affinity than prednisolone. The bioavailability of budesonide is low due to its extensive first pass hepatic metabolism by CYP3A4, resulting in a low probability of systemic side effects. Entocort capsules are filled with enteric coated granules that dissolve when the pH is above 5.5, resulting in budesonide release into the distal ileum and colon.

Anti-proliferative Drugs

Purine Analogs

Azathioprine (Imuran®) and **6-mercaptopurine (6-MP, Purinethol®)** are purine anti-proliferative

agents used for the induction and maintenance of remission in IBDs. Their inhibitory effect on DNA and RNA synthesis results in the apoptosis of proliferating cells, including T cells. Following administration, azathioprine is non-enzymatically converted to 6-MP, which is subsequently converted into several metabolites through the action of hypoxanthine-guanine phosphoribosyltransferase (HGPRT). The active metabolites, 6-thioguanine nucleotides and 6-methylmercaptopurine, inhibit the enzyme amidophosphoribosyltransferase (ATase), required for purine synthesis. Proliferating T cells treated with 6-MP have rapid reductions in intracellular ATP concentrations, leading to decreased catabolism of glucose and glutamine. Purine analogs can cause bone marrow depression, leading to leukopenia and increased susceptibility to infections. Adverse effects of purine analogs can be exacerbated by allopurinol through the inhibition of xanthine oxidase, a major enzyme in the catabolism of 6-MP metabolites.

Methotrexate (Rheumatrex®) is a competitive inhibitor of dihydrofolic acid reductase, suppressing purine and pyrimidine biosynthesis.

This inhibits DNA replication in T cells and other actively proliferating cells including tumors and intestinal mucosa. In addition to its effects on DNA synthesis, methotrexate has anti-inflammatory properties by inducing the production of IL-10, IL-1 receptor antagonists, and adenosine secretion. These properties make methotrexate effective at inducing and maintaining remission in CD. Similar to purine analogs, methotrexate can result in bone marrow depression and leukopenia. Supplementation with folate may be able to reduce the risk of leukopenia and susceptibility to infections without impairing its anti-inflammatory functions.

Monoclonal Antibodies

Cytokine Targets

TNF produced by immune cells is a pharmacologic target in IBD. Initially synthesized as a plasma membrane-bound protein, **TNF- α converting enzyme (TACE)** cleaves the extracellular domain to release soluble TNF. The effects of soluble and membrane-bound TNF are mediated through TNF receptor I and II, respectively. Many cell types express TNF receptors, the activation of which results in the nuclear translocation of NF- κ B, pro-inflammatory cytokine (IL-1, IL-6) production, leukocyte activation, fibroblast collagen production, endothelial adhesion molecule expression, and liver acute phase response proteins. These effects of TNF can promote intestinal tissue damage during IBD. Cellular responses to TNF are complex, as signaling pathways downstream of its receptors can promote either survival or apoptosis. Three monoclonal antibodies are used therapeutically to neutralize soluble and membrane-bound TNF, preventing it from interacting with its receptors. These treatments are typically used for patients that have inadequate responses to conventional anti-proliferative agents. In addition to neutralization, the full-length antibodies **infliximab** and **adalimumab** can lyse cells containing membrane-bound TNF in the presence of complement, destroying cellular sources of this cytokine. **Certolizumab pegol (Cimzia[®])** is an

antibody fragment with the variable region conjugated to polyethylene glycol and does not induce complement-mediated lysis. Anti-TNF antibodies have been shown to induce apoptosis in lamina propria-resident T cells, providing another mechanism through which they protect against IBD. Due to important roles for TNF in host defense, anti-TNF antibodies may increase susceptibility to infections and allow for the reactivation of latent infections. Adverse effects are most likely to occur in patients concurrently taking other immunosuppressive agents such as corticosteroids, anakinra, abatacept, rituximab or natalizumab. A large percentage of patients on anti-TNF therapy become non-responsive over time, resulting in treatments targeting other inflammatory pathways.

Interleukin-12 is a heterodimeric cytokine consisting of p35 and p40 subunits encoded by different genes. The p40 subunit is also used to form IL-23 when it dimerizes with the p19 subunit. Both IL-12 and IL-23 have been implicated in pre-clinical models of IBD. These cytokines are produced by antigen presenting cells including DCs and macrophages. Receptors for IL-12 and IL-23 are expressed on CD4⁺ T cells, CD8⁺ T cells, $\gamma\delta$ ⁺ T cells, NK cells and innate lymphoid cells. Stimulating CD4⁺ T cells with IL-12 or IL-23 results in their differentiation into inflammatory T_H1 and T_H17 subsets, respectively. Inflamed sections of the GI tract in IBD patients have elevated numbers of CD4⁺ T cells. In addition, IL-23 may promote intestinal inflammation by stimulating innate lymphoid cells. **Ustekinumab** is a human IgG1k monoclonal antibody against IL-12/23p40, preventing its binding to IL-12R β 1 (Fig. 5.11). Thus, ustekinumab neutralizes the function of IL-12 and IL-23, both of which signal through IL-12R β 1. This treatment is used for moderate to severe CD, including patients that have become non-responsive to anti-TNF therapy. Patients receiving ustekinumab should not receive live vaccines.

Integrin Targets

Integrins are adhesion molecules on the surface of leukocytes that interact with their ligands

expressed on vascular endothelium, allowing circulating leukocytes to adhere to the vascular endothelium and migrate into the underlying tissue. Integrins are heterodimers consisting of α and β subunits. A subset of memory T cells express $\alpha_4\beta_7$, resulting in their migration to the GI tract. The ligand for $\alpha_4\beta_7$ is MAdCAM-1, which is expressed on intestinal endothelial cells. The $\alpha_4\beta_7$ /MAdCAM-1 interaction facilitates T cell transmigration into the intestinal lamina propria, contributing to the pathogenesis of CD. **Natalizumab (Tysabri®)** recognizes α_4 subunits of integrins, inhibiting the interaction of $\alpha_4\beta_7$ with MAdCAM-1 on GI vascular endothelial cells (Fig. 6.8). In addition, natalizumab inhibits $\alpha_4\beta_1$ from interacting with vascular cell adhesion molecule-1 (VCAM-1) on activated vascular endothelium. By preventing the transmigration of leukocytes from endothelial to parenchymal tissue compartments, natalizumab reduces intestinal inflammation in IBD patients. **Vedolizumab (Entyvio®)** is specific for $\alpha_4\beta_7$ with no effect on $\alpha_4\beta_1$ integrins, and is used to decrease T cell accumulation into the intestine. Due to their inhibitory effects on leukocyte migration, these antibody therapies may increase susceptibility to infections.

