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Influence of the Gastrointestinal Microbiota on Development of the Immune System in Young Animals

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Abbreviations for scientific terms

Ang4: Angionin-4; APC: antigen-presenting cell; CD: cluster of differentiation; DC: dendritic cell; DGGE: denaturing gradient gel electrophoresis; FAE: follicle-associated epithelium; GALT: gut-associated-lymphoid tissue; GF: germ-free; GIT: gastrointestinal tract; IEL: intraepithelial lymphocytes; IFN: interferon; Ig: immunoglobulin; IL: interleukin; LPS: lipopolysaccharide; LTA: lipoteichoic acid; MHC: major histocompatibility complex; NDO: non-digestible oligosaccharides; NF: nuclear factor; NK: natural killer cell; PAMPs: pathogen-associated molecular patterns; PCR: polymerase chain reaction; PlgR: polymeric immunoglobulin receptor; PP: Peyer's patches; SC: secretory component; SFB: segmented filamentous bacteria; slgA: secretory immunoglobulin A; TGF: transforming growth factor; Th: T helper cell; TLR: Toll-like receptor; TNF: tumor necrosis factor.

Abstract

The gastrointestinal tract (GIT) of adult mammals is colonized by a complex and dynamic community of microorganisms. Most protection against potential pathogens occurs via a mucosal immune system involving mechanisms of innate immunity as well as a secondary lymphoid organ, the gut-associated lymphoid tissue (GALT). However, the bacterial community also supports its host against invasion by potential pathogens, by a mechanism called 'colonization resistance'. Young animals need time to develop both a complex bacterial community and their immature GIT immune system, and until such developments have taken place, they are vulnerable to the presence of potential pathogens in their GIT. Initial protection against invading pathogens is provided by milk and colostrum, which contain antibodies and other bioactive components. At weaning, with the introduction of solid food and deprivation of the mother's milk, the young must also cope with a rapidly changing microbiota. The colonizing microbiota not only provides colonization resistance to potentially pathogenic bacteria. It also has a major role in the development of

the intestinal immune system, both in terms of GALT development and mucosal immunity, and the induction of oral tolerance. Studies using gnotobiotic animal models have revealed that the presence of even limited numbers of the indigenous microbiota may influence the GIT immune system. Regulation of the composition of the GIT microbiota, e.g. by the use of pre- and probiotics, offers the possibility to influence the development of mucosal, and also systemic immunity.

Introduction

The bacterial community which inhabits the mammalian GIT is characterized by its high density and diversity. The colon contents support at least 400 different species, with numbers as high as 10^{10} and 10^{11} culturable bacteria/g of digesta (Savage, 1977; Mackie and White, 1997). These bacteria are constantly interacting with each other, and with the host, comprising a highly complex ecosystem of which comparatively little is known. It is only recently that new techniques in molecular biology are allowing the detection of microbial species that are either difficult or as yet impossible to culture (Vaughan *et al.*, 2000; Tannock, 2001).

The resident microbiota confers many benefits to the intestinal physiology of the host and is, therefore, an example of a truly symbiotic relationship (Hooper and Gordon, 2001). Some of these benefits include the metabolism of nutrients and organic substrates, and the contribution to the phenomenon of colonization resistance. The latter is the ability of the GIT bacterial community to resist invasion of the host by exogenous microorganisms (Van der Waaij *et al.*, 1971; Berg, 1996). Further benefits become visible in the period after birth, when the complexity of the intestinal environment increases considerably while changing from an exclusively milk-containing diet to an adult diet after weaning (Rumbo and Schiffrin, 2005). During this period, the intestinal microbiota plays a crucial inductive role in intestinal development. Studies in gnotobiotic animals have shown that association of germ-free rodents with a single bacterial species has a profound impact on the anatomical, physiological, and immunological development of the host. This includes microbicidal protein production, development of intestinal epithelium, vasculature and GALT (Shroff *et al.*, 1995; Stappenbeck *et al.*, 2002; Hooper, 2004). Bacterial colonization of the GIT is also essential for the development of oral tolerance, i.e. the systemic unresponsiveness to commensal bacteria and food proteins to avoid chronic inflammation. In the germ-free state, this mechanism does not exist, but develops rapidly after colonization (Sudo *et al.*, 1997; Shi and Walker, 2004).

With the ban on dietary antibiotics as growth promoters within the European Union, animal nutritionists are seeking alternatives to these promoters, particularly for young animals. The beneficial effects of bacteria on

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the immune system have been proposed as one theory supporting the use of probiotic bacteria as an alternative to antibiotics in improving animal health and protection against infectious agents (Simon *et al.*, 2003). Probiotics are live microorganisms that may beneficially affect the GIT balance, going far beyond their conventional nutritional effect (Fuller, 1992). A number of studies have reported immune-stimulating effects of different bacterial species, such as production of microbicidal proteins, or an enhanced antibody response (Forchielli and Walker, 2005). They may also lead to alleviation of intestinal inflammatory responses by regulation of cytokine production (Isolauri *et al.*, 2001).

