Over the past century, we have seen great advances in the discovery of disease etiologies and pathogenesis. We have also learned that many diseases are complex, and definitions of pathogenesis based simply on pathogen virulence do not always explain disease progression. A preeminent example of this complexity is the role of Helicobacter species in peptic ulcer disease of humans. Helicobacter pylori infection, although usually asymptomatic, may induce gastritis with damage resulting from both pathogen- and host-mediated factors. The discovery of this disease etiology and associated host response has redefined the treatment of gastritis of humans. Likewise, valuable new insights into the pathogenesis of intestinal diseases of animals are being discovered rapidly. Thus, it is of importance as practitioners to develop an integrated view of disease pathogenesis, considering pathogen virulence factors and host responses. In this article, the author has attempted to describe the hosts' contributions to inflammatory diseases of the bowel. It is hoped that these ideas will foster new ideas in intestinal disease management.

Inflammation is the progression of vascular changes in response to injury leading to accumulation of fluids and leukocytes in the extracellular space. Endothelial cell activation leads to an increased permeabil-

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From the US Department of Agriculture, Agricultural Research Service, National Animal Disease Center, Bacterial Diseases of Livestock Unit, Ames, Iowa
ity of capillaries and venules, resulting in leakage of fluids and leukocytes from these small vessels. Trauma, toxins, or chemical mediators such as cytokines, histamine, leukotrienes, and bradykinins elicit activation of endothelial cells. Although inflammation is fundamentally a protective response, excessive inflammation may result in hypersensitivity, fibrosis, or other harmful reactions. Within the intestinal mucosa, a physiologic steady state between reactivity to luminal antigens and downregulation of inflammation provides protection and gut homeostasis, respectively. Stimulation with certain microbes and their toxins may alter this balance, resulting in inflammatory diseases of the bowel.

IMMUNOBIOLOGY OF THE INTESTINAL TRACT

The intestinal mucosa has two opposing functions, uptake of nutrients and exclusion of pathogens. Ideally, potential pathogens never cross the epithelial barrier. Mechanisms used to exclude pathogens include degradative enzymatic activity, an electrostatically charged glycocalyx, luminal flow, a harsh gastric pH, competition for nutrients and receptors, elaboration of cryptidins and lysozyme by Paneth cells, and adaptive immune responses. The intestinal epithelium, whose main function is to absorb nutrients and secrete degradative enzymes, is also a first line of defense against many enteric pathogens. In addition to their nutritive functions, epithelial cells provide a physical barrier to pathogens and are an important component of the innate immune system.79 These nonclassical immune cells express major histocompatibility class (MHC) I and II antigens and receptors (Toll-like) on their surface that enable them to detect bacterial products and initiate a host response by secretion of chemokines that recruit leukocytes, especially lymphocytes.13, 44 Resident and recruited lymphocytes recognize foreign antigen in association with MHC or nonclassical antigen presentation molecules on epithelial cells, thereby linking innate and adaptive immune responses at the onset of microbial invasion of the intestines.

Cells other than leukocytes provide repair and resistance mechanisms. Neural cells produce chemical mediators (e.g., neurokinins) that induce inflammation and increase gut peristalsis. Epithelial cells and fibroblasts produce growth factors and cytokines that induce inflammation and repair of damaged mucosal tissues, with repair generally preceded by tissue destruction and remodeling. Transforming growth factor-β (TGF-β), secreted by mononuclear cells and platelets, activates fibroblasts and other connective tissue cells to produce collagen and matrix proteins. TGF-β, a potent immunosuppressive cytokine, also has a role in dampening inflammation within the gut. Although TGF-β is key for repair, the suppressive effects of this cytokine may allow persistence of certain microorganisms such as Mycobacterium avium subsp. paratuberculosis, the causative agent of Johne's disease.66 Other growth factors such as epidermal growth factor and keratinocyte growth factor stimulate epithelial cell proliferation, leading to repair of damaged intes-
Epithelial cell replacement is obviously an important component of healing for infections that induce epithelial cell damage and loss. Although repair mechanisms may be elicited by direct interaction with the insulting agent, they are often mediated by factors elaborated by mononuclear cells (e.g., cytokines and growth factors). Therefore, the intimate interaction between immune and nonclassical immune cells is necessary for the maintenance of homeostasis within the intestinal tract.