Thalidomide (Thalomid®) suppresses pro-inflammatory cytokine production from immune cells and may be used in patients that fail to respond to anti-TNF therapy. Thalidomide is well known for causing embryo-fetal toxicity resulting in congenital limb abnormalities and should

never be taken during pregnancy. Nonetheless, its anti-inflammatory properties are unrelated to embryo-fetal toxic effects, and thalidomide has been shown to induce a clinical response and remission in 70% and 55% of IBD patients, respectively. The most common side effects associated with thalidomide use in IBD patients include peripheral neuropathy and sedation.

Celiac Disease

Celiac Disease is a widely occurring autoimmune disorder of the small intestine that is prevalent in up to 1% of the world's population. In recent years, the incidence of the disease has been steadily increasing, and it is not just confined to industrialized countries in Western Europe or the United States, but has been found in populations throughout the world including North Africa, the Middle East, and India. The defining characteristic of celiac disease is a chronic inflammatory reaction in susceptible individuals to derivatives of the dietary component gluten, which are found in grains including wheat, barley, and rye. This reaction results in severe impairment and enteropathy of the small intestine, leading to villous atrophy, and the malabsorption of nutrients. Patients with celiac disease often present with either classic symptoms such as weight loss, chronic diarrhea, malabsorption, or failure to thrive (which is rare), or non-classical symptoms

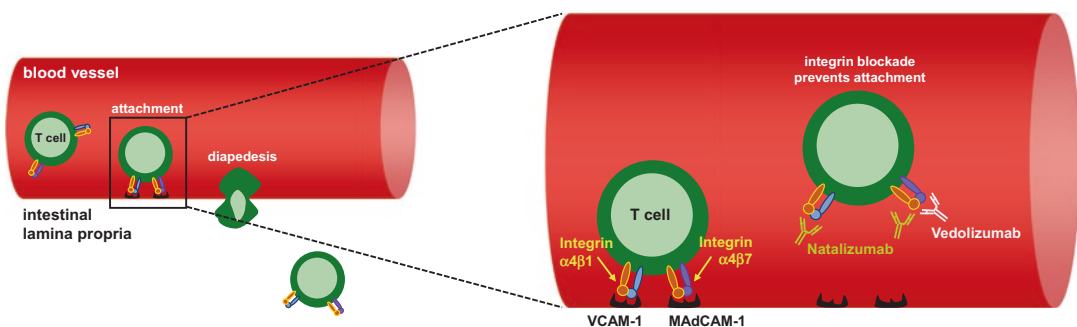


Fig. 6.8 Mechanism of action for anti-integrin antibodies. α_4 integrins are important for the migration of circulating T cells to the GI tract by facilitating attachment to VCAM-1 ($\alpha_4\beta_1$) and MAdCAM-1 ($\alpha_4\beta_7$) on intestinal blood vessels. The monoclonal antibodies natalizumab and vedolizumab neutralize α_4 integrins, preventing T cell attachment and entry into the GI tract

such as bloating, constipation, abdominal pain, iron deficiency, chronic fatigue, and osteoporosis. Both intestinal and extra-intestinal manifestations of celiac disease have been reported. Although celiac disease was first described in 1888, its relation to gluten components was only established in 1953, and the immunological basis of the disease is only now beginning to be appreciated.

Immune Mechanisms in the Pathogenesis of Celiac Disease

The primary immune triggers in the development of celiac disease are peptides generated from gluten components that induce CD4 T cell reactions to autoantigens in susceptible individuals. Gluten is a major component of the cereals wheat, rye, and barley, and is widely used in bread-making due to its elastic properties. The main components

of gluten include glutenins, prolamins and gliadins in wheat, secalines in rye, and hordeins in barley. The presence of various glutamine and proline residues in gluten results in its incomplete digestion by intestinal gastric, pancreatic, and brush-border enzymes, giving rise to the formation of large, undigested peptides up to 33 amino acids in length. In susceptible individuals, gliadins interact with intestinal epithelial cells and disassemble the tight junctions between enterocytes (Fig. 6.9). This results in leakiness and impairment of the epithelial cell barrier, leading to transcytosis (probably via secretory IgA) of gluten components including gliadin peptides from the intestinal lumen to the lamina propria. Here, the gliadin peptides are deamidated by enzymes such as tissue transglutaminase. Deamidation increases the immunogenicity of these peptides in susceptible individuals, favoring their binding to the HLA molecules DQ2 and DQ8 on antigen presenting cells. The subsequent