An improved understanding of the complex relationship between the indigenous intestinal microbiota and the host immune system will help to establish strategies to improve host health. This is of special importance in the period after birth, or at stressful moments in animal lives. For piglets, interest has focussed on the time of abrupt weaning, which has always been a source of economic losses for pig farmers.

Development of the intestinal microbiota from birth

After birth, the intestinal microbiota takes some time before developing a stable community (Gaskins, 2001). Colonization is a complex process of natural selection and ecological succession. It depends on various factors, some of which are of host origin, such as the genome and physiology of the animal, while others are of microbial origin, such as interactions between bacterial species (Konstantinov *et al.*, 2004b).

During the first few weeks of life, microbial succession in the GIT of humans (Favier *et al.*, 2002), pigs (Moughan *et al.*, 1992), chickens (Barrow, 1992) and calves (Smith, 1965) is remarkably similar, even though animal species are usually exposed to greater numbers of bacteria from fecal and environmental sources, compared with humans. After birth, the germ-free GIT is rapidly colonized by anaerobic and facultative anaerobic bacteria. Culture studies have indicated that in general, humans are initially colonized by species showing a high reductive potential (e.g., *Enterobacter*). They metabolize oxygen, thus indirectly encouraging the growth of anaerobic bacteria including lactobacilli and bifidobacteria, *Bacteroides* and clostridia (Mackie *et al.*, 1999; Teitelbaum and Walker, 2002). Favier *et al.* (2002) investigated the succession of bacterial communities in human neonates, by monitoring 16S rRNA gene diversity in fecal samples using PCR/DGGE. The first colonizers belonged to *Escherichia coli* or *Clostridium*, followed after a few days by *Bifidobacterium*, which then remained predominant in breast-fed infants. After weaning, *Clostridium*, *Ruminococcus*, *Enterococcus* and *Enterobacter* spp. appeared, and microbial DGGE profiles became more complex and also more stable with increasing age.

In suckling piglets, on the other hand, the population of fecal bifidobacteria seems to be numerically low (Mikkelsen *et al.*, 2003), or even absent (Konstantinov *et al.*, 2004a). Lactobacilli, however, establish early in the piglet's intestine, and, although succession does occur throughout the pig's lifetime, they remain a predominant member of the small intestinal microbiota (Tannock *et al.*, 1990; Naito *et al.*, 1995; Stewart, 1997). At weaning, when

it occurs early, the transition from milk to a solid diet leads to dramatic changes in the composition of the microbial community during the 7–14 days after weaning (Hillman, 2001). According to Ewing and Cole (1994), numbers of lactobacilli and other beneficial bacteria decrease in times of stress, as do their beneficial effects, allowing potential pathogens such as coliforms to increase. Franklin *et al.* (2002) found that lactobacilli populations in different GIT sections (jejunum, ileum, cecum) declined to lower levels in early-weaned pigs (17 days), compared with piglets weaned at 24 days.

Mucosal immunity

The mucosal immune system is directly exposed to the external environment and stimulated by antigens consisting of commensal and potentially pathogenic bacteria, dietary antigens, and viruses (Elson, 1985; Mayer, 1997; 2000; Nagler-Anderson and Shi, 2001). The basic mechanism of mucosal immunity is innate, non-specific immunity represented by processes that protect the host immediately after exposure to pathogens (Tlaskalova-Hogenova *et al.*, 2004). Non-specific mechanisms include e.g. natural killer cells (NK) and activated macrophages, but also soluble mediators such as cytokines and 'complement', a system of proteolytic enzymes, which plays an important role in the elimination of microbes (Fearon, 2000; Janeway, 2001; Beutler, 2004).

The intestinal epithelium provides an essential part of innate immunity, as it must control the access of potential antigens and pathogens. It is supported by tight junctions which join intestinal epithelial cells, and restrict the passage of even very small (2 kDa) molecules (Madara, 1998). Further protection is provided by the generation of antimicrobial substances, including inorganic disinfectants (e.g. hydrogen peroxide and nitric oxide), large antimicrobial proteins (e.g. lysozyme and lactoferrin), or small antimicrobial peptides (e.g. defensins). The β -defensins are synthesized by epithelial cells lining the mucous membranes of, e.g., the GIT. On the other hand, α -defensins are produced in granules of neutrophils and Paneth cells of the small intestine (reviewed by Zhang *et al.*, 2000; Zasloff, 2002; Lehrer, 2004). The present understanding of defensin antimicrobial mechanisms is that the peptides disable susceptible organisms by disrupting structural elements of the target cell membranes, and that permeabilization of the bacterial envelope is linked to microbicidal activity (Lehrer *et al.*, 1989; Selsted and Ouellette, 2005).

