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# Host defense in the piglet gut: A brief review

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The intestinal mucosa possesses an extensive surface area and is exposed to a large and diverse number of microorganisms and potentially antigenic proteins throughout the lifetime of a pig. The mucosal epithelium is capable of assimilating vital nutrients while simultaneously excluding ingested pathogens and other harmful materials. In mature swine, an array of gastrointestinal defense processes act rapidly and collectively to intercept, neutralize or eliminate harmful antigens and microbes before they can invade the mucosal surface. These defenses involve a complex interplay between components of the innate and adaptive immune systems, mucosal epithelial cells, and resident microorganisms represented in the large and diverse gut microbiome. Neonatal pigs, on the other hand, are in the process of developing this sophisticated intercellular communication network and rely primarily on passively-acquired immunity from the sow as they develop mucosal immunocompetence and optimum gut function during the first few days and weeks of life. Therefore, the newborn piglet intestine is vulnerable to infection by many different microbial pathogens, and diarrhea associated with these infections remains a challenge to the swine industry (Lay et al., 2002). The purpose of this article is to briefly review some key developmental events pertinent to intestinal defense against mucosal pathogens that occur in the piglet prior to weaning.

## Establishment of the gut microbial community

The gastrointestinal tract of the fetal pig is sterile, but after birth quickly becomes populated with ingested bacteria, *Archaea*, yeasts, protozoa and fungi from the sow and the animal's immediate environment. The gut microbial community is highly dynamic in the pre-weaned piglet and does not stabilize until after weaning (3–4 weeks after birth), a time during which the gut microbial community seems to transition from a microbial ecosystem to a super-organismal ecosystem (Thompson et al., 2008). Commensal microorganisms occupy unique ecological niches within the intestine which are vertically stratified (i.e. some organisms reside in the intestinal lumen, others in the mucus overlying intestinal epithelial cells, and still others, such as *Bacteroides*, associate with enterocytes) and the symbiotic relationships they establish with the host are influenced by dietary signals (Buddington and Sangild, 2011). This commensal microbial population is essential in the development and maintenance of mucosal immunity in pigs and other animals. Resident bacteria also prevent the mucosal colonization of pathogenic bacteria by the process of competitive exclusion (Bailey et al., 2001). Colonization of the intestine is associated with intestinal vasculogenesis and the turnover of the intestinal epithelium (Willing and Van Kessel, 2007). Commensal flora acting in part through Toll-like pathogen recognition receptors (TLRs), seem to dampen intestinal injury as well as allergic reactions to food antigens. On a more holistic scale, the gut microbiome may modulate energy balance and even brain development in animals (Wall et al., 2009; Greiner and Backhed, 2011; Heijtz et al., 2011). There is recent evidence that the gut microbiome of swine may be influenced by husbandry practices (Lamendella et al., 2011). The judicious use of antibiotics in newborn piglets is warranted as the selection pressure they introduce on resident bacteria could destabilize the enteric microbial community and affect the development of the gut-brain axis. Stress exposure in suckling piglets is a concern as well. Chemical mediators of the stress response (e.g. catecholamines, ACTH), which is functional in newborn swine, may alter interactions between intestinal bacteria and mucosal epithelial cells in the pig (Klemcke and Pond, 1991; Lyte et al., 2011). Moreover norepinephrine, released in response to acute stress, increases the vectorial secretion of secretory immunoglobulin A (SIgA) towards the mucosal surface in porcine intestinal explants; SIgA plays an important role in regulating the density, composition, and function of luminal bacteria (Schmidt et al., 2007; Hansen et al., 2010).

## Development of barriers to infection

Immediately after birth, the piglet gut is permeable to proteins and readily absorbs gamma-globulins (IgGs) and other macromolecules from the sow's colostrum. Gut "closure" to macromolecules occurs within 24 hours of birth (Weström et al., 1984). With the onset of lactation, the ingestion of sow's milk containing IgA as well as numerous cytokines and growth factors confers passive immune protection to intestinal mucosal surfaces (Butler et al., 2009).

Epithelial growth factors present in colostrum and milk stimulate epithelial cell growth and differentiation, which are important factors in maintaining mucosal integrity in the suckling piglet. Epithelial cells are held together by a complex array of specialized membrane proteins to form tight junctions. These tight junctions physically exclude proteins and other macromolecules as well as microbes in the intestinal lumen but are selectively permeable to ions, water, and neutrophils. There is recent evidence that barrier function is maintained by the intestinal microbial community through its interactions with gut epithelial cells (Chowdhury et al., 2007). Tight junctional permeability is regulated by a variety of inflammatory, immune and neuroactive substances. For example, inflammatory mediators and certain bacterial or fungal exotoxins (e.g. zonula occludens toxin from *Vibrio cholerae*; trichothecene toxins from *Fusarium*) can increase epithelial tight junction permeability and thereby weaken barrier function (Moeser et al., 2007; Gopalakrishnan et al., 2009). Neutrophils also modify the mucosal barrier and, in response to pathogen- and epithelium-associated chemoattractants, cross the epithelium to attack bacteria in the lumen (Foster et al., 2003). Intestinal epithelial cells themselves can take up and process luminal antigens in humans and rodents, but probably not in swine (Haverson et al., 2000). However, 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 (Wells et al., 2011).