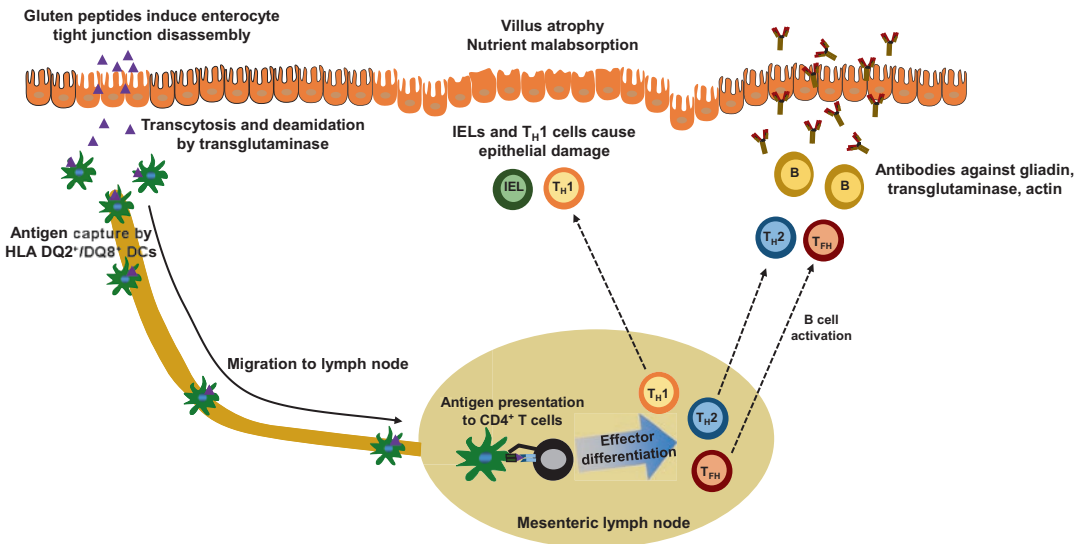


Fig. 6.9 Role of immune cells in celiac disease. In susceptible individuals, gluten-derived peptides (purple) cause the disassembly of enterocyte tight junctions, resulting in their translocation into the lamina propria where the enzyme transglutaminase deamidates the peptides. Dendritic cells (DCs) acquire this antigen and migrate to lymph nodes where they interact with T cells. Expression of HLA DQ2 or DQ8 by the DC increases the likelihood of antigen presentation. T cells recognizing gluten-derived peptides and receiving other signals required for activation will undergo effector differentiation into T_H1 , T_H2 and T_{FH} subsets. B cell activation is associated with their production of antibodies recognizing gliadin, transglutaminase or actin. T cells migrating to the intestinal lamina propria promote inflammation in the presence of gluten, as shown. In this way, chronic exposure to gluten over time will result in villus atrophy and nutrient malabsorption in the GI tract in susceptible individuals

activation of naïve CD4 T cells against these peptides results in immune sensitization and the production of antibodies against gliadin peptides and the autoantigens tissue transglutaminase and actin. T_H1 cells producing IFN- γ , T_H2 cells and T_{FH} cells have been found to be implicated in the pathogenesis of celiac disease.

In addition to the adaptive immune response in the lamina propria, innate immune responses mediated by IELs also appear to be important for the formation of celiac lesions. These cells are able to acquire receptors characteristic of NK cells, including NKG2D, in an IL-15-dependent manner and directly contribute to epithelial cell damage. In fact, an increased density of IELs in celiac lesions is a hallmark of celiac disease.

While genome-wide association studies have identified non-HLA loci that may predispose to the development of celiac disease, the most important genetic risk factors are the genes encoding the α and β chains of HLA DQ2 and DQ8 molecules, respectively. Nearly all patients with celiac disease harbor specific variants of these genes, named *HLADQA1* and *HLADQB1*. Greater than 90% of celiac patients are DQ2-positive, with most of the others being DQ8-positive. The presence of these loci is not sufficient for the development of disease, however, as only 1–3% of individuals expressing these loci develop disease. This suggests a role for additional environmental factors in the development of disease, such as the early introduction of large doses of gluten to children, birth by cesarean section, various infections including rotavirus in children, *campylobacter* in adults, respiratory infections, antibiotic and proton pump inhibitor use, and microbial dysbiosis. With respect to the latter, increased concentrations of *Bifidobacterium bifidum* were found to be present in the feces of adults with celiac disease compared with healthy controls. Similarly, children with celiac disease were reported to have a greater proportion of duodenal gram-negative bacteria compared to healthy controls. Thus, genetic and environmental factors both contribute to celiac disease.

The presentation of celiac disease may be extremely heterogeneous. While patients with classic symptoms are easily diagnosed, those with atypical symptoms such as EIMs, silent or latent forms of the disease may be harder to diagnose. The latter typically exhibit serum IgA antibodies against tissue transglutaminase and harbor genetic risk factors without developing symptoms. Patients with refractory celiac disease, who exhibit persistent symptoms despite a gluten-free diet for a period greater than 1 year, are particularly challenging to treat. Patients with classical symptoms usually present in childhood, while those with atypical symptoms usually present as adults. Examples of EIMs associated with celiac disease include dermatitis herpetiformis (an inflammatory cutaneous disease resulting in diffuse, polymorphic lesions consisting of erythema, urticarial plaques, herpetiform vesiculae and blisters), type 1 diabetes, autoimmune thyroid disorders, autoimmune hepatitis, liver conditions, and other neurological conditions. The link between celiac disease and autoimmunity is further supported by the role of HLADQ2 and HLADQ8 genetic risk factors in thyroid disorders and antibodies to autoantigens such as actin in dermatitis herpetiformis. Other manifestations such as hepatic damage could occur due to increased entry of toxins and other factors into portal veins due to the defective intestinal barrier. Secondary manifestations caused by nutrient malabsorption can occur in celiac patients. Examples of these include osteoporosis due to insufficient calcium absorption or malignancies resulting from intestinal damage.

