Immunology of the Gut – Taking the Good with the Bad

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Take-Home Message

Mucosal surfaces establish a barrier between the external environment and the internal body and function to interact with the immune system to maintain the health of the whole body. The gastrointestinal tract is the largest mucosal surface of the body and is in a constant state of immune stimulation due to continual contact with dietary antigens and microorganisms. A rapid pro-inflammatory response is critical to initiate defense mechanisms against pathogens; however, just as critical is tight regulation of the immune response so that immune cell activation does not proceed unchecked. For livestock production the primary goal is maintaining mucosal immune homeostasis which is a delicate balance between a healthy activated mucosal immune system and an over-activated pro-inflammatory immune system. Research has begun to look more closely at the interaction between factors that affect gut immune stimulation such as microbial interactions and gut permeability. Although intestinal microbiota may improve animal digestive efficiency, the sheer abundance of microbiota in close proximity with intestinal tissues poses a health risk. As well, a permeable mucosal barrier is essential for the gut to function as an absorptive organ but gut permeability similarly poses a risk to immune stimulation. A greater understanding of these interactions can be used to develop immune-specific strategies to improve overall animal health and productivity.

Immune activation versus livestock production

Mucosal surfaces establish a barrier between the external environment and the internal body and function to interact with the immune system to maintain the health of the whole body. For livestock production, activation of the systemic immune system is undesirable because nutrients are directed away from saleable-tissues (i.e. milk, lean tissue) and catabolism of those tissues may be required to support immune system activation. The systemic immune response, once activated, is hypermetabolic and capable of causing protein malnutrition in a few days' time equivalent to that which could develop in a few weeks' time under starvation conditions (Long, 1977). Excited macrophages utilize ATP at a rate comparable to heart muscle functioning at maximal capacity (Newsholme and Newsholme, 1989). One of the first responses to a systemic infection is reduced feed intake. This sepsis-induced anorexia may act to reduce the risk of further infection (Kyriazakis et al., 1998); however, tissue catabolism then becomes necessary to support the increased demands of mounting the immune response. For livestock production a quantifiable estimate of the metabolic cost of immune system activation on loss of saleable tissue is desired. Unfortunately, an accurate measure of the metabolic cost of maintaining a competent immune system is unlikely (Lochmiller and Deerenberg, 2000). Resting metabolic rate (Baracos et al., 1987), weight gain and protein accretion (Spurlock et al., 1997; Williams et al., 1997a), and tests of immune competence (Bayyari et al., 1997; Williams et al., 1997b) have all been used as surrogates to quantitatively estimate the metabolic cost of immune activation. A 33% increase in metabolic rate, assessed as heat production, was reported following endotoxin-induced fever and antipyresis in sheep (Baracos et al., 1987) and in pigs, chronic immune system stimulation decreased body protein accretion 23% and increased serum alpha-1-acylglycoprotein concentration 50% (Williams et al., 1997a,b).

Changes to livestock production systems (i.e. indoor production, age segregated housing, all-in all-out) and genetic selection have resulted in dramatic advances in animal growth and efficiency (i.e. lean growth, milk output). Simultaneously genetic improvements in productivity has inadvertently selected for reduced robustness or disease resistance (Qureshi and Havenstein, 1994; Bayyari et al., 1997; Knap, 2005) resulting in immune systems with a lesser capability for rapid response or lower maximal response capacity (Bayyari et al., 1997). In livestock production, immune activation is unavoidable; stressors associated with standard production practices (i.e. weaning, tail docking, or castration) and housing and environmental conditions activate the immune system and increase susceptibility to pathogen exposure. As such, a clear understanding of factors affecting immune activation and homeostasis is necessary to develop immune-specific strategies to improve overall animal health and productivity.

A rapid pro-inflammatory response is critical to initiate defense mechanisms against pathogens; however, tight regulation of the immune response is necessary to ensure immune cell activation does not proceed unchecked. The gastrointestinal tract is the largest mucosal surface of the body and is one of the first lines of defense to keep the systemic immune system inactivated. Due to continual contact with dietary antigens and microorganisms, the gut-specific immune system is in a constant state of immune activation in an effort to maintain homeostatic balance between pro- and anti-inflammatory immune responses (Turner, 2009); a nutrient expensive, but necessary, cost to maintaining a healthy animal. An organ system experiencing an activated immune response isn't typically considered in a healthy state but for the gut, a healthy immune system is one that is constantly activated minimizing systemic immune stimulation.