The mucosal epithelium with its tight junctions constitutes an important physical barrier, but chemical barriers to infection also exist in neonatal pigs. For instance, the low pH of the gastric contents plays a key role in reducing the numbers of ingested pathogenic bacteria that could infect the intestinal mucosa. 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; Sangild et al., 1995). Beyond the stomach, non-immune protection is provided by a host of anti-microbial peptides whose synthesis can be induced even in newborn animals (Campeotto et al., 2010). In pigs, these peptides include a wide variety of cathelicidins that are mainly found in leukocytes and some defensins, which can be secreted by mucosal epithelial cells. In addition to their broad-spectrum antimicrobial activities, these peptides may regulate aspects of innate and acquired immunity (Sang and Blecha, 2009). Mucin glycoproteins are also present in the swine intestine at birth (Deplancke and Gaskins, 2001). They are secreted by goblet cells located in the intestinal crypts and polymerize to form mucus. With the assistance of water movement linked to active anion secretion (see below), mucus is hydrated and moves from the crypts to extensively coat the intestinal epithelium. High-viscosity mucus limits the movement of microbes and large molecules such as antigenic proteins towards the epithelial 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 may also act as a reservoir for some host defense molecules, including SIgA (McGuckin et al., 2011). 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).

Host defense peptides and proteins such as mucin, antimicrobial peptides and SIgA are released from epithelial cells situated in the intestinal crypts. Active secretion of chloride and bicarbonate anions by crypt epithelial cells is accompanied by the movement of water into the intestinal lumen. In swine, active anion secretion in the small and large intestines develops during suckling (Pácha, 2000). This process of ion and water secretion facilitates the hydration and movement of defense molecules from the deep crypts towards the gut lumen. Anion secretion can be stimulated by several enteropathogens as well as inflammatory and immune mediators.

The extrusion and mixing of host defense molecules at the surface of the intestinal mucosa is facilitated by the movements of the muscularis mucosae, a thin band of smooth muscle lying just beneath the intestinal mucosa. It is present at birth in swine, but whether or not it displays mechanical activity at this time is unknown (Georgieva and Gerov, 1975). Activity in this musculature appears to be linked through the developing enteric nervous system to epithelial secretion of ions and fluid (Uchida and Kamikawa, 2007). Mixing and propulsive activities of smooth muscle (muscularis propria) in the gastrointestinal tract are present at birth and serve to remove pathogenic microorganisms from the intestinal lumen (Groner et al., 1990). Indeed, the density of microorganisms in the intestinal lumen increases in dysmotility states (Di Lorenzo et al., 1995). On the other hand, enteric infections decrease intestinal propulsion in neonatal pigs (Burrows and Merritt, 1984).

## Post-natal development of intestinal immune function

Cells participating in first 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. TLRs on epithelial cells and other cell types in the intestine detect a variety of bacterial and viral constituents, and 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 (Wells et al., 2011). Several different types of TLRs appear to be expressed in the intestinal tract of newborn swine, where they play a role in the development of Peyer's patches (Shimazu et al., 2009).

Peyer's patches in the swine intestine include the continuous Peyer's patch in the ileum, whose precise function is unknown, and smaller, discrete Peyer's patch follicles in the jejunum. These latter structures fully form in the first two weeks of post-natal life and function to initiate the induction of humoral immune responses to orally-administered foreign antigens. In mature pigs, the lymphoepithelial cells covering jejunal 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 IgA-expressing plasma cells (Rothkötter, 2009). $IgA^+$-plasma cells are not present in the newborn piglet intestine, but are seen in increasing numbers within two to three weeks after birth (Butler et al., 2009). $IgA^+$-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. As it passes through epithelial cells, IgA appears to be capable of neutralizing viruses present within infected 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.

As passive immunity declines with increasing piglet age, functional T lymphocytes begin to populate the intestinal villi and lamina propria of the piglet intestine between one and four weeks after birth (Brown et al., 2006). In addition to their role in adaptive immune function in the gut, a subset of regulatory T cells is capable of releasing immunosuppressive cytokines, such as interleukin 10 and transforming growth factor-*beta* and function to restrain inappropriate immune responses (Bailey et al., 2001). Some of these immunosuppressive T cells express TLRs and are activated by lipopolysaccharide, and it is possible that resident microflora participate in modulating oral tolerance to otherwise harmless foreign antigens (Smith and Nagler-Anderson, 2005).

## Summary

After birth, the piglet intestine begins to develop a formidable array of overlapping physical and chemical barriers to microbial infection, some of which depend upon the establishment of a resident microbial community with which it eventually forms a symbiotic relationship. Until the piglet gut can mount sophisticated gut immune responses involving effective crosstalk between a number of different cell types it must rely upon maternal colostrum and milk as critical factors that confer passive immune protection and enhance the development of enteric defense mechanisms (Zabielski et al., 2008). During this "critical window" encompassing the first few days of life, the piglet gut remains susceptible to pathogenic microorganisms that surmount its defensive capabilities. Husbandry practices, including diet (i.e. colostrum and milk ingestion), housing and stressor reduction, remain an important determinant of a piglet's success in maintaining health and defending itself from gastrointestinal infections over its lifetime.

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