Celiac Disease Treatment

The most widely prescribed and effective treatment for celiac disease is the adoption of a gluten-free diet. Typically, clinical improvement is observed within weeks, and physiological improvement of intestinal tissues occurs within 1–2 years (Fig. 6.10). Early diagnosis and treatment in pediatric celiac disease is especially

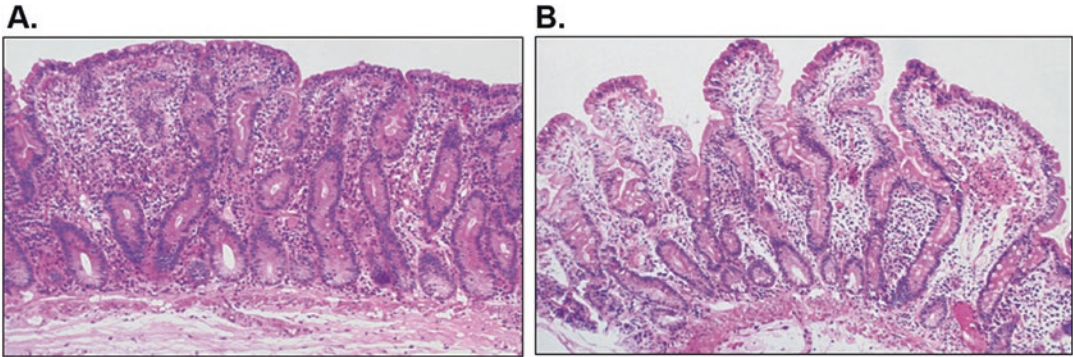


Fig. 6.10 Normalization of biopsy-proven changes in celiac disease after implementation of a gluten-free diet. (a) Biopsy-proven changes of untreated celiac disease. The villi are “flattened” and rudimentary, while the crypts are expanded and hyperplastic with increased numbers of epithelial cells and an increased mitotic index. The cellularity of the lamina propria is enhanced, and there is an increased number of plasma cells and lymphocytes. (b) Following implementation of a gluten-free diet, the villi are elongated, crypts are shortened, and the cellularity of the lamina propria is much reduced. Credit: Adapted from Hugh J. Freeman. *Adult Celiac Disease and Its Malignant Complications*. Gut and Liver, Vol. 3, No. 4, December 2009, pp. 237–246. CCLI attribution

beneficial, preventing the induction of many secondary conditions. Pharmacologic treatment options, especially in patients with refractory celiac disease, are limited and include immunosuppressants such as corticosteroids, azathioprine, and cyclosporine. Since the benefits derived from these are transient, a number of other pharmacologic agents are currently being evaluated. These investigational treatments include gluten degrading enzymes and the zonulin inhibitory octapeptide larazotide. Zonulin is a protein involved in the degradation of epithelial tight junctions. Inhibiting its function with larazotide has been found to be particularly promising, decreasing symptom scores and autoantibody levels during clinical trials. Other immunotherapeutic options being evaluated include the use of anti-IL-15 antibodies to induce apoptosis of IELs and tolerogenic vaccines containing gluten peptides.

Food Allergies

Food allergies are adverse health effects arising from an immune response that occurs reproducibly on exposure to a given food. The incidence of food allergy has been dramatically increasing throughout the developed world and constitutes a

substantial public health burden in affected nations. It is estimated that approximately 3–6% of the population in the United States has a food allergy. The induction of food allergy occurs due to a breakdown of oral tolerance, resulting in immune sensitization to offending allergens and the activation of mast cells, T_H2 cells, and eosinophils. The most common allergen-containing foods include milk, egg, peanuts, wheat, soy and fish. In sensitized individuals, exposure to these foods can manifest in a range of clinical symptoms that involve the gastrointestinal tract, skin, lungs, and in severe cases, anaphylactic shock. Skin reactions include urticaria and angioedema manifesting as itching and hives, while gastrointestinal symptoms include intestinal spasms, vomiting, and diarrhea. Systemic anaphylaxis, resulting from allergen absorption into the bloodstream and systemic mast cell and basophil activation, affects several organ systems including the skin, GI, lung, and cardiovascular systems. This leads to a severe drop in blood pressure and is accompanied by tightening of the throat and difficulty breathing and swallowing, requiring immediate treatment with injected adrenaline or epinephrine.

Currently, food allergies can only be managed through allergen avoidance or treating the symp-

toms. While most allergies are identified during childhood, 15% are first diagnosed in adults. Many patients with food allergy naturally outgrow them over time; however, this depends on the causative allergen. For example, patients frequently outgrow hen and cow milk allergies, while peanut and tree nut allergies typically persist for life.

The development of food allergy has been linked to genetic susceptibilities as well as extrinsic factors that have the potential to modulate the immune response. Genetic susceptibility includes polymorphisms in a number of immune-related genes including IgE, the TCR, FoxP3, IL-4, IL-10, and IL-13. Extrinsic factors tend to be multifactorial, including early exposure to allergens and infections, microbial diversity within the GI tract, predisposing conditions including eczema due to cutaneous sensitization to food allergens, the type of diet, and vitamin D deficiency. A number of hypotheses have been postulated to explain how environmental factors regulate susceptibility to food allergies, including the dual-allergen hypothesis, the hygiene hypothesis, and the vitamin D hypothesis.

Mechanisms of Food Allergy

Although the term ‘food allergy’ is widely used among the public to refer to adverse reactions to food, the development of food allergy is a consequence of the breakdown of immunological tolerance to specific ingested antigens. A failure in oral tolerance is defined as the state of active systemic unresponsiveness to ingested food antigens. Adverse reactions not due to immune responses are referred to as food intolerances. The breakdown of tolerance can result in the development of IgE-mediated reactions to the ingested food, as well as non-IgE-mediated reactions such as eosinophilic esophagitis, allergic proctocolitis, and food-protein induced enterocolitis. Mixed reactions involving both IgE-dependent and IgE-independent mechanisms may be present during some types of food allergies.

Acute episodes of food allergy manifest as immediate hypersensitivity reactions that are triggered by the IgE-mediated activation of mast cells and basophils in target tissues (Fig. 6.11).