A significant barrier to antigen entry is also provided by the mucin glycoproteins that line the surface epithelium in the GIT (McNabb and Tomasi, 1981; Deplancke and Gaskins, 2001). Bacteria and viruses become trapped in the mucus layer and are expelled by the peristaltic process of the gut, thereby preventing potential pathogens and antigens from gaining access to the underlying epithelium, a process called non-immune exclusion. Mucins also serve as a reservoir for secretory IgA (sIgA).

Gut-associated lymphoid tissue (GALT)

The GALT is a secondary lymphoid organ, which can be divided into inductive and effector sites. The different lymphoid tissues found in the intestine are summarized in Table 1.

Table 1. Lymphoid tissues found in the intestine.		
Type of lymphoid tissue	Distribution	Characteristics
<i>Organized lymphoid tissue (inductive site)</i>		
Small isolated lymphoid follicles	Throughout the wall of small and large intestines	- Mainly occur singly or in small groups; - Appearance of microscopic PP
Peyer's patches <i>Mouse</i> : discrete PP (6–12) with few follicles along small intestine (40–57) <i>Human</i> : several PP in ileum, 180–240 in jejunum <i>Pig</i> : Isolated, discrete PP in jejunum (14–27) and upper ileum; one continuous PP in the terminal ileum (1.0–2.0 m)	Submucosa along the small intestine	- Macroscopic lymphoid aggregates; - Consist of collections of large B cell follicles and intervening T cell areas; - Lymphoid area separated from the intestinal lumen by follicle-associated epithelium with M cells; - Sites of antigen sampling from gut lumen and induction of immune responses; - Expanded role in production of systemic B cells and primary antibody repertoire in some species
Mesenteric lymph nodes		- Largest lymph nodes of the body; - Crossroads between peripheral and systemic immune systems
Cryptopatches • <i>Mouse</i> : 1500 • <i>Human</i> : none • <i>Pig</i> : not yet found	Intestinal mucosa	- Occur as small clusters of lymphocytes between intestinal crypts; - Proposed sites for T and/or B cell differentiation
Appendix	Large intestine	- Accumulation of B cell follicles in terminal part of cecum
<i>Diffuse lymphoid tissue (effector site)</i>		
Intraepithelial lymphocytes	Mucosal epithelium, single cells between intestinal epithelial cells	- Almost exclusively T cells; - Protection against entry and dissemination of foreign antigen
Diffuse lymphocytes	Lamina propria	- B and T cells
PP, Peyer's patches. Compiled after Kraehenbuhl and Neutra (1992), Griebel and Hein (1996), Mowat and Viney (1997), Hein (1999), Pabst and Rothkötter (1999), Brandtzaeg (2003a), MacDonald (2003), Cheroutre (2004) and Wang <i>et al.</i> (2004).		

The inductive sites, mainly the Peyer's patches (PP), consist of aggregations of lymphoid follicles. Their surface is covered by a unique epithelium (termed follicle-associated epithelium, FAE) which contains, interspersed between enterocytes, a specialized epithelial cell type, known as M cells (Brandtzaeg, 2003a). The brush border glycocalyx that characterizes villus enterocytes is absent from the apical surface of the M cell. It is replaced by microfolds (hence 'M' cells) that are more accessible to luminal antigens (Owen and Jones, 1974; Hathaway and Kraehenbuhl, 2000). M cells use trans-epithelial vesicular transport to carry antigens in the underlying GALT.

In the PP, antigens are presented by antigen-presenting cells (APCs, macrophages and dendritic cells) to both immature T and B cells (Kraehenbuhl and Neutra, 1992). Activated T cells preferentially differentiate into CD4⁺ helper cells which, aided by dendritic cells (DCs) and secretion of cytokines such as transforming growth factor (TGF)- β and interleukin (IL)-10, induce the differentiation of antigen-specific B cells to predominantly IgA-committed plasmablasts (Brandtzaeg *et al.*, 1999). The GALT-derived B cell blasts proliferate and differentiate further on their way through the mesenteric lymph nodes and the thoracic duct into the bloodstream. Then they migrate preferentially to the mucosal effector sites (i.e. lamina propria and intraepithelial regions, but not PP). Here they complete their terminal differentiation to IgA-producing plasma cells – a process called 'homing' (Kraehenbuhl and Neutra, 1992). A schematic depiction of the functional organization of the GALT with inductive and effector sites, is shown in Fig. 1.