The intestinal microbiota play an important role in nutrient digestion efficiency through degradation of undigested nutrients such as plant polysaccharides, which can be a source of nutrients for the host and the microbiome. However, the sheer abundance of microbiota in close proximity with intestinal tissues poses a health risk. Opportunistic invasion of host tissues by microbes stimulate the host immune response and can pose a significant health risk to the host (Hooper et al., 2012). Alternatively, development of a functioning gut immune system is dependent on interactions with intestinal bacteria (Round and Mazmanian, 2009). Germ-free animals are an excellent model to study the impact of microbial colonization on immune development. Phenotypic differences in immune development observed in germ-free animals, relative to conventionally-housed animals, include fewer Peyer's patches, plasma cells, and intestinal epithelial lymphocytes. A thinner lamina propria, fewer CD8+ cells with reduced cytotoxicity and fewer T_{reg} cells with reduced suppressive capacity was also reported (Round and Mazmaniam, 2009). A homeostatic relationship is necessary to maintain a healthy balance between microbiota-host interaction and immune stimulation (Hooper et al., 2012). Under constant contact with dietary antigens and microorganisms the gut immune system controls exposure by minimizing contact through the synthesis of a mucus layer and by confining penetrant antigens and bacteria to intestinal sites and limiting their contact with the systemic immune system (Hooper et al., 2012). Dendritic cells, macrophages, secreted bacterial-specific IgA, and lymphocytes all act to confine penetrant antigens.

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The gut immune system

The gut immune system is highly developed with a broad range of distinct components that act in concert to manage the continual pressure of microbial and antigen contact, including epithelial cells, tight junction proteins, the mucus and unstirred layer, antigen presenting cells (i.e. macrophages), lymph tissue, T- and B-cells, and cytokines.

The intestinal epithelium provides a barrier against luminal commensal and pathogenic microorganisms and dietary antigens. A critical component of this barrier is the creation of selective mucosal permeability through the use of mucus, an unstirred layer and tight junctions. Mucins secreted by intestinal goblet cells coat the apical membrane and reduce bulk fluid flow creating an unstirred layer which limits direct contact of large particles and bacteria with epithelial cells (Turner, 2009). It is suggested that this unstirred layer further acts to slow nutrient absorption and minimize the loss of nutrients that are released following digestion by brush border membrane enzymes due to diffusion into the intestinal lumen (Turner, 2009). Thickness of the mucosal layer can be an indicator of antigen exposure. Segregated early weaning was used as a means to improve piglet performance by minimizing exposure to antigens immediately post-weaning. Pigs weaned to an off-site nursery had a thinner intestinal mucus layer and greater digestive enzyme activity than pigs weaned to a conventional on-site nursery (Tang et al., 1999). Although a minimal mucus layer has not been established the role of mucin in disease development is being investigated (Heazlewood et al., 2008).

A permeable mucosal barrier is essential for the gut to function as an absorptive organ but gut permeability similarly poses a risk to immune stimulation. Tight junctions play an important role in regulating selective intestinal permeability. They are composed of a variety of proteins which function to maintain cell-to-cell proximity and intercellular communication (i.e. cadherin-1, β-catenin) and to create pores through which selective permeability, based on size or charge, can occur (i.e. claudins, occludin; Turner, 2009). An increase in tight junction permeability increases antigen access to underlying mucosal tissue leading to mucosal immune cell activation (Turner, 2009). The release of pro-inflammatory cytokines such as TNF and IFNɣ play an important role in modifying tight junction barrier function (Turner, 2009) such that TNF-specific antibodies has been used as therapy to remedy barrier dysfunction in patients with Crohn's disease (Suenaert, et al. 2002). Production-associated factors (i.e. weaning, heat, and housing) can also negatively impact intestinal barrier function. For example, heat stress reduced intestinal barrier integrity based on increased endotoxin permeability and reduced transepithelial resistance (Pearce et al., 2013). Although increased tight junction permeability in the absence of other epithelial cell damage may not lead to a disease state (Turner, 2009), tight junction permeability does contributes to intestinal inflammation (Hollander, 1988).