Studies with experimental models of inflammatory bowel disease (IBD) have demonstrated that inflammation within the gut often results from a dysregulation of the balance between chronic activation of the mucosal immune system and suppressed reactivity to autoantigens. Intestinal integrity is maintained at the level of the epithelial cell and by production of nonspecific antimicrobial peptides. Disruption of either adhesion molecules that ensure a normal turnover rate of intestinal epithelial cells or intestinal trefoil factor (a nonspecific antimicrobial peptide) results in loss of intestinal epithelial integrity and ensuing tissue changes (e.g., IBD of humans). Loss of intestinal epithelial integrity (leading to IBD) may also result from either antigen-specific reactivity with cross reactivity directed to autoantigens or from bystander inflammatory processes directed at removal of pathogens. IBD of humans is characterized by two diseases of unknown cause, Crohn's disease and ulcerative colitis. Although both diseases are considered entities of IBD, the causes of these two diseases remain unresolved. Because food animals rarely exhibit IBD as occurs in humans, this article focuses on immune mechanisms of inflammation in food animals caused by enteric pathogens.

MUCOSAL IMMUNOLOGY OF THE INTESTINAL TRACT

Adaptive immune responses of intestinal lymphocytes to foreign antigen result in either tolerance to the antigen or a response directed at eliminating the antigen. Although tolerance is a state of specific nonreactivity, it is still defined as an adaptive immune response. Indeed, loss of tolerance often leads to excessive inflammation. Specific reactivity directed at eliminating the pathogen is evoked either before invasion (e.g., immune exclusion) or after invasion (e.g., immune elimination). Secretory immunoglobulin (Ig) A produced by B cells within the intestinal mucosa and secreted into the intestinal lumen provides a specific mechanism of immune exclusion without inflammation. Mucosally derived IgA is not, however, limited to activity within the intestinal lumen. During transcytosis to the apical surface of the epithelial cell, IgA can bind proteins within endosomes where low pH exposes novel pathogen-associated antigens not exposed extracellularly. Conversely, responses that are directed at eliminating pathogens once they have invaded the intestinal mucosa often lead to inflammation. In addition to clearance of the invading organism, these inflammatory responses may lead to loss
of tolerance to commonly encountered luminal antigens (e.g., normal gut flora or food antigens) and excessive tissue injury. Indeed, several mutant strains of mice that spontaneously develop IBD fail to develop disease when they are raised in a germ-free environment. Additionally, mice with a defined flora (e.g., modified Schaedler’s flora) infected with the porcine pathogen *Brachyspira hyodysenteriae* develop serum antibody responses to commensal bacteria of the intestinal tract, whereas control, noninfected mice do not (Michael J. Wannemuehler, PhD, personal communication, 2000). Likewise, infection of gnotobiotic pigs with *B. hyodysenteriae* does not induce intestinal inflammatory lesions as seen with conventionally reared pigs. Lesion formation is likely dependent on inflammatory responses directed at otherwise harmless commensal bacteria. Thus, intestinal inflammatory responses may be evoked by invasion of pathogens (e.g., *Salmonella* sp., rotavirus, coronavirus, helminths, and so forth) or as a result of an aberrant immune response to commensal bacteria (e.g., swine dysentery).

Immune reactivity to food antigens, although uncommon with food-producing animals, may also occur. Certain calves fed a soybean milk replacer developed a hypersensitive response to soy proteins, resulting in intestinal inflammation. Similar reactions to soy proteins are described for early weaned pigs. Thus, as with humans, loss of tolerance or a failure to establish tolerance to commensal microbes and to food antigens may lead to inflammatory diseases of the bowel of food animals.