However, there are also other cell types capable of transporting antigen across the epithelial barrier

(Rescigno *et al.*, 2001). Dendritic cells may extend their dendritic-like processes through epithelial tight junctions and sample luminal antigen directly. However, the principal function of DC appears to be activation of T cells (Banchereau and Steinman, 1998). Circulating precursor DC enter peripheral tissues as immature DC, where they capture microbial or viral antigens, thereby acting as sentinels at the front line of host defence. Following antigen capture, the immature DC leave the tissues and migrate to lymphoid organs, where after maturation, they display antigen-derived peptides on their major histocompatibility complex (MHC) molecules, which in turn, select circulating antigen-specific T cells (Palucka and Banchereau, 1999).

Intestinal DC can retain small numbers of live commensals for several days. This allows DC to selectively induce IgA, which helps to protect against mucosal penetration by commensals. The commensal-loaded DC are restricted to the mucosal immune compartment by the mesenteric lymph nodes, which ensures that the immune responses to commensal bacteria are induced locally. This prevents any induction of either a systemic immune response or a damaging inflammatory response. So, IgA production, and probably intestinal T cell responses, can be selectively induced by DC loaded with commensal bacteria, and this increased local secretion of IgA limits the penetration of commensal bacteria (Macpherson and Uhr, 2004).

Secretory IgA (sIgA)

The most abundantly produced immunoglobulin in mammals is IgA, which is secreted mainly across mucous membranes, e.g., of the intestine. This 'secretory' form of

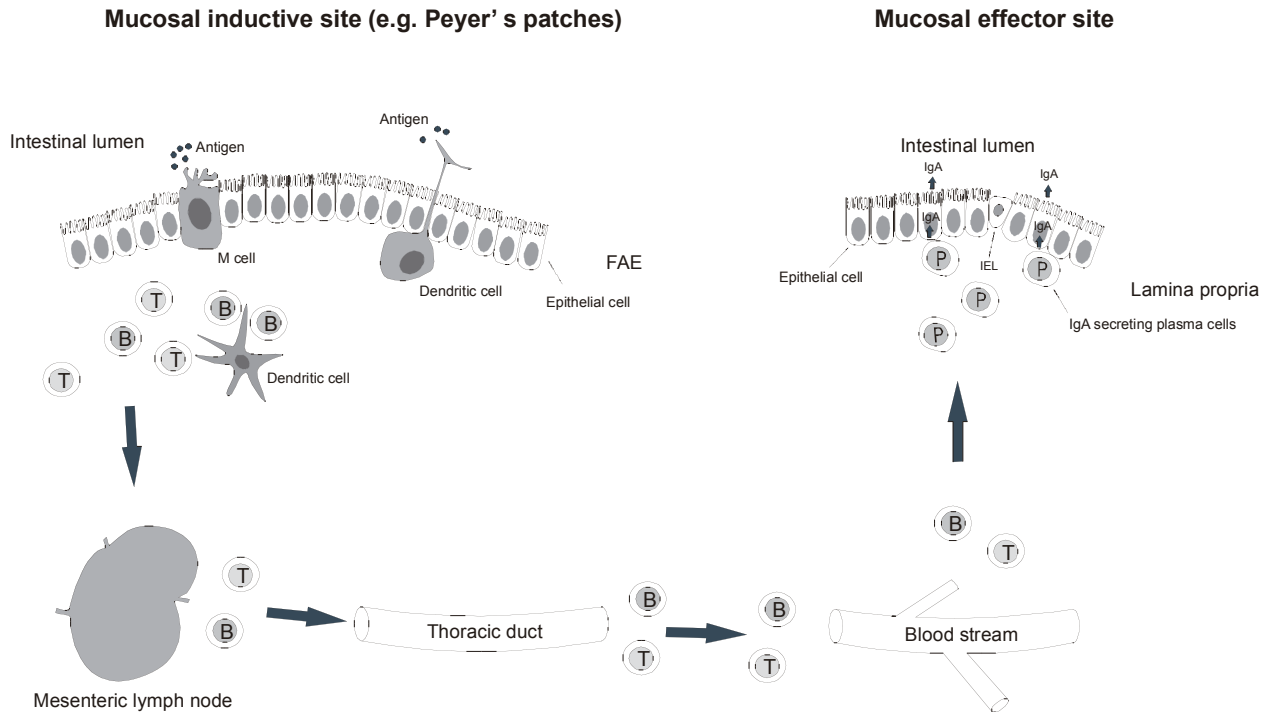


Fig. 1. Schematic functional organization of the GALT, which is divided into inductive (Peyer's patches) and effector sites (lamina propria). Antigen transport across the epithelium occurs through M cells and dendritic cells. After being primed in the GALT, B and T cells differentiate further on their way through the lymph nodes and the blood and migrate to the mucosal effector sites. B: B cell; FAE: follicle-associated epithelium; IEL: intra-epithelial lymphocyte; Ig: immunoglobulin; P: plasma cell; T: T cell. Based on Nagler-Anderson (2001) and Brandtzaeg (2003a).