Like other immune systems of the host, the gut immune response includes both innate and adaptive responses with the innate response activating the adaptive response. Activation of an innate immune response occurs through the recognition of microbial-associated molecular patterns (MAMPs) found on commensal and pathogenic microorganisms (Didierlaurent et al. 2002). Intestinal epithelial cells contain transmembrane receptor proteins called toll-like receptors (TLRs) that sense MAMPs, as well as dietary antigens, which stimulate secretion of antimicrobial proteins such as defensins and lysozymes by Paneth cells (Duerkop et al., 2009). It is unclear the exact mechanism used by epithelial cells to distinguish between commensal and pathogenic microorganisms but suggestions include monitoring the densities of MAMP concentration or bacterial density-dependent immune activation (Duerkop et al., 2008) and lower expression of TLRs associated with LPS recognition (i.e. TLR4) resulting in lower responsiveness to MAMPs than other mucosal tissue (Didierlaurent et al., 2002). Another possible mechanism is though bacteria-directed immune responses. A gram-negative

commensal *Bacteroides* species stimulated the release of an anti-microbial peptide with specificity towards certain gram-positive bacteria rather than 'self-specificity' (Cash et al. 2006) suggesting that symbiotic bacteria may direct innate immune responses as a means of environmental protection (Round and Mazmanian, 2009). Macrophages, another innate immune cell, located in the lamina propria rapidly phagocytose and kill penetrant bacteria when microbial breach of the mucosal surface occurs (Duerkop et al., 2009).

Highly orchestrated negative feedback loops between the innate and adaptive immune responses cooperate to regulate mucosal interactions with antigens (Duerkop et al., 2009). Dendritic cells (innate immune cells) sample the environment within the unstirred layer and along the apical epithelial surface, migrate to mucosal lymph tissue, interact with T- and B-cells and induce B-cell differentiated plasma cells to produce bacteria-specific IgA secreted into the intestinal lumen (Duerkop et al., 2009). Each B-cell is programmed to produce one antibody type targeted to a specific antigen. The T-cells differentiate into cell types (i.e. CD4+, CD8+) with different functions and cytokine patterns (Watzl et al., 2005).

Immune homeostasis relies on a balance between pro-inflammatory responses to dietary antigens and bacterial products and anti-inflammatory negative feedback mechanisms. Increased paracellular permeability may allow dietary antigen or bacteria to enter the lamina propria where they are picked up by antigen presenting cells that results in the differentiation of effector T helper cells to T_H1 or T_H2 cells which trigger release of pro-inflammatory cytokines (TFN, IFNɣ, and IL-13) which can stimulate further paracellular permeability. Simultaneously, epithelial cells sense bacteria using MAMPs (i.e. lipopolysaccharide) through TLRs which stimulates release of pro-inflammatory cytokines (Duerkop et al., 2009). However, antigen presenting cells can promote up-regulation of regulatory T (T_{Reg}) cell differentiation which release IL-10 and transforming growth factor-β, cytokines that act as negative feedback signals for production of T_H1 cells. Through dendritic cell sampling of bacteria at the mucosal surface and migration to lymphoid tissue, IgA+ B-cells move to the lamina propria where bacteria-specific IgA is secreted and aids in inhibiting bacterial penetration (Duerkop et al., 2009). Epithelial cell damage triggers the release of epithelial cell-derived pro-inflammatory cytokines TGF and retinoic acid which also enhance T_{Reg} cell differentiation (Turner, 2009) as a means to initiate epithelial cell repair mechanisms. In the healthy animal, T_{Reg} cells are dominant over T_H1 or T_H2 cells (Eison et al., 2005) allowing for a rapid, but controlled, gut immune activation and immune response homeostasis. The intraepithelial lymphocyte is an adaptive immune cell, located on the basolateral side of the epithelial tight junction that plays a critical role in maintaining immune homeostasis (Duerkop et al., 2009). They primarily consist of cytotoxic and suppressor T-cells that aid in removal of damaged epithelial cells and play a role in development of oral tolerance (Watzl et al., 2005).

