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Defense of gut mucosal surfaces in the weaned pig: An overview

David R. Brown, PhD
Department of Veterinary & Biomedical Sciences, University of Minnesota College of Veterinary Medicine

With its extensive surface area and critical role in the absorption of nutrients, the intestinal mucosa is exposed to a great quantity and variety of potentially antigenic substances throughout the lifetime of the pig. In defending the mucosal surface, the intestine must clearly distinguish between harmless and harmful foreign antigens that are introduced during the process of feeding and drinking. Moreover, it must coexist with and even support several hundred species of bacteria which comprise the microbial flora of the gut, but exclude pathogenic microorganisms. A diverse and overlapping array of gastrointestinal defense processes act rapidly to intercept, neutralize or eliminate harmful antigens and microbes before they invade the mucosal surface. Several of these defense systems are linked through antigen-presenting cells and cellular receptors in the gut-associated lymphoid tissue, the largest collection of immune cells in the body, to promote long-term adaptive immune responses to noxious agents. First-line defense processes at the mucosal surface act to exclude harmful antigens and microbes that could otherwise enter submucosal tissue and evoke inflammation or hypersensitivity reactions, as these reactions can compromise mucosal barrier function. In general, they mature relatively early in swine development. My intent is to review briefly some of the key surface defenses of the gastrointestinal mucosa, emphasize recent findings on this topic and indicate how they may relate to the ingestion of nutrients and pathogens in weaned pigs.

Role of gastrointestinal secretomotor function

Food and microbes that enter the oral cavity initially encounter salivary enzymes, immunoglobulins and other substances as well as commensal microbes that function in feed digestion and mucosal protection. Subsequently, stomach acid plays a key role in killing bacteria capable of infecting the gastric and intestinal mucosa. However some common intestinal pathogens like *Salmonella* have adapted to acidic environments. In pigs, hydrochloric acid secretion by gastric parietal cells develops immediately after birth and rises nearly seven-fold by five weeks of age (Xu and Cranwell, 1990).

Mechanical activity of smooth muscle in the gastrointestinal tract is linked through intestinal nerves to epithelial cell secretion of ions and fluid; together, these processes dilute and purge pathogenic microorganisms from the intestinal lumen. Decreases in intestinal propulsion have been correlated with overgrowth of luminal bacteria. Movements of the intestinal microvilli that are mediated by the muscularis mucosae, a thin band of smooth muscle lying just beneath the intestinal mucosa, may additionally contribute to the extrusion and mixing of mucus and other protective substances on the surface of the intestinal mucosa. Intestinal transit in pigs has been shown to increase after weaning (Snoeck et al., 2004) and both gastric emptying and intestinal motility are influenced by diet and feed structure (Ruckenbusch and Bueno, 1976; Bardon and Fioramonti, 1983; Boudry et al., 2004a).

Active secretion of chloride and bicarbonate anions by epithelial cells in the intestinal crypts is accompanied by the movement of water into the intestinal lumen. This process may assist in the elimination of luminal pathogens as mentioned above, but it also facilitates the hydration and movement towards the lumen of defense molecules, such as mucin, antimicrobial peptides and secretory immunoglobulin A (sIgA), that are released by crypt epithelial cells. Anion secretion is stimulated by several enteropathogens as well as inflammatory and immune mediators. In swine, active anion secretion in the small and large intestines develops during suckling (Pácha, 2000) and undergoes changes at the onset of weaning (Boudry et al., 2004b).

Mucosal barrier function

Intestinal epithelial cells are held together by a complex array of specialized membrane proteins to form tight junctions. Epithelial tight junctions mediate the selective permeability of the epithelial barrier to ions, water, and phagocytic cells, but maintain a barrier against mucosal penetration of antigens, microbes and toxins in the intestinal lumen. They also control aspects of epithelial cell growth and differentiation, which are important in maintaining mucosal integrity. Tight junctions are not a passive component of the mucosal barrier, but rather are regulated by a variety of inflammatory, immune and neuroactive substances. Inflammation has been shown to decrease epithelial tight junction permeability and thereby weaken barrier function (Berkes et al., 2003). In swine,

weaning triggers long-lasting increases in mucosal barrier function (Boudry et al., 2004b). Intestinal epithelial cells can take up and process luminal antigens in humans and rodents, but probably not in swine (Haverson et al., 2000). Neutrophils modify the mucosal barrier and, in response to pathogen-elicited, epithelial chemoattractants, cross the epithelium to stimulate active anion secretion and destroy microbes in the lumen. Dendritic cells represent an important cellular link between innate and adaptive intestinal immunity. Recently, antigen-presenting dendritic cells have been shown to express tight junction proteins and appear to be capable of spanning the epithelial layer in order to sample luminal contents without disrupting barrier function (Granucci and Ricciardi-Castagnoli, 2003).