IgA constitutes a dimer of two monomeric IgA molecules with an attached secretory component (SC). Dimeric IgA is synthesized by plasma cells present in mucosa-associated lymphoid tissues and is then transported across the epithelium, via the polymeric immunoglobulin receptor (pIgR). The SC is a fragment of the pIgR that is left attached to sIgA after its transport to the apical surface of the epithelial cells (Macpherson *et al.*, 2001). The role of sIgA in excluding antigen from entering the epithelium has long been appreciated (Brandtzaeg, 2003a). For example, in the lumen, antigen exclusion is provided by free sIgA which mainly functions as an inhibitor of bacterial/viral adherence and penetration of the underlying epithelium (Cunningham-Rundles, 2001; Macpherson *et al.*, 2001). It has also been suggested that, by use of the pIgR, IgA actively transports antigen out of the lamina propria to the apical surface of the enterocytes (Robinson *et al.*, 2001).

Recognition of potential pathogens by the host

Cells of the innate immune system, such as DC and macrophages, provide broad innate protection against microorganisms that are newly encountered by the host. Activation of innate host defence depends on specific recognition of microbial signature molecules called pathogen-associated molecular patterns (PAMPs) (Janeway and Medzhitov, 2002). They are shared by large groups of microorganisms, such as lipopolysaccharides (LPS) on Gram-negative bacteria, and peptidoglycan and lipoteichoic acids (LTA) on Gram-positive bacteria (Barton and Medzhitov, 2002). In the gut mucosa, monocytes and particularly DC in the intra-epithelial and sub-epithelial layers are specialized for detecting microbial

pathogens. Both cell types recognize PAMPs through pattern-recognition receptors that are either secreted or expressed on the immune cell surface (Medzhitov and Janeway, 2002). These molecules include the Toll-like receptor (TLR) family. Human monocytes and DC express all known TLR (TLR1 to TLR10) (Kadowaki *et al.*, 2001). Activation of TLR initiates a signal transduction cascade which can lead to activation of nuclear factor κ B (NF- κ B), which, in turn, controls the expression of several genes involved in the inflammatory response (Janeway and Medzhitov, 2002), up-regulation of co-stimulatory molecules on APC, and the production of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)- α , IL-1 and IL-6. Cytokines are produced by cells of the innate immune system, such as macrophages and DC, in response to pathogens, and can affect distinct cells, such as DC precursors and B and T cells, to induce an adaptive immune response (Belardelli and Ferrantini, 2002). In this way, the gut epithelium forms a link between the innate and adaptive immune systems.

It is thought that the sequestration of indigenous microbiota by surface epithelia plays an important role in preventing TLR activation by commensals (Gewirtz *et al.*, 2001; Sansonetti, 2002). Using adult rodent models, a recent study has shown that under normal conditions, commensals will interact mainly with TLR expressed by intestinal epithelial cells. This may contribute to a cytoprotective epithelial response and promote barrier integrity and repair, thus preventing bacterial translocation (Rakoff-Nahoum *et al.*, 2004). Expression of at least some TLR, most notably TLR4, has been described in both human and mouse intestinal epithelium (Cario *et*

et al., 2000; 2002; Ortega-Cava *et al.*, 2003). Thus, TLR function as sensors of microbial infection, and are critical for the initiation of inflammatory and immune defence responses. However, they also seem to play a major role in maintaining intestinal epithelial homeostasis. However, it is not known to what extent this mechanism operates in newborns (Rumbo and Schiffrin, 2005).

Protection after birth

Role of milk and colostrum

In contrast to humans and rodents, which benefit from a transplacental passage of maternal serum antibodies during embryonic development, the multi-layered placenta in pigs prevents the transit of maternal Ig into the fetus (Butler, 1998; Salmon, 1999). Thus, first protection of the neonate piglet is provided by colostrum and milk.

In pigs, systemic humoral immunity is transmitted through colostrums conveying mainly IgG, and lower concentrations of IgA and IgM (Klobasa and Butler, 1987; Klobasa *et al.*, 1987). Furthermore, a local humoral immunity, via sIgA, is transmitted mainly by milk (lactogenic immunity) until weaning. The specificity of these antibodies is believed to be acquired through the 'entero-mammary' pathway: during lactation, IgA⁺ B lymphocytes migrate from the intestine to the mammary glands, where they produce sIgA antibodies against the mother's previous and present intestinal microbiota (Roux *et al.*, 1977). Since the antigenic specificity of the antibodies reflects the maternal experience of environmental antigens, immunity acquired through colostrum and milk will protect the piglet against these antigens, but not against novel antigens (Rooke and Bland, 2002). In humans, it has been shown that free secretory component, which is abundant in breast milk, may, on its own, block epithelial adhesion, and thereby limit infection by enterotoxigenic *E. coli* (De Oliveira *et al.*, 2001). Free SC is released into secretions when the polymeric Ig receptor is transcytosed without being bound to IgA (Mullock *et al.*, 1980; Phalipon and Corthésy, 2003).