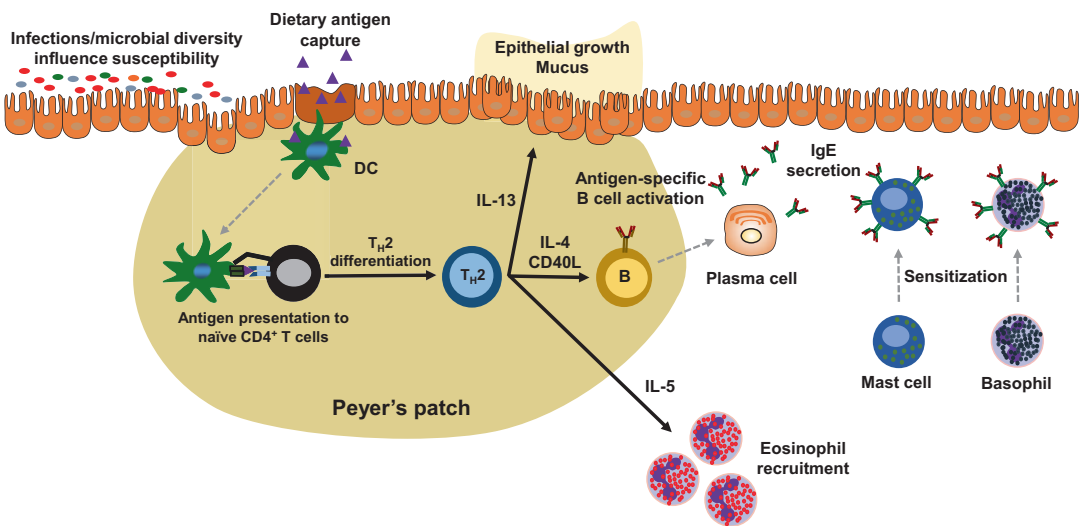


Fig. 6.11 Immune cell activation during food allergies. Susceptibility to food allergies is influenced by microorganisms in the GI tract. DCs that acquire dietary antigens and become activated can induce the differentiation of allergen-specific CD4 T cells into Th2 cells that produce cytokines in the presence of the allergen. B cells activated under these conditions produce antigen-specific IgE antibodies that sensitize mast cells and basophils. Re-exposure to the allergen will result in their degranulation and subsequent tissue damage. IL-13 produced by Th2 cells induces intestinal epithelial cell growth and mucus production, while IL-5 recruits eosinophils to the GI tract. Thus, Th2 cells have a pathogenic role in the context of food allergies

Exposure to food allergens in sensitized patients results in the crosslinking of specific IgE molecules on mast cells and basophils, leading to their degranulation and release of preformed mediators including histamine, tryptase, and chymase, triggering vasodilation and smooth muscle contractions in the GI tract. Subsequently, mast cells and basophils produce substances (leukotrienes, prostaglandins, platelet activating factor (PAF)) that potentiate vasodilation, vascular permeability, smooth muscle contraction, mucus production and the activation of nociceptive nerves involved in pruritis. Cytokines secreted by these cells (IL-4, IL-5, IL-13) further propagate the inflammatory reaction, promoting the proliferation and survival of T_H2 cells and eosinophils. Specific roles for T_H2 cells, mast cells, basophils, and eosinophils in allergic responses are described in Chap. 4. The ensuing myriad of physiological reactions includes angioedema, oral pruritis, intestinal spasms, vomiting and diarrhea. These reactions are mediated by mast cell and eosinophil activation upon the recognition of allergens by IgE antibodies on sensitized cells. The cytokines produced by activated T_H2 cells (IL-3, IL-4, IL-5, IL-9, IL-10, IL-13) further drive these reactions. For instance, IL-3, IL-9, and IL-10 act as growth factors for mast cells, IL-4 promotes T cell survival and B cell activation, IL-5 induces eosinophilia, and IL-13 promotes epithelial cell turnover, smooth muscle contraction, and mucus secretion. Lastly, pro-inflammatory factors from the intestinal microbiota or diet can induce DC activation, resulting in their loss of a tolerogenic phenotype and induction of T_H2 cell effector differentiation against food-derived antigens rather than T_{reg} differentiation. In addition, cells that are already committed to the T_{reg} lineage cells can begin to produce IL-4 while still retaining the expression of FoxP3. Altogether, T cell responses are shifted towards an inflammatory T_H2 phenotype. Through these mechanisms, interactions between environmental, cellular and physiological processes drive acute inflammatory responses to ingested food antigens.

In contrast to acute episodes of food allergy, chronic allergic processes are responsible for the

pathology of proctocolitis, food-protein induced enterocolitis, and eosinophilic esophagitis. While allergen-specific IgE is produced during these conditions, the pathological responses are primarily mediated by T cells. Eosinophilic esophagitis in response to food allergens is a chronic inflammatory condition characterized by epithelial cell hyperplasia. Patients typically present with gradual onset of gastro-esophageal reflux, dysphagia (difficulty swallowing), eosinophilic infiltrates in the esophagus, and esophageal strictures (narrowing of the esophagus). Patients have difficulty eating and express an aversion to various types of foods. The disease is driven by T_H2 cells producing IL-4, IL-5, IL-13, and chemokines promoting the inflammatory reaction. In particular, IL-5 promotes the proliferation and survival of eosinophils, which are responsible for the tissue damage and fibrosis associated with the disease. Epithelial cell-derived cytokines such as TSLP and IL-33 also play a role, promoting sensitization to various food allergens and T_H2 responses. In addition to eosinophils and T cells, mast cells have been observed in the esophagus of these patients. Allergic proctocolitis is another example of a non-IgE-mediated food allergy. This occurs in susceptible infants fed with formula containing cow milk proteins and results in rectal bleeding that is driven by mucosal eosinophils rather than IgE antibodies. When left untreated, infants can exhibit chronic emesis, diarrhea and failure to thrive. Similarly, in food protein-induced enterocolitis syndrome, patients exhibit eosinophil and T_H2 cell-mediated inflammation in the absence of IgE-mediated sensitization to specific food allergens, driving severe gastrointestinal reactions such as vomiting. The mechanisms responsible for these processes still remain to be elucidated.