Nutritional modulation of the gut immune response

Considering the need to maintain homeostatic balance of the gut immune system and the lower maximal response capacity in commercial livestock the question must be asked: what can be done to support the homeostatic metabolism of the gut-specific immune system such that in the healthy state the energetic cost to immune system activation is minimized but sufficient to allow a rapid, aggressive activation to eliminate the insult and return to a healthy state?

Feed additives such as antibiotics, plant extracts, oligosaccharides, organic acids, and microbial fermentation products have all been studied for their potential to modulate the immune response or microbial populations in an effort to enhance animal growth. These additives may also have the potential to enhance basal immune function and potential for disease resistance. The exact mechanism of how these additives modulate the immune response is not known but three

primary mechanisms have been proposed: 1) specific bacterial-dependent modulation of cytokine and antibody production, 2) short-chain fatty acid production along with enhanced fatty acid binding to leukocyte receptors, and 3) interaction with leukocyte carbohydrate receptors (Watzl et al., 2005). One such additive that has received considerable attention, particularly for application to human nutrition and obesity prevention is resistant starch. Pigs fed resistant starch had increased circulating levels of the 3 main short-chain fatty acids, higher relative abundance of butyrate-producing microbial species and lower colonic cell expression of genes associated with adaptive and innate immune response pathways (Haenen et al., 2013). In cell culture butyrate suppresses lymphocyte proliferation, inhibits T_H1 lymphocyte cytokine production and increases IL-10 production (Säemann et al., 2000) suggesting that dietary supplementation with butyrate may also provide immunomodulatory effects. The addition of sodium butyrate to weaned pig diets improved feed efficiency and increased stomach, but not small intestinal or hindgut, butyrate concentration indicating the observed effect was not due to a direct effect of butyrate on the intestinal epithelium (Manzanilla et al 2006). Thus it is likely that the immunomodulatory effects of butyrate are more likely to be observed with the use of dietary additives that increase bacterial production of butyrate rather than supplementation with butyrate alone. For example, β-glucans, fungal and plant cell wall polysaccharides, have immunomodulating action that may in part be regulated by a bacterial-dependent mechanism given that β-glucans are typically considered indigestible carbohydrates fermented by intestinal microbes (Xu et al., 2013). In chicks, supplementation with β-glucans improved macrophage proliferation, greater antibody production, more persistent hypersensitivity response, and higher percentage of CD4+, CD8+, and CD4+/CD8+ intraepithelial lymphocytes (Guo et al., 2003). Phagocytic cells express carbohydrate receptors sensitive to β-glucans that can activate cytotoxic reactions (Watzl et al., 2005) suggesting multiple modes of action for β-glucans modulation of the immune response.

A major challenge with understanding the immune response in relational to nutritional modulation is concluding whether the observed response is negative or positive relative to overall host immune status because the cells involved in immune activation are also cells involved in maintaining homeostasis (Brown et al., 2006). An increase in T helper cells (i.e. CD4+) and MHC class II bearing cells is suggested to indicate an increase in presentation of foreign antigens, subsequent cytokine production and hence cellular and humoral immune activation (Tonegawa, 1985; Matis, 1990). However, the population of CD4+, CD8+, and CD25+ cells in jejunal tissue was greater in segregated early weaned pigs relative to conventionally weaned pigs (Brown et al., 2006). The authors conclude that although this would suggest the jejunal epithelial cells of the SEW pigs encountered more antigen the increase in CD4+ and CD8+ T cells may indicate greater development of oral tolerance through T_H3 cell (CD4+CD25+) production of interleukin-4 and TGF-β, cytokines involved in down-regulation of the immune response (Mowart and Weiner, 1999). A further complication is that a vast array of cell types are involved within a given immune response but evaluation methods often involve assessment of a few specific cell types which may result in apparently contradictory responses. For example, in a line of turkeys selected for superior weight gain total blood lymphocyte numbers and toe-web hypersensitivity response to phytohemagglutinin-P (PHA-P) was lower but lymphocyte mitogenic activity to PHA-P was higher compared to a randombred control line (Bayyari et al., 1997). The authors concluded the lack of correlation between hypersensitivity response and mitogenic activity may reflect the different aspects of cellular immune response measured by each test. Similarly, the higher antibody production and percentage of activated T-cells in chicks supplemented with β-glucans may appear to indicate an immune challenge and hence a negative effect of β-glucan supplementation (Guo et al., 2003). However, in the absence of differences in growth performance the response may imply an improved baseline immune status such that chicks supplemented with β-glucan have a greater capacity to resist a disease challenge. Another challenge in understanding, or evaluating the gut immune response,