Porcine milk also contains other factors, which support resistance of the neonate against infections. These include maternal cells such as phagocytes, lymphocytes and epithelial cells, as well as antimicrobial substances, e.g. lactoferrin and lysozyme (reviewed by Wagstrom *et al.*, 2000). Lactoferrin has a number of effects including microbicidal, immunostimulatory, and efficiently anti-inflammatory, by turning off the production of numerous pro-inflammatory cytokines such as IL-1 β , IL-6, TNF- α , and IL-8 (Mattsbj-Baltzer *et al.*, 1996; Håversen *et al.*, 2000; Ellass *et al.*, 2002). During neonatal colonization and the subsequent expansion of the intestinal microbiota, it is particularly important to have considerable amounts of a protein such as lactoferrin in the gut, which is bactericidal and also prevents the induction of cytokines that cause clinical symptoms, energy consumption and inflammation (Hanson *et al.*, 2003).

In sow's milk, there are also considerable amounts of polyamines, which are of fundamental importance with regard to cell proliferation and differentiation (Johnson, 1988), but which also seem to be involved in the

maturation of the intestinal immune system. For example, oral administration of spermine to neonatal mice affected differentiation of the intraepithelial lymphocytes (Ter Steege *et al.*, 1997). Furthermore, it has been suggested that polyamines are involved in the maturation of the glycosylation of the enterocytes (Biol-N'garagba and Louisot, 2003). Intestinal glycosylation is relevant to the implantation of the microbiota as the glycan structures at the enterocytes' surface may determine the adhesion of specific pathogens (Jones and Freter, 1976).

Milk can also contain components such as glycoproteins, glycolipids, mucins and oligosaccharides (Newburg, 1999), some of which exhibit antimicrobial activity, but which may also act as growth promoters for bifidobacteria (Kunz and Rudloff, 1993). For example, the proliferation of *Bifidobacterium bifidum* and lactobacilli in the lower GIT is promoted by certain glycopeptides and glycoproteins, including caseins in human milk (Bezkorovainy and Topouzian, 1981; Liepke *et al.*, 2002). Human milk oligosaccharides may also act as specific 'bifidogenic factors', supporting the survival of these bacteria (Beerens *et al.*, 1980). They can also function as receptor analogues that inhibit the binding of enteric or respiratory bacterial pathogens, or their toxins, to epithelial cells (Kunz *et al.*, 2000). Compared to other host species, human milk is considered to be unique in terms of its complex oligosaccharide content (Rudloff and Kunz, 1997). Although only present in small quantities in porcine milk, oligosaccharides may exert similar biological effects (Xu, 2003).

Development of the structure of the mucosal immune system

In humans, PP and other structures of the mucosa-associated lymphoid tissue are already well developed at birth. However, they do not contain any secondary follicles with germinal centres signifying B cell activation, until some weeks after birth. This reflects their dependency on exogenous stimuli (Brandtzaeg *et al.*, 1991; Brandtzaeg, 2003b). The lamina propria contains very few immunoglobulin-containing cells (Russell *et al.*, 1990), which are mainly IgM⁺ and almost never IgA⁺ (Iwase *et al.*, 1987; Russell *et al.*, 1990). Fetal GIT contents or neonatal secretions contain no or only low levels of sIgA, but relatively more IgM (Petit *et al.*, 1973; Gleeson *et al.*, 1982; Mellander *et al.*, 1984). This is due to the fact that IgM, in the absence of dimeric IgA, can bind to SC and be transported out into mucosal secretions (Hanson *et al.*, 1999).

In the piglet, although considerable development of the systemic immune system has already taken place, the cells and structure of the mucosal immune system are almost completely absent, or immature, at birth (Gaskins, 1998). Four major phases of development of the mucosal immune system have been identified as shown in Table 2.