As stated previously, foreign antigens traversing the intestinal epithelial barrier evoke either tolerance or inflammation. Antigens may also be actively sampled from the intestinal lumen. Specialized epithelial cells, microfold or M cells, pinocytose luminal antigens. These antigens are delivered intact to antigen-presenting cells, B cells, and macrophages stationed within basilar folds of M cells (Fig. 1). Antigen-presenting cells then deliver the antigens to Peyer’s patches and other lymphoid aggregates. Antigens are processed and presented in association with MHC molecules for αβ T cells or intact without processing for B cells or γδ T cells. αβ T cells express α and β T-cell receptor chains on their surfaces, whereas γδ T cells express γ and δ T-cell receptor chains on their surfaces. αβ T cells are well-characterized T cells, whereas γδ T cells are a less-characterized subset of T cells. γδ T cells are often associated with epithelial surfaces and are considered a first line of defense against invading pathogens. Higher percentages of γδ T cells are detected within the peripheral blood of cattle and pigs as compared with percentages within the peripheral blood of humans and rodents.

Both T and B cells recognizing antigen delivered from M cells proliferate and produce cytokines or become cytotoxic (T cells) or produce antibody (B cells). Progeny of these antigen-specific intestinal lymphocytes often traffic to other mucosal areas through the systemic circulation (e.g., common mucosal immune system). Therefore, B cells originating within the intestinal mucosa may eventually localize in the
Lymphocytes and macrophages migrate into the invagination of the basolateral surface of M cells and sample endocytosed antigens that have been transported into the basolateral pocket via vesicles. The apical surface of M cells lacks closely packed microvilli and the enzyme-rich coat of absorptive enterocytes yet efficiently binds and transports microorganisms, particles, and antigens to underlying antigen-presenting cells. (Adapted from Neutra MR, Kraehenbuhl JP: The role of transepithelial transport by M cells in microbial invasion and host defense. J Cell Science 17:209–215, 1993; with permission from Company of Biologists Ltd.)

Figure 1. An M (microfold) cell. Lymphocytes and macrophages migrate into the invagination of the basolateral surface of M cells and sample endocytosed antigens that have been transported into the basolateral pocket via vesicles. The apical surface of M cells lacks closely packed microvilli and the enzyme-rich coat of absorptive enterocytes yet efficiently binds and transports microorganisms, particles, and antigens to underlying antigen-presenting cells. (Adapted from Neutra MR, Kraehenbuhl JP: The role of transepithelial transport by M cells in microbial invasion and host defense. J Cell Science 17:209–215, 1993; with permission from Company of Biologists Ltd.)

Mammary gland and secrete antibody into milk, providing lactogenic immunity to newborns.

The type of antigen presented, responding cell type, and local environment contribute to the nature of an ensuing response. For instance, a porcine B cell specific for *Escherichia coli* shigatoxin likely produces shigatoxin-specific secretory IgA if it encounters its specific antigen in a TGF-β, interleukin (IL)-5 rich environment (e.g., the intestinal mucosa). Conversely, certain mycobacterial antigens, especially if encountered early in the course of a *Mycobacterium* spp. infection of cattle, often induce interferon (IFN)-γ production by T cells resulting in a predominant IgG2 response by B cells. With the *Mycobacterium* example, the nature of the antigen evoked an IFN-γ/IgG2 response, whereas with the porcine shigatoxin example, the environment of the gut mucosa biased the response to IgA production. Because the gut mucosa is under constant antigenic stimulation, it is not surprising that the overall bias of the cytokines produced within the gut mucosa favor a less inflammatory outcome (e.g., IL-10, TGF-β, IL-5). A constant barrage of inflammatory responses at gut mucosal surfaces would compromise intestinal metabolic function. Indeed, it is postulated that an overactive Th 1 (e.g., IFN-γ, IL-12) response may be the mechanism underlying the development of chronic intestinal inflammation in multiple models of IBD.
Proinflammatory or acute-phase cytokines are produced by mononuclear cells (IL-1, IL-6, and tumor necrosis factor [TNF]-α) and epithelial cells (IL-1 and IL-6) of the intestinal mucosa in response to bacterial cell wall products (e.g., lipopolysaccharide, muramyl dipeptide, and β-glucan) or other mediators of inflammation (e.g., toxins and superantigens). Genes for these cytokines are consistently upregulated in the intestinal mucosa of human IBD patients. Transient increases in production of acute-phase cytokines within domestic animals infected with various pathogens have also been described. Experimental infection with the porcine enteric pathogens, *Salmonella typhimurium* or *Brachyspira hyodysenteriae*, or administration of *Clostridium perfringens* enterotoxin induces increased serum levels of TNF-α as compared with serum levels of TNF-α in control animals. Chronic enteric disease of domestic animals may also evoke a sustained acute-phase response. For instance, mononuclear cells isolated from cattle with subclinical Johne’s disease produce greater amounts of TNF-α as compared with amounts from cattle with clinical paratuberculosis or with amounts from noninfected cattle. The TNF-α response by mononuclear cells from subclinically affected cattle likely reflects a predominance of a cell-mediated, IFN-γ response. *Mycobacterium avium subsp. paratuberculosis* (M. para) organisms also stimulate production of IL-1 and IL-6 in vitro (e.g., by isolated peripheral blood mononuclear cells) and in vivo (e.g., by cells located within the intestinal mucosa). Induction of acute-phase cytokine responses by enteric pathogens of food animals is not surprising, as it is likely that any ensuing inflammation is a direct result of IL-1, IL-6, and TNF-α production.