Role of mucus

Mucin glycoproteins are secreted by goblet cells located in the intestinal crypts. They polymerize to form mucus and, with the assistance of water movement linked to active anion secretion, extensively coat the mucosal surface. High-viscosity mucus serves to restrict the movement of microbes and large molecules such as antigenic proteins towards the mucosal surface. By virtue of its carbohydrate groups, mucus can act as a high-affinity binding site for several classes of enteropathogens and commensal flora. It might also act as a reservoir for some host defense molecules. Mucus production and breakdown as well as the number of goblet cells are regulated by both microbial and host-related factors (Deplancke and Gaskins, 2001). In pigs, diet and the viscosity of luminal contents have been shown to change goblet cell numbers and mucin production (More et al., 1987; Piel et al., 2005).

Role of antimicrobial peptides

Innate immune processes span the animal kingdom, and small peptides with broad-spectrum antimicrobial activity first discovered in plants and insects were subsequently found to be produced in mammals, including the pig (Zhang et al., 2000). Neutrophils contain a wide variety of these highly-charged peptides, which act on the phospholipid-rich surfaces of bacteria, parasites or fungi to form pores and collapse the large ionic gradients necessary for the survival of these organisms. Through this mechanism, antimicrobial peptides act in a manner different from conventional antibiotic drugs and have little or no toxic effects on host cells. In addition to neutrophils, which can transmigrate across the intestinal epithelium, Paneth cells located in the crypt region of the intestinal mucosa produce and release antimicrobial peptides which then act at the mucosal surface. The inability of animals to produce these peptides has been associated with a decreased resistance to food-borne pathogens (Ouellette, 2005). Paneth cells also secrete bacteriolytic enzymes such as lysozyme that protect the mucosa from pathogen invasion.

Pathogen recognition receptors link innate and adaptive immunity

Cells participating in the front-line defense against pathogenic microbes must have the ability to recognize conserved, pathogen-associated molecular patterns, such as the presence of lipopolysaccharide or lipopeptides on bacterial surfaces. Cell surface receptors capable of recognizing many different types of pathogen-related motifs were first discovered in fruit flies and soon found to be expressed in mammals as well; at least ten of these mammalian *Toll*-like receptors (TLRs) have been identified so far. TLRs on epithelial cells and other cell types in the intestine detect a variety of bacterial and viral constituents. For example, bacterial lipopeptides, lipopolysaccharide, the *Salmonella* flagellar protein flagellin, and bacterial DNA specifically stimulate TLRs 2, 4, 5 and 9, respectively. TLRs are linked to intracellular signaling pathways which promote programmed cell death, the production of cytokines and antimicrobial peptides, increased epithelial tight junction integrity, and mucosal inflammation (Abreu et al., 2005). Recent studies show that TLRs 2 and 9 appear to be highly expressed in mesenteric lymph nodes and Peyer's patches of the porcine small intestine where they may play a role in the induction of mucosal immune reactions to luminal bacteria (Shimosato et al., 2005; Tohno et al., 2005).

Another class of pathogen-recognition receptors, originally associated with disease resistance in plants, have been discovered recently in mammals. At least two different NOD proteins are expressed in the cytoplasm of intestinal epithelial cells, Paneth cells and leukocytes that recognize either bacterial peptidoglycan (Gram-negative bacteria) or peptidoglycan-derived muramyl dipeptide (Gram-positive and –negative bacteria) . It has been hypothesized that these NOD proteins detect invasive bacteria and convey this information through signaling pathways identical to those linked to TLRs (Abreu et al., 2005).