Effects of weaning on mucosal immunity

Weaning is associated with alterations in intestinal morphology, such as villous atrophy and crypt hyperplasia in the small intestine (Kelly *et al.*, 1992), together with a

Period	Components of the mucosal immune system
Birth	Low numbers of macrophage and granulocytes in villous and crypt regions (not yet functionally mature?) PP consist of primordial follicles surrounded by few T cells Low numbers of CD4 ⁺ and CD8 ⁺ T cells Low numbers of intestinal MHC class II ⁺ cells Ig-containing cells are rare or absent
First two weeks	Intestine becomes colonized with lymphoid cells (express the CD2 surface marker but not CD4 or CD8) PP begin to organize (a relatively 'adult architecture' is reached by 10–15 days)
Two to four weeks	Mucosa becomes colonized by CD4 ⁺ cells (mainly in lamina propria) CD8 ⁺ cells are still largely absent Small numbers of B cells appear, mainly expressing IgM
Five weeks onwards	CD8 ⁺ cells appear in intestinal epithelium IgA ⁺ B cells appear (IgA becomes the predominant isotype) Intestinal architecture is comparable to that of the mature animal by 7 weeks

CD: cluster of differentiation; Ig: immunoglobulin; MHC: major histocompatibility complex; PP: Peyer's patches. Compiled after Allen and Porter (1973), Butler *et al.* (1981), Bianchi *et al.* (1992), Stokes *et al.* (1992), Vega-López *et al.* (1995), Pabst and Rothkötter (1999) and Vega-López *et al.* (2001).

reduction in brush-border enzyme activities, resulting in an impaired absorptive capacity of the small intestine (Hampson and Kidder, 1986; Hampson and Smith, 1986).

In addition, activation of the GIT immune system during weaning has been reported. For example, the decreasing ratio of villous height to crypt depth after weaning is associated with an increase in plasma concentrations of the pro-inflammatory cytokine IL-1 (McCracken *et al.*, 1995). Cytokines are produced by immune cells, but also by intestinal epithelial cells, thereby signalling the onset of the host innate and adaptive immune responses. Uncontrolled synthesis of pro-inflammatory cytokines can have a strong influence on gut integrity and epithelial functions, including permeability to macromolecules and transport of nutrients and ions (McKay and Baird, 1999). Pié *et al.* (2004) observed an early response in gene expression of inflammatory cytokines in the gut associated with weaning, which might contribute to anatomical and functional disorders in the GIT.

Solano-Aguilar *et al.* (2001) surveyed the phenotypes of lymphocytes isolated from blood, lymph nodes, and lymphoid associated structures of the pig small intestine. Weaning had a significant effect on the numbers of CD4⁺, CD8⁺ or CD4⁺/CD8⁺ T cells. Age of the pig before and after weaning resulted in significant changes in lymphocytes isolated from mesenteric lymph nodes and ileal sites. That weaning time may exert an impact on the relative percentages of differentiated effector cells is in accordance with other authors (Hampson, 1986; McCracken *et al.*, 1999). The results of Solano-Aguilar *et al.* (2001) emphasized the importance of weaning time on immune system development and thus their potential as key management factors in swine production systems.

Manzano *et al.* (2002) observed a rapid development of intraepithelial lymphocytes (IEL), as well as lamina propria and PP lymphocyte populations during the weaning period in mice. In PP lymphocytes the main change was a higher percentage of B cells, whereas the most notable change in the IEL and lamina propria lymphocytes was a higher percentage of T cells, especially in IEL. Changes in the specific phenotypes of the intestinal lymphocyte population at weaning appear to be related to the maturation of the intestinal immune system.

Role of intestinal microbiota in shaping immunity

In pigs, development of the mucosal immune system takes place over a period of several weeks. However, this development also seems to be largely dependent on microbial exposure (Bianchi *et al.*, 1992; Vega-López *et al.*, 1995; Pabst and Rothkötter, 1999). This has become obvious by studies investigating germ-free animals, which have an undeveloped mucosal immune system. The secondary lymphoid organs, i.e., spleen, lymph nodes and PP, are poorly developed in germ-free rodents in comparison to conventional rodents with an indigenous GIT microbiota (Bealmeier, 1981; Berg, 1983; Berg, 1996). For example, PP contain few germinal centres, as well as greatly reduced numbers of IgA-producing plasma cells and lamina propria CD4⁺ T cells (Macpherson *et al.*, 2001; 2003). In germ-free piglets aged 39 and 59 days, ileal and jejunal PP were significantly shorter than in age-matched control animals (Pabst *et al.*, 1988). At six weeks of age, the vast majority of lymphocytes present in PP of conventional pigs are B cells, whereas in germ-free pigs, T cells predominate (Rothkötter and Pabst, 1989; Pabst and Rothkötter, 1999). In a study of Rothkötter *et al.* (1994), with germ-free piglets of 45 days of age, the cell yield and subset patterns were comparable with those in five-day old normal animals. The number of intraepithelial T lymphocytes also shows an increase that is significantly related with age and exposure to microbial antigen (Rothkötter *et al.*, 1999; Vega-López *et al.*, 2001).