The combined effects of the acute-phase cytokines mentioned above and other inflammatory mediators such as eicosanoids, nitric oxide, and neuropeptides released in response to enteric infections contribute to a local and systemic inflammatory response. Macrophages stimulated by bacterial products produce IL-1, IL-6, and TNF-α. These cytokines activate vascular endothelium, increasing permeability of small blood vessels. Increased vascular permeability leads to leakage of cells and serum containing antibody and complement proteins. IL-1, in addition to its actions on vascular endothelium, activates leukocytes and is responsible for the induction of fever. IL-1 and IL-6 can reduce appetite. IL-6 is also important for the activation of B cells for the production of antibody. Excessive production of proinflammatory cytokines, especially TNF-α, leads to cachexia or shock. Although excessive responses are detrimental, regulated acute-phase responses are essential for prompt activation of innate and specific immune reactions.

Chronic infection of cattle with *M. para* results in a remarkable influx of macrophages into the gut mucosa (Fig. 2). This influx of macrophages is especially remarkable in that other obvious indications of inflammation (e.g., edema, neutrophilic infiltrates, or crypt abscesses) are lacking. As stated previously, however, the response to *M. para* antigens at specific stages of disease includes the induction of acute-
Figure 2. Section of ileum from an adult cow with clinical Johne's disease. The histologic section was stained by the Ziehl-Neelson method to detect acid-fast organisms (stained dark). The arrow points to macrophages infected with *M. avium* subsp. *paratuberculosis* (original magnification ×16). (Courtesy of Mitchell V. Palmer, DVM, PhD, and Judith R. Stabel, PhD, National Animal Disease Center, Ames, IA.)

Phase cytokines. Cattle with subclinical disease and sheep or goats with tuberculoid-type lesions have a significant proinflammatory cytokine response, whereas cattle with clinical disease do not display a significant proinflammatory cytokine response. Lesions associated with end-stage paratuberculosis lack the associated histologic changes (e.g., edema and neutrophilic infiltrates) indicative of an active inflammatory response (e.g., IL-1, IL-6, and TNF-α production). It is likely that this organism (or chronic infection with this organism) induces cytokines such as TGF-β or IL-10 that suppress inflammation later in the course of disease. The outcome is an environment in which numerous macrophages are present for the mycobacterium to reside within; yet inflammatory mediators necessary to activate macrophages to kill the bacilli are not produced. In this case, suppression of proinflammatory cytokines leads to an inability of the host to clear the pathogen. In sharp contrast to paratuberculosis, infection of young pigs with the spirochete *B. hyodysenteriae* results in acute colitis mediated by acute-phase cytokines such as IL-1, IL-6, and TNF-α (Fig. 3). Administration of anti-inflammatory agents or antibodies that block trafficking of leukocytes to the lesion diminishes disease expression. With this example, an overzealous acute-phase response is detrimental to the host.
Figure 3. Section of colon (original magnification ×25) from a pig experimentally infected with Brachyspira hyodysenteriae. Note mild edema of the lamina propria (heavy arrow) and many neutrophils. Also, note epithelial necrosis with numerous neutrophils and cellular debris within the intestinal lumen (thin arrow). G = gland lumina. (Courtesy of Robert A. Kunkle, DVM, PhD, and Thaddeus B. Stanton, PhD, National Animal Disease Center, Ames, IA.)