The exponential increase in the development of food allergies in recent decades implicates a role for environmental factors in sensitization to food allergens. Such factors include commensal microbes and their metabolites, adjuvants in foods including peanuts, and other dietary components. The role of microbial factors has been extensively studied. While germ-free mice are prone to developing food allergies, certain species including

Clostridium confer protection against allergen sensitization. In susceptible individuals, the intestinal microbiota present at birth has been linked to food allergies. Factors that may regulate the diversity of microbial species include the route of birth delivery (vaginal or caesarian section), infections, diet, and antibiotics. Short chain fatty acids produced by intestinal microorganisms activate certain G-protein coupled receptors (GPCRs) on epithelial cells, mast cells and T cells, inducing their differentiation into T_{reg} s. The mas-related G-protein-coupled receptor member X2 is a recently described GPCR on mast cells and binds to peptides and quinolone compounds. Activation of this receptor induces mast cell degranulation in the absence of IgE, implicating its potential role in food allergies.

Three hypotheses have been proposed to account for the factors contributing to food allergies, termed the dual-allergen hypothesis, vitamin D hypothesis, and hygiene hypothesis. Variables contributing to these hypotheses are summarized as the '5Ds'- dry skin, diet, dogs, dribble, and vitamin D.

The dual-allergen hypothesis (dry skin, diet) postulates that allergic sensitization to food allergens occurs in early life *via* disrupted skin barrier function, resulting in increased skin permeability to food antigens. In this way, transdermal sensitization leads to food allergies in the absence of oral consumption. This hypothesis is supported by multiple lines of evidence in both animal and human studies, including patients with eczema (atopic dermatitis). Mutations in skin barrier proteins such as filaggrin have been linked to both atopic dermatitis and food allergy. Mouse studies have shown that epicutaneous sensitization with allergens stimulates epithelial cells to secrete TSLP and IL-33, leading to the activation of T cells and eosinophils which drive the allergic reaction (described in Chap. 5). Additionally, the Learning Early About Peanut Allergy (LEAP) study was designed to induce oral tolerance in infants in order to prevent the development of peanut allergies. Data from this randomized controlled trial suggested that early introduction of peanuts lowers the risk of developing an allergy to this food later in life. This was in contrast to

guidelines issued in 2000 by the American Academy of Pediatrics and other organizations world-wide, which recommended to delay the introduction of solid allergenic foods to children. Several other trials, including the Enquiring About Tolerance (EAT) study, have since examined the effects of early introduction of other allergens including hen egg protein. Although these trials have yielded mixed results, most data supports early exposure to allergenic foods. Currently, North American guidelines recommend early peanut consumption in infants with atopic dermatitis in order to lower the risk of developing peanut allergies.

The Hygiene Hypothesis (dogs, dribble) postulates that allergies can result from a lack of exposure to infections or other microorganisms during early childhood. The tenets of the Hygiene Hypothesis are described in detail in Chap. 4. It is believed that the absence of infectious or other microorganisms during early childhood results in individuals having T cells that are biased towards differentiating into T_H2 cells following their activation during allergic sensitization. On the other hand, exposure to helminth worms can help to confer protection against IgE-mediated allergies involving mast cells and eosinophils. As described above, recent evidence suggests the microbiome can help to shape immune responses during allergies. Other factors associated with increased microbial exposure, such as living on a farm, attending daycare, having pets, vaginal delivery during birth, and the presence of older siblings, have been found to be protective.

Lastly, a number of studies suggest a role for vitamin D deficiency in the development of allergic responses, with some of these showing higher rates of food allergies in individuals living further from the equator compared to those living closer. Similarly, higher rates of food allergies are observed in individuals born during the Fall or Winter in contrast to those born during the Spring or Summer. These studies suggest that a lack of vitamin D by lieu of equatorial distance and/or seasonal birth may predispose to the development of food allergy. More direct evidence comes from the Australian HealthNuts study, which demonstrated a correlation between low levels of

vitamin D in infants and the risk of developing food allergies. A number of mechanisms have been postulated to account for the role of vitamin D in preventing food allergies. Vitamin D is known to support intestinal T_{reg} responses and may also be important for the differentiation of effector T cell subsets. Further studies are needed to more fully understand its beneficial effects.

Did You Know: *A Recently Discovered Allergy Makes Some People Allergic to Steak?*

Commentary by Kaitlin Armstrong

Mammalian-meat allergy is an allergic reaction to meat characterized by hives and anaphylaxis hours after consuming the allergen. Unlike common food allergies (e.g. peanuts), mammalian-meat allergy is triggered by a lone star tick bite and can break an established tolerance—causing people to develop this allergy after eating meat their entire lives without mishap. This phenomenon was first observed in the 1990s but it wasn't until 2009 that the tick bite was identified as the sensitizing agent, shedding some light on the immunological mechanism of this novel allergy. The lone star tick bite functions similarly to a vaccine containing an antigen and an adjuvant. While the disaccharide galactose-alpha-1,3-galactose (alpha-gal) serves as the antigen, an enzyme in the mouth of the tick (dipeptidyl-peptidase) is the adjuvant, increasing the magnitude of immune responses against alpha-gal. The alpha-gal antigen is not present in the human body but is in the cell membranes of non-primate mammals, resulting in our immune system identifying it as a foreign antigen upon meat consumption. The reason why humans lost the enzyme required for producing this sugar is uncertain; however, it may be linked to protection against malaria infections. The malaria virus is coated in alpha-gal residues, resulting in a survival advantage for humans recognizing alpha-gal as a non-self antigen.

One of the most intriguing questions related to the rise in mammalian-meat allergy is why people have started developing this allergic response in the past 30 years even though ticks have been around for centuries. This phenomenon has been observed in the United States, Australia, Europe and elsewhere. One of the proposed reasons for this reaction is the idea that changes in the diet and antibiotic use have altered the human gut microbiome in such a way that increases susceptibility to developing allergic reactions against alpha-gal. Humans normally have circulating antibodies that recognize alpha-gal due to its production by intestinal microbiota. These naturally occurring antibodies are IgG or IgM, which do not produce allergic responses. Tick-derived compounds along with changes in the human microbiome may help to increase the likelihood of IgE class switching. Overall, the surge in cases of mammalian-meat allergy is an example of how environmental factors help to shape susceptibility to allergies.