is that under different conditions, an ingredient that is considered immunogenic in the gut may provide immune protection or aid in minimizing a response to antigen. For example, glycinin, the major storage protein in soybeans, induces intestinal inflammatory response and transient hypersensitivity, including increased mucosal mast cell number and intestinal IgE antibodies in young pigs (Li, et al., 1990; Sun et al., 2008). As such, in practical diet formulation inclusion of soybean meal (SBM) in nursery diets is typically kept below 20% to minimize the risk of intestinal inflammation and risk of diarrhea. However, nursery pigs fed diets containing 29% SBM had lower serum PRRS virus load, haptoglobin, and tumor necrosis factor-α and greater growth than pigs fed diets containing 17.5% SBM during a PRRS infection suggesting dietary SBM modulated the systemic immune response (Rochelle et al., 2014). This apparent immunogenic protection conveyed by SBM in a disease state is a recent development and the relationship between the gut response to SBM and systemic immune modulation is unknown.

The gut immune system is complex in its ability to regulate pro- and anti-inflammatory responses with a vast array of highly integrated cellular components. As a result, interpretation of *in vitro* and *in vivo* immune responses can be difficult. Correlating cellular immune responses with whole body responses will improve data interpretation and application of results to practical feeding strategies. Continued assessment of the interaction between dietary components, immune system metabolism, and animal performance is necessary for the development of immune-specific strategies to improve overall animal health and productivity.

References

Baracos, V.E., Whitmore, W.T., and Gale, R. 1987. The metabolic cost of fever. Can J Physiol Pharmacol 65:1248-1254.

Bayyari, G.R., Huff, W.E., Rath, N.C., Balaog, J.M., et al. 1997. Effect of genetic selection of turkeys for increased body weight and egg production on immune and physiological responses. Poult Sci. 76:289-296.

Brown, D.C., Maxwell, C.V., Erf, G.F., Davis, M.E., et al. 2006. The influence of different management systems and age on intestinal morphology, immune cell numbers and mucin production from goblet cells in post-weaning pigs. Vet Immunol Immunopath 111:187-198.

Cash, H.L., Whitman, C.V., Behrendt, C.L., and Hooper, L.V. 2006. Symbiotic bacterial direct expression of an intestinal bactericidal lectin. Science 313:1126-1130.

Didierlaurent, A., Sirard, J-C., Kraehenbuhl, J-P., and Neutra, M.R. 2002. How the gut senses its content. Cell Microbiol 4:61-72.

Duerkop. B.A., Vaishnava, S., and Hooper, L.V. 2009. Immune responses to the microbiata at the intestinal mucosal surface. Immun Rev. 31:368-376.

Elson, C.O., Cong, Y., McCracken, V.J., Dimmitt, R.A., et al. 2005. Experimental models of inflammatory bowel disease reveal innate, adaptive, and regulatory mechanisms of host dialogue with the microbiota. Immunol Rev. 206:260-276.

Guo, Y., Ali, R.A., and Quereshi, M.A. 2003. The influence of b-glucan on immune responses in broiler chicks. Immunopharm Immunotox 25:4461-472.

Haenen, D., Souza de Silva, C., Zhang, J., Jan Koopmans, S., et al. 2013. Resistant starch induces catabolic but suppresses immune and cell division pathways and changes the microbiome in the proximal colon of male pigs. J Nutr 143:1889-1898.

Hollander, D. 1988. Crohn's disease – a permeability disorder of the tight junction? Gut 29:1621-1624.

Hooper, L.V., Littman, D.R., and Macpherson, A.J. 2012. Interaction between the microbiota and the immune system. Science 336:1268-1273.