In summary, induction of acute-phase cytokines after invasion of the intestinal mucosa by pathogens is essential for the initiation of an immune response. These cytokines activate endothelial cells of blood vessels, initiating the cascade of events that eventually leads to elaboration of serum factors and leukocytes into gut mucosal tissues. Although proinflammatory cytokines are beneficial and necessary for clearance of certain pathogens, excessive production of IL-1, IL-6, and TNF-α may lead to harmful inflammatory reactions within the gut mucosa.

SERUM FACTORS

On inflammation of the gut wall, acute-phase cytokines activate vascular endothelium, causing leakage of serum into the affected area.
Within this serum, specific (e.g., IgG and IgM) and nonspecific (e.g., C-reactive protein and the complement protein, C3b) opsonins bind to the surface of foreign organisms or particles, promoting phagocytosis of these antigens by polymorphonuclear cells and macrophages. Serum-derived IgG or IgM capable of directly neutralizing virus or toxin (if specific for epitopes on the virus or toxin) is also contained in the sera leaking into the tissues. Antigen-specific, serum-derived IgG or IgM may also activate the classical complement pathway leading to cell lysis (e.g., membrane attack complex) and the production of opsonins (e.g., C3b and iC3b) and anaphylatoxins (e.g., C5a, C3a, and C4a). The alternative complement pathway may also be activated by direct binding of serum-derived C3b to microbial surfaces. The production of anaphylatoxins promotes inflammation and chemotaxis of phagocytic cells to the affected area. These responses, although beneficial for the innate clearance of invading organisms, often result in tissue destruction and loss of function.

Autoreactive antibody is detected in the sera of a significant percentage of human patients with IBD. The most commonly reported antigens reactive with these autoantibodies are perinuclear antineutrophil cytoplasmic proteins, lactoferrin, and colonic epithelial cell antigens (e.g., tropomyosin). Cross reactivity of these autoreactive sera with nonpathogenic *E. coli* antigens suggests sensitization by the normal intestinal flora and a breach of tolerance. Enteric pathogens such as *Campylobacter jejuni*, *S. typhimurium*, and rotavirus also may induce autoimmune reactivity. The role of autoreactive antibody in enteric inflammatory diseases of domestic animals, however, is unclear.

**NEUTROPHILS**

Holstein cattle with bovine leukocyte adhesion deficiency have chronic respiratory and enteric infections. The underlying genetic defect of this disorder is a mutation in the gene encoding CD18, resulting in a nonfunctional CD18 molecule. CD18 (coexpressed with CD11a, CD11b, or CD11c as a heterodimer) is an adhesion molecule necessary for the extravasation of neutrophils. Cattle with this defect develop a neutrophilia caused by the inability of these cells to tightly adhere to the blood vessel endothelium. The end result is an inability of neutrophils to traffic to areas of inflammation. Because cattle with this defect are prone to bacterial infections of the intestinal tract, it is postulated that neutrophils are necessary for certain enteric defense mechanisms of cattle.

Infection of weanling pigs with *S. typhimurium* or *S. choleraesuis* results in multifocal erosions, villous atrophy, and neutrophilic infiltrates of the small intestine and colon. Within these lesions, *Salmonella* bacilli, although commonly seen within macrophages, are rarely observed within neutrophils. Using mouse models, it has been determined that virulence mechanisms enabling *Salmonella* spp. growth within macro-
phages do not protect them from killing by neutrophils. It is, therefore, hypothesized that neutrophils are the primary cells involved in host defense against nontyphoid Salmonella and that macrophage invasion is the principal strategy employed to evade host defense mechanisms. Conversely, administration of monoclonal antibodies that prevent extravasation of neutrophils (e.g., anti-CD18 antibodies) or depletion of neutrophils (e.g., with antibodies specific for murine neutrophils) prevents colitis induced by infection of mice with the swine dysentery pathogen, B. hyodysenteriae. The spirochete still colonizes the mucosa of these mice; thus, it is postulated that disease results from the host response as opposed to direct effects of the bacterium. In this case, the ensuing colitis is likely initiated by acute-phase cytokines produced after neutrophil migration into the ceca of infected mice.