Food Allergy Treatment

Strict avoidance of allergenic foods is currently the only realistic option for patients with food allergy. Patients at risk of developing severe reactions such as anaphylaxis must carry epinephrine auto-injectors for immediate self-treatment. Elimination diets, which consist of avoiding specific foods based on the patient's clinical history, are often utilized to identify the offending allergen. While mild allergic reactions can be controlled by over-the-counter drugs such as anti-histamines, anaphylactic shock requires the administration of epinephrine. The effects of epinephrine are rapid, reversing mast cell-mediated responses in various target tissues by binding β_1 and β_2 -adrenergic receptors. This results in the restoration of bronchodilation, repair of tight junctions between epithelial cells, increases in

systolic blood pressure, and the enhancement of myocardial function. Inhaled salbutamol and corticosteroids may be used as add-on medications during adrenaline therapy.

Although allergen avoidance is currently the only option available for preventing food allergies, a number of immunotherapeutic options are currently being examined. This includes oral allergen immunotherapy where patients are

exposed to increasing concentrations of the allergen until a state of tolerance is achieved. Sublingual therapy and epicutaneous therapy, where patients are epicutaneously treated with allergens such as peanuts for several weeks, are also under investigation. Finally, the effects of biologics such as omalizumab, cytokine blockers or cytokine receptor blockers are also being examined.

From Bench to Bedside: Interferon- α and Hepatitis C

In 1957, two British scientists, Alick Isaacs and Jean Lindenmann, published a seminal paper in the Proceedings of the Royal Society (Biological Sciences). They had been focused for several years on understanding how inactivated influenza virus can interfere with the growth of live influenza viruses in the chorioallantoic membranes of chick embryos. Years of research by several investigators had established that the interference activity could not be attributed to the dose of the virus used, type of influenza strain, method of viral inactivation, time interval between inoculations, or the blockage of known cell surface receptors. To investigate this more fully, the British scientists suspended chick embryo membranes in buffered salt solution, allowing them to observe and manipulate the cells and fluid separately outside of the intact chick embryo. The scientists surprisingly discovered that some of the interference activity remained in the fluid despite complete uptake of the virus by cells. They termed the interference factor “interferon” and proceeded to establish the kinetics of its release through an elegant set of experiments.

In short course, interferons were found to be produced by many cells and tissues in diverse animals and its initial classification was based on the type of cell that produced it—leukocyte, fibroblast, or lymphoblastoid cells. Then in the 1960s, Finnish scientist Kari Cantell succeeded in extracting large scale batches of interferon- α (IFN- α) from human leukocytes, which could be partially purified and used in clinical trials. However, these preparations consisted of crude purified extracts that contained IFN- α at levels of less than 1% of the total extract. Thus, it could not be established whether the effects of the extract in clinical trials were due to the interferons present or other contaminating proteins in the extract. Purification of interferons would not occur for at least another two decades until 1978, with the advent of reverse-phase and normal-phase high performance liquid chromatography. In the next few years, the laboratories of Alan Waldmann and Sidney Pestka spent considerable time and effort characterizing the chemical and immunological properties of IFN- α and interferon- β (IFN- β). They developed several techniques and cytopathic assays that confirmed the anti-viral effects of interferon and revealed the existence of a number of different interferons, establishing that IFN- α belonged to a family of interferon proteins. Simultaneously, Wellcome Pharmaceuticals in the UK developed novel ways of extracting large quantities of interferon from lymphoblastoid cell cultures for use in clinical trials as an anti-cancer agent. Finally, in 1980, the biotechnology company Genentech, in collaboration with Hoffman-LaRoche, announced the production of large-scale quantities of leukocyte and fibroblast interferon for use in clinical trials using recombinant DNA technology. Subsequently, they succeeded in cloning immune interferon or type II interferon (IFN- γ) using bacterial, yeast, and mammalian cell culture.

Today, at least seven interferon species are known to exist in humans. Six of these belong to the Type I interferon family, including IFN- α and IFN- β . Only IFN- γ is classified as a Type II interferon. Following their discovery, many of the cell surface receptors for interferons were also discovered, as well as the downstream JAK-STAT signaling pathway.

It is now known that the type I interferons have a breadth of biological activities, including the inhibitory anti-viral effects that were first demonstrated by Isaacs and Lindenmann. Other biological activities include the stimulation of cytotoxic T cells and natural killer cells, increased expression of tumor-associated antigens and MHC I, induction of pro-apoptotic genes (TRAIL, caspases), repression of anti-apoptotic genes (BCL2), and the inhibition of angiogenesis. The Food and Drug Administration (FDA) has approved several type I interferon formulations for a number of diseases and cancer. The first approval was for IFN- α 2a and IFN- α 2b (allelic versions of IFN- α 2) in 1986 for the treatment of hairy cell leukemia. Ten years later, IFN- β 1a and IFN- β 1b were approved for treating relapsing-remitting multiple sclerosis. Additional diseases that IFN- α is approved for include malignant melanoma, follicular lymphoma, genital warts, AIDS-related Kaposi sarcoma, and chronic hepatitis B and C. IFN- γ is approved for chronic granulomatous disease and malignant osteopetrosis.

Hepatitis C virus (HCV) is an insidious chronic infection of the liver that has become a global public health concern and affects 2.7–4.0 million people in the United States. Initial infection with HCV is asymptomatic and 15–45% of patients are able to eliminate it. In the majority of infected patients, HCV infection becomes chronic, contributing to increasing rates of cirrhosis, hepatocellular carcinoma, and liver transplantations. HCV is a small RNA virus belonging to the family *Flaviviridae*. Seven genotypes are known to exist, along with 52 subtypes. Of these, genotype 1 is responsible for 70% of HCV infections in the U.S. Unfortunately, it is also the genotype that is most insensitive to treatment with IFN- α . Following HCV infections, type I interferons turn on a number of interferon-stimulated genes, leading to the inhibition of viral replication, activation of NK cells and increased viral resistance. HCV succeeds at establishing chronic infections by expressing NS3/4A, a non-structural serine protease that suppresses interferon signaling. Due to the high replication rates of HCV and lack of proof-reading mechanisms for its viral RNA-dependent RNA polymerase (NS5B protein), the virus is able to mutate its antigens and escape the immune response, resulting in patients being infected with several quasi-species.