The colonization of germ-free reared animals with known microorganisms provide valuable models for investigating the effects of microbial exposure on the immune system of the host. Such experimental colonization might seem artificial, but similar colonization occurs in every neonate within days of its birth (Mackie *et al.*, 1999; Macpherson and Harris, 2004). Studies using such 'gnotobiotic' ('known life') animals confirm the importance of microbial exposure for the proper development of mucosal immunity and of the intestinal epithelia. Table 3 summarizes some studies investigating the effects on mucosal immunity of colonizing germ-free mice. For some of these colonization studies, segmented filamentous bacteria (SFB) have been used, which appear in high numbers shortly after weaning in a wide range of species e.g. mice (Davis and Savage, 1974), and pigs

Table 3. Studies using gnotobiotic animals for investigation of microbial influence on immunological parameters.		
Bacterial colonization	Effects	Reference
Association of GF mice with fecal microbiota of conventionally reared mice	- Increase in $\alpha\beta$ IELs, level of conventional mice reached one month after microbial colonization - $\gamma\delta$ IELs: no change during colonization process	Umesaki <i>et al.</i> , 1993
Mono-association of GF mice with SFB	- Increase in number of $\alpha\beta$ IELs - Increased cytolytic activity of IEL - Induction of MHC II class molecules	Umesaki <i>et al.</i> , 1995
Mono-association of GF mice with <i>Morganella morganii</i>	- Hypertrophy of PP including germinal centre reactions - Specific IgA responses	Shroff <i>et al.</i> , 1995
Mono-association of GF mice with SFB	- Rise in germinal centre reactions of PP - Increase of levels of natural IgA in PP (up to 63% of that of conventional mice) - SFB-specific IgA: less than 1.4% of total IgA - Increase of proportion of activated CD4 ⁺ in PP to level of conventional mice	Talham <i>et al.</i> , 1999
Association of mice: -with SFB -with clostridia -with SFB and clostridia	- Small intestine: increase in number of $\alpha\beta$ IEL - Ileum, colon: increase in number of IgA-producing cells in lamina propria as compared to <i>Clostridium</i> -associated mice - Small intestine: no increase in $\alpha\beta$ IEL - Numbers and phenotypes of IEL in the small and large intestine became similar to those in conventionalised mice	Umesaki <i>et al.</i> , 1999
CD: cluster of differentiation; GF: germ-free; IEL: intraepithelial lymphocytes: the T cell receptor that recognizes antigen consists of α and β chains (most common form), or γ and δ chains (less common); IgA: immunoglobulin A; MHC: major histocompatibility complex; PP: Peyer's patches; SFB: segmented filamentous bacteria.		

(Sanford, 1991), and which disappear weeks later (Snel *et al.*, 1998). They show a preference for attachment to the epithelium covering the lymphoid tissue of the PP (Klaasen *et al.*, 1992; Meyerholz *et al.*, 2002), and are thought to play a significant role in the stimulation of the mucosal immune system (Klaasen *et al.*, 1993; Talham *et al.*, 1999; Suzuki *et al.*, 2004).

Recent work has shown that the association of germ-free rodents with commensal bacteria (*Bacteroides thetaiotaomicron*) also results in major changes of gene expression of intestinal epithelial cells. Genes involved in lipid absorption, intestinal barrier function, xenobiotic metabolism, gut motility, postnatal intestinal maturation, and angiogenesis were found to have been affected by *B. thetaiotaomicron* (Hooper *et al.*, 1999; 2000; Hooper and Gordon, 2001).

Angionin-4 (Ang4) is a Paneth cell protein, the expression of which is developmentally regulated during early postnatal life. It is induced during weaning, and exhibits potent bactericidal activity that establishes its role in epithelial host defence. In conventional mice, Ang4 expression increases dramatically during weaning, while germ-free animals never reach high Ang4 expression levels. This suggests the relevance of intestinal microbiota for induction of Ang4 expression during weaning. Also, colonization of adult germ-free mice with intestinal microbiota, or with *B. thetaiotaomicron* alone, stimulated conventional adult Ang4 expression levels (Hooper *et al.*, 2003). This reveals a mechanism by which intestinal commensal bacteria may shape innate immunity, and influence the composition of their own evolving microbial community during postnatal development (Hooper, 2004).

Oral tolerance

The ability of the mucosal immune system to distinguish between harmful and harmless antigens is essential to

mount a protective immune response and to prevent the induction of mucosal pathology (Garside and Mowat, 2001). It has been shown that oral administration of soluble antigens induces systemic non-responsiveness to peripheral antigen challenge. The state of antigen-specific systemic unresponsiveness with the maintenance of mucosal sIgA responses has been termed oral tolerance. Oral tolerance presumably evolved to prevent hypersensitivity reactions to food proteins and bacterial antigens in the mucosal microbiota (Weiner, 