Neutrophils are critical effector cells in the host response to bacteria, fungi, and parasites. Phagocytosis and enhanced killing by way of activation of the respiratory burst and release of degradative enzymes from within preformed granules are well-known functional responses by these important cells of the innate immune system. It has only recently been determined that neutrophils are also a rich source of IL-12. Early IL-12 production biases the ensuing immune response toward a type 1 (e.g., IFN-γ) response. Early recruitment of IL-12–producing neutrophils into areas of inflamed gut may be critical for the efficient clearance of invading organisms such as Cryptosporidium parvum, M. para, and Salmonella spp. Unfortunately, trafficking of IL-12–producing neutrophils into the lesion likely interferes with the initiation of type 2 responses and may even evoke an excessive inflammatory response. Indeed, many mouse models of IBD are type 1–mediated, and neutrophils are likely key inducers of this unfavorable response.

**REACTIVE METABOLITES**

Within inflamed mucosa of human patients with IBD, increased levels of prostaglandins, thromboxane B, and platelet-activating factor are detected. These lipid mediators of inflammation are produced through the breakdown of membrane phospholipids and induce constriction of smooth muscle (e.g., increased gut peristalsis), activation of vascular endothelial cells, and chemotaxis of leukocytes. Increased levels of mucosal eicosanoids are also detected after infection with Cryptosporidium parvum, rotavirus, and Salmonella spp. and have been implicated in the mechanism of diarrhea induction with each of these diseases. As with most mediators of inflammation, production and release of lipid mediators of inflammation are beneficial in the clearance of pathogens; however, excessive production often leads to unnecessary tissue injury.

Phagocytic cells use reactive oxygen metabolites and nitric oxide to kill pathogens that have been engulfed into the phagolysosome. Production of nitric oxide by bovine macrophages is greatly enhanced by lipopolysaccharide, IFN-γ, and TNF-α. Mononuclear cells from cattle
with subclinical paratuberculosis produce superoxide anions and nitric oxide in response to M. para antigens; however, these substances have little, if any, effect on the intracellular killing of M. para. Nitric oxide is, however, essential for the proficient killing of S. typhimurium and is likely involved in the clearance of C. parvum infection. Although generally considered beneficial, excessive amounts of reactive oxygen metabolites and nitric oxide within the intestinal mucosa may be detrimental and have been associated with IBD of human patients.

CELL-MEDIATED RESPONSES

T lymphocytes provide two effector functions: elaboration of cytokines and cytotoxicity. Cytotoxic responses by T cells located within the intestinal mucosa are essential for the removal of cells infected with intracellular pathogens such as viruses, protozoa, or bacteria. Cytotoxic responses may be antigen specific (e.g., by CD8\(^+\), \(\alpha\beta\) T-cell receptor [TCR]\(^+\) cells) or nonspecific (e.g., by natural killer or \(\gamma\delta\) TCR\(^+\) cells). Antigen-specific responses generally require prior sensitization and clonal expansion to provide sufficient numbers of specific cells for a potent response, whereas nonspecific responses by definition do not require prior sensitization. It has been determined recently that \(\gamma\delta\) TCR\(^-\) cells within the intestinal epithelium (e.g., intraepithelial lymphocytes) recognize antigens expressed on the surface of stressed or damaged epithelial cells (e.g., MHC class I chain-related gene A, MHC class I chain-related gene B, and heat shock proteins). Once these cells recognize stressed or damaged cells, they respond either by direct cytolytic action of the affected cell or by the production of cytokines such as IFN-\(\gamma\). Because of their location and nature of antigen recognition, \(\gamma\delta\) TCR\(^+\) intraepithelial lymphocytes are considered an important first line of defense against invading pathogens at the intestinal surface.

Cytokines produced by lymphocytes activate macrophages, promote antibody production by B cells, and induce inflammation. Studies with mice have shown that functional subsets of T cells, based on their cytokine production profile, either augment resistance or susceptibility to disease. In addition, cytokines produced by antigen-presenting cells, especially monocytes or macrophages, promote a bias in the subset of T cells responding to a particular antigen. T-cell responses characterized by the production of IFN-\(\gamma\) and IL-12, termed type 1 responses, are generally effective against intracellular pathogens such as Mycobacterium spp. Conversely, type 2 responses, characterized by the production of IL-4 and IL-10, are generally effective for helminth infections. These responses are cross-regulatory in that type 1 responses limit type 2 responses and vice versa. Polarized type 1 and type 2 responses by human, pig, and cattle T cells are not as well characterized as are those in mice. Studies examining responses of humans to Mycobacterium leprae infection, however, have shown that a polarized type 1 or type 2 immune response dramatically affects the clinical outcome of disease.
Thus, the type 1/type 2 paradigm is not just a phenomenon of the mouse immune system.