Kyriazakis, I. Tolkamp, B.J., and Hutchings, M.R. 1998. Towards a functional explanation for the occurrence of anorexia during parasitic infections. Anim Behav 56:265-274.

Li, D.F., Nelssen, J.L., Reddy, P.G., Bleecha, F., et al. 1990. Transient hypersensitivity to soybean meal in the early-weaned pig. J Anim Sci 68:1790-1799.

Lochmiller, R.L. and Deerenberg, C. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88:87-98.

Long, C.L. 1977. Energy balance and carbohydrate metabolism in infection and sepsis. Am J Clin Nutr 30:1301-1310.

Manzanilla, E.G., Nofrarias, M., Anguita, M., Castillo, M., et al. 2006. Effects of butyrate, avilamycin, and a plant extract combination on the intestinal equilibrium of early-weaned pigs. J Anim Sci 84:2743-2751.

Matis, L.A. 1990. The molecular basis of T cell specificity. Annu Rev Immunol 8:65-82.

Mowart, A. and Weiner, H. 1999. Mucosal immunology. In: Ogra, P. Mestecky, J., Lamm, M., Strober, W., et al. (Eds), Inflammation and mucosal cytokine production. 2nd ed. Acad Press, San Diego, CA p. 601-609.

Newsholme, P. and Newsholme, E.A. 1989. Rates of utilization of glucose, glutamine and oleate and formation of end-products by mourse peritoneal macrophages in culture. Biochem J 261:211-218.

Pearce, S.C., Mani, V., Weber, T.E., Rhoads, R.P., et al. 2013. Heat stress and reduced plane of nutrition decreases intestinal integrity and function in pigs. J Anim Sci 91:5183-5193.

Qureshi, M. and Havenstein, G.B. 1994. A comparison of the immune performance of a 1991 commercial broiler with a 1957 randombred strain when fed "typical" 1957 and 1991 broiler diets. Poult Sci. 73:1805-1812.

Rochelle, S.J., Alexander, L.S., Boyd, R.D., van Alstine, W.G., et al. 2014. Effects of dietary soybean meal concentration on growth performance and immune response of pigs during a porcine reproductive and respiratory syndrome virus challenge. Midwest ASAS Annual Meeting, Des Moines, IA. March 14-17, 2014. Abstr. 84.

Round, J.L. and Mazmanian, S.K. 2009. The gut microbiota shapes intestinal immune responses during health and disease. Nature Rev: Immunol 9:313-323.

Säemann, M.D., Böhmig, G.A., Österreicher, C.H., Burtscher, H., et al. 2000. Anti-inflammatory effects of sodium butyrate on human monocytes: potent inhibition of IL-12 and up-regulation of IL-10 production. FASEB J 14:2380-2382.

Suenaert, P., Bulteel, V., Lemmens, L., Noman, M., et al. 2002. Anti-tumor necrosis factor treatment restores the gut barrier in Crohn's disease. Am J Gastroenterol 97:2000-2004.

Sun, P, Li, D., Li, Z., Dong, B., et al. 2008. Effects of glycinin on IgE-mediated increase of mast cell numbers and histamine release in the small intestine. J Nutr Biochem 19:627-633.

Tang, M., Laarveld, B., van Kessel, A.G., Hamilton, D.L., et al. 1999. Effects of segregated early weaning on postweaning small intestinal development in pigs. J Anim Sci 77:3191-3200.

Tonegwa, S. 1985. The molecules of the immune system. Sci Am 253:122-131.

Turner, J.R. 2009. Intestinal mucosal barrier function in health and disease. Nature Rev: Immunol 9:799 – 809.

Watzl, B., Girrbach, S., and Roller, M. 2005. Inulin, oligofructose and immunomodulation. Br J Nutr 93 Suppl1:S49-S55.

Williams, N.H., Stahly, T.S., and Zimmerman, D.R. 1997. Effect of level of chronic immune system activation on the growth and dietary lysine needs of pigs fed from 6 to 112 kg. J Anim Sci 75:2481-2496.

Xu, X., Xu, P., Ma, C., Tang, J., et al. 2013. Gut microbiota, host health, and polysaccharides. Biotech Adv 31:318-337.

Notes