In the early 2000s, pegylated IFN- α was found to have a significantly increased half-life compared to recombinant IFN- α , extending its anti-viral activity. The purine analog ribavirin was also used to inhibit the replication of flaviviruses including HCV. The combination of ribavirin and pegylated IFN- α was successful at maintaining a sustained anti-viral response, improving survival, and lowering the risk of hepatocellular carcinoma in 75% of non-genotype 1-affected patients and 50% of genotype 1-infected patients. However, the enhanced half-life of IFN- α also contributed to serious side effects including fatigue, myalgia, fevers, and other symptoms associated with acute viral infections.

A number of HCV medications are currently available including inhibitors of the NS5A, NS5B, and NS3/4A proteins. These have substantially shortened the course of HCV therapy and resulted in ‘cures’ for many patients without the use of recombinant IFN- α and ribavirin. Although the continued need for IFN- α therapy during HCV infections has been questioned, studying the relationship between this highly subversive virus and IFN- α enabled us to appreciate the role of type I interferons in protecting against viruses.

Summary

The mucosal immune system of the GI tract plays a pivotal role in defending us from infectious pathogens and maintaining tolerance to ingested food and beneficial commensal bacteria. Abrogation of these tolerogenic mechanisms due to genetic, environmental, or immunologic factors can lead to abnormal proinflammatory immune responses that result in disease. These include inflammatory bowel diseases such as UC

and CD, Celiac Disease, and food allergies. The goal of current research is to better understand the immune mechanisms involved in the pathogenesis of these diseases with the hope of developing novel therapeutic approaches for reversing inflammation. Approaches such as fecal microbial transfer or immunosuppressive cytokines have the potential to modulate the immune system by inducing regulatory T cells and suppressing the activation and proliferation of effector T cells.

Case Studies and Practice Questions

Provided by Charles Babcock, Pharm.D.

Case Study 1

Ethan Boyce is a 34 year-old male who was recently seen at a local urgent care clinic for a flare-up of his IBD. He stated that he has been suffering from IBD for several years, but is not sure if he has UC or CD. He has been taking two brown capsules called Delzicol[®] several times a day. However, during the last week he has had several bouts of diarrhea each day which has become unbearable. There does not appear to be visible blood in his stool. He states that he has not been able to eat much over the last few weeks due to intense pain every time he eats. Because of this, he has lost 6 lb over the last few weeks. Ethan does not have any family members with a history of IBD. He reports smoking about a pack a day for the last 10 years and has been taking Advil[®] liquid gels during the last few weeks for the pain in his stomach. He also states that he noticed some red scaly patches on his arms that he thinks started earlier this week. He can't think of any other changes in his diet or lifestyle that could have caused the sudden diarrhea and stomach pains.

1. Based on Ethan's prior history and current symptoms, which type of IBD is he most likely suffering from?
2. Which of Ethan's current medications medication is likely to cause a flare-up of his IBD?
3. A subsequent colonoscopy later revealed the presence of disease in several areas of the intestines. This included the small intestine, the ileocecal region, and the colon. These areas were interspersed by sections that otherwise appeared to be normal. What type of IBD is the colonoscopy indicative of?
4. The physician prescribes a corticosteroid product intended to reduce the inflammation in Ethan's intestines. Which of the following products is the best choice for Ethan?
 - (a) Hydrocortisone enema
 - (b) Hydrocortisone suppositories
 - (c) Budesonide capsules
 - (d) Budesonide rectal foam

5. Since the mesalamine that Ethan has been taking has not been extremely effective, the GI doctor would also like to initiate changes in his therapy. Which of the following drugs represent some therapeutic options for Ethan? Select all that apply.
- (a) Daily prednisone
 - (b) Methotrexate
 - (c) Adalimumab
 - (d) Ustekinumab

Case Study 2

Brenda Rothchild is a 27 year-old female who presents to your clinic with severe abdominal cramping and diarrhea. She states that the pain appears to intensify when she has a bowel movement, which she has had a lot of lately. She reports 8–10 bowel movements in the last 2 days despite taking over-the-counter Imodium® and Motrin®. When asked, she mentions that her most recent stools have had some bright red material, which she thought might be blood. Her only other current complaint is the aphthous ulcer in her mouth that recently flared up. She does not have a family history of any gastrointestinal problems other than her mother having been treated for heartburn, and she has never smoked. A bowel perforation x-ray did not show a perforated colon.

- 6. Based on Brenda's presentation, can you determine the type of IBD she may have?
- 7. The results of a colonoscopy revealed continual inflammation and hemorrhage limited to the colon and rectum. Inflammation was not found in any other part of the gastrointestinal tract. A biopsy was taken and sent to the lab for pathological analysis. Based on the results from the colonoscopy, is it possible to determine the type of IBD that Brenda has?
- 8. The pathology report reveals the presence of numerous crypt abscesses without aphthoid ulcers. Does this determine the type of IBD Brenda has?
- 9. Further analysis of the pathology report revealed a depletion of goblet cells. However, no signs of mesenteric lymph node involvement, fistulas, or non-caseating granulomas were observed. Based on this information, is it possible to determine the type of IBD Brenda has?
- 10. Why did Brenda have aphthous ulcers in her mouth?
- 11. What is a good first line medication class to start Brenda on to induce remission?
- 12. What other class of drugs may be added to the treatment regimen to enhance the onset of remission and reduce flareups? Hint: This drug class should not be used long-term and can be given either orally or systemically.
- 13. If Brenda continues to have flareups after beginning treatment, what are some other types of drugs that could be considered?
- 14. Brenda has heard that some IBD drugs can cause a severe viral infection of the brain. She wants to avoid these drugs at all costs. What is the drug class and the drugs that Brenda wishes to avoid?

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