The most notable function of CD8+ T cells is to lyse cells expressing foreign antigen presented in the context of MHC class I antigens. A clonally expanded population of CD8+ T cells specific for the pathogen of interest is generally required for an efficient response. CD8+ T cells, in addition to their cytotoxic activity, also secrete cytokines (e.g., IFN-γ) that activate macrophages and enhance antigen presentation. Like CD4+ T cells, subsets of CD8+ T cells produce discrete cytokine patterns termed Tc1 and Tc2. The exact roles of each of these discrete subsets in the response to enteric pathogens are not yet clear; however, CD8+ T cells are capable of producing anti-inflammatory cytokines such as IL-10. Production of IL-10 within the intestinal mucosa would likely dampen inflammation, especially if induced by pathogens evoking type 1 (e.g., IFN-γ) responses. Clearance of pathogens by CD8+ T cells resulting in removal of foreign antigen would also result in reduction of inflammation.

Clear roles for CD8+ T cells in the response to enteric pathogens are described. For instance, CD8+ T cells are necessary for the proficient clearance of rotavirus and coronavirus infection of mice. Infection of calves with Cryptosporidium parvum leads to an increase in CD8+ T cells at areas of C. parvum colonization; thus, it is hypothesized that CD8+ T cells are involved in the clearance of this protozoan, at least in calves. Likewise, CD8+ T cells have a role in immunity to Eimeria infections of chickens. Salmonella typhimurium infection of mice results in the generation of expanded numbers of CD8+ T cell clones specific for S. typhimurium antigens that are important in both primary and secondary responses to this pathogen. It is probable that most, if not all, intracellular enteric pathogens of food-producing animals induce expansion of antigen-specific CD8+ T cells that play a role in clearance of the pathogen.

CD8+ T-cell responses to extracellular pathogens, conversely, are rare. Classically, CD8+ T cells recognize antigen in association with MHC class I antigens located on the surface of antigen-presenting cells, which entails loading of endogenously processed antigen (peptides of approximately 8 to 12 amino acids in length) into the cleft of MHC class I molecules within the endoplasmic reticulum of antigen-presenting cells. Because antigens from extracellular pathogens are processed through the exogenous pathway, it is rare that proteins from these microbes reach the cytosol where they can be processed and presented on MHC class I molecules. Despite these limitations, B. hyodysenteriae-specific CD8+ T cells are detected within the peripheral blood and colonic lymph nodes of pigs vaccinated with a B. hyodysenteriae bacterin or infected with the spirochete. B. hyodysenteriae is a noninvasive, extracellular pathogen. Resolution of infection and protection by vaccination appear to be linked with the appearance of a unique subset of CD8+ T cells within the peripheral blood. These cells express CD8 as a homodimer (e.g., CD8αα) as opposed to a heterodimer (e.g., CD8αβ), as occurs on most CD8-expressing cytotoxic T cells. This popu-
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lation, although rare in the peripheral blood, is relatively common within the enteric mucosa of many mammalian species. The exact mechanism of protection afforded by these cells is unclear. It is likely that they provide an anti-inflammatory or regulatory function within the colonic mucosa. Vaccination of pigs with the *B. hyodysenteriae* bacterin, therefore, facilitates the expansion of CD8αα-expressing cells that traffic to the colonic mucosa after infection with *B. hyodysenteriae*. Once in the colonic mucosa, these cells inhibit inflammation evoked by the spirochete. Thus, vaccination biases the host response to a less inflammatory, more favorable reaction than occurs in nonvaccinated pigs.