Role of commensal microflora

The density of commensal bacteria in the intestinal tract increases 100- to-1,000-fold from the upper small intestine to the colon. Intestinal epithelial cells and these microflora continually interact and it is likely that resident bacteria prevent the mucosal colonization of pathogenic bacteria. It is now known that these commensals are essential in the development and maintenance of mucosal immunity in pigs and other animals (Bailey et al., 2001). Moreover, commensal flora acting in part through TLRs, appear to dampen intestinal injury as well as allergic reactions to food antigens. Recent evidence suggests that

they may modulate energy balance in the host as well (Backhed et al., 2004).

Secretory immunoglobulin A: First-line, acquired immune defense

In contrast to innate immune processes, may of which are phylogenetically old, adaptive immunity arose in fish and higher vertebrates. The induction of humoral immune responses to orally-administered foreign antigens is initiated in discrete Peyer's patches in the porcine upper small intestine, which form in the first two weeks of postnatal life. The lymphoepithelial cells covering Peyer's patch domes rapidly transport luminal antigens and microbes to underlying antigen-presenting cells and T lymphocytes, with the subsequent production, trafficking and migration to the lamina propria of immunoglobulin A (IgA)-expressing plasma cells (Rothkötter et al, 1999). IgA+-plasma cells and epithelial cells expressing the polymeric Ig receptor (pIgR) lie in close proximity in the crypt regions of the small and large intestinal mucosae. Dimeric IgA released from plasma cells is transported to the mucosal surface after its binding to pIgR on the basolateral aspect of epithelial cells and endocytotic transport through these cells. During its intracellular transit, recent studies have shown that it appears to be capable of neutralizing viruses present within infected epithelial cells. The secreted IgA is cleaved from the pIgR at the apical surface of the epithelial cell, but remains attached to the Ig binding domain of the receptor, otherwise known as secretory component. Secretory IgA is stable in the harsh proteolytic environment of the intestinal lumen and inhibits colonization and invasion of enteropathogenic bacteria ("immune exclusion"). With its attached secretory component, it can bind to and reside in the mucus blanket to await pathogen entry. It may also complex with proinflammatory cytokines to decrease mucosal inflammation. Going full circle, the lymphoepithelial cells of Peyer's patch domes (at least in mice and humans) appear to express IgA receptors which mediate the uptake of secretory IgA-antigen complexes at these sampling sites in the intestine.

Oral tolerance

Weaning in swine is often associated with a transient allergic reaction to food antigens, such as those from soybeans. Older animals do not display this degree of mucosal hypersensitivity and are said to have undergone "oral tolerance". The phenomenon of oral tolerance to food antigens and resident microflora has been recognized for at least a century, but the intestinal immune mechanisms underlying it remain a subject of active investigation. T lymphocytes, which begin to populate the intestinal villi and lamina propria of the piglet intestine between 2 and 4 weeks of postnatal life, appear to play an important role in suppressing inappropriate immune responses (Bailey et al., 2001). In particular, regulatory T cells capable of releasing immunosuppressive cytokines, such as interleukin 10 and transforming growth factor-*beta*, act in this capacity. Some of these immunosuppressive T cells express TLR4 and are activated by lipopolysaccharide, and it is possible that resident microflora participate in modulating intestinal tolerance (Smith and Nagler-Anderson, 2005).

Bailey et al. (2001) have proposed that post-weaning diarrhea in swine may be due to a transient imbalance between regulatory influences and mucosal immune effector function. In this model, piglets having an excess of regulatory (immunosuppressive) influence might be predisposed to microfloral overgrowth, whereas those with heightened effector function could be prone to food allergies. Development of the intestinal immune system during this critical period of time would eventually attain a balance in the system, such that these inappropriate immune reactions would be transient in otherwise normal pigs.

Summary

Defense of the intestinal mucosa against pathogens is achieved through the integrated operation of several, overlapping mechanisms above, in and below the mucosa, which function to prevent microbial infection and maintain mucosal barrier function. Dendritic cells which span the epithelial layer and pattern-recognition receptors expressed by intestinal epithelial cells represent important links between innate and acquired intestinal immunity. Mucosal defense processes occur early in swine development and appear to be modified to some extent by diet and feed structure. It is an important and complex area of research that would clearly benefit from additional investigations aimed at optimizing digestive health in pigs.

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