The ability of type 2 cytokines to regulate inflammation has been shown in several disease models. For example, mucosal inflammatory responses can be inhibited by administering recombinant bacteria that secrete anti-inflammatory cytokines such as IL-10. With this approach, an ensuing immune response to a particular intestinal pathogen would be biased to a type 2 response (e.g., IL-4, IL-5, and IL-13) that is less inflammatory than a type 1 response (e.g., IFN-γ and IL-12) and increases sIgA production. Unfortunately, it may lead to a less favorable outcome if animals become infected with intracellular enteric pathogens such as *M. para* or *Salmonella* spp. This method is not likely to be of use in the near future for prevention of inflammatory diseases of food-producing animals because of the current availability of effective antibiotics and the risk of serious side effects. Increasing concerns with antibiotic resistance, however, warrant exploration of similar approaches as a future alternative.

B cells within the lamina propria, Peyer’s patches, or other lymphoid aggregates of the gut mucosa provide significant antimicrobial protective mechanisms through antibody production and presentation of antigens to T cells. Human B cells produce, on average, 5 grams of sIgA every day. The large energy demand of chronic immune stimulation (e.g., production of sIgA) has been viewed as an unnecessary drain on average daily gains of food-producing animals, especially pigs. On the contrary, effective maturation of the intestinal immune system requires chronic stimulation by a microbial flora, leading to chronic immune stimulation. Current strategies to restrict unnecessary immune stimulation include strict biocontainment to limit access of pathogens (e.g., transmissible gastroenteritis virus) and housing pigs in clean environments. Another reasonable approach to this problem is to augment intestinal immune maturation of young animals by administration of probiotics that also inhibit colonization of pathogens, thereby managing the microflora to promote maturation and inhibit inflammatory responses that result in diminished performance and increased health costs. As stated previously, these organisms may be genetically altered to also deliver anti-inflammatory agents.

T-cell immune responses are essential in limiting the severity of paratuberculosis infection. Antibody production affords little, if any, protection against this intracellular pathogen. Antigen presentation and costimulatory functions by B cells, however, can support T-cell
responses.\textsuperscript{20, 79} Antigen presentation/costimulation of T cells by B cells often result in type 2 immune responses characterized by secretion of IL-4, IL-5, IL-6, and IL-10.\textsuperscript{16, 19} Several of these cytokines (e.g., IL-6 and IL-10) are potent B-cell growth factors.\textsuperscript{12, 47} Cattle with clinical Johne's disease have excessive numbers of peripheral blood B cells.\textsuperscript{72} Expansion of B cells may reflect a substantial increase in IL-10 production by \textit{M. para}-infected macrophages. Increased antigen presentation by the expanded pool of B cells would also favor a type 2 immune response to \textit{M. para} antigen and result in decreased killing of \textit{M. para} with progression to clinical disease. With other pathogens, it has been suggested that increased production of type 2 cytokines, especially IL-10, occurs later in the course of disease to dampen the immune response.\textsuperscript{12} A closer examination of the effects of IL-10 on B-cell function may elucidate mechanisms of clinical progression of Johne's disease.

**SUMMARY**

During the past century, research on animal diseases has focused on the characterization of specific etiologies and disease control strategies. Many diseases affecting domestic animals have been successfully controlled using various methods, including vaccination, management, vector control, or antimicrobial agents. A number of microorganisms have proven resistant to these efforts. Control of these organisms requires the development of new strategies. As practitioners and researchers, we need to consider approaches that encompass the entire realm of disease expression from molecular to immune responses and interactions with other functional systems (e.g., endocrine, neurologic, and vascular systems). We need a basic understanding of effective immune responses enabling the tailoring of vaccines to produce the desired response. This tailoring of host responses is augmented by the use of vaccines that use host growth factors, cytokines, or costimulatory molecules to bias the ensuing response. Intestinal microbial flora of food-producing animals can be managed to optimize health and minimize colonization by pathogenic organisms, especially zoonotic agents. New systems for the delivery of cytokines and other factors that favor optimal intestinal health and homeostasis need to be researched and evaluated. With time, it is likely that our clients and the consumers will be less tolerant of antibiotic usage. They will be more aware of the zoonotic potential of many microbes that colonize food animals. Food safety issues will be a continuing concern, as will the protection of our water supply from contamination from feedlots and pasture runoff. We are in the dawn of a new century, and, it is hoped, a new era of discovery of enteric disease pathogenesis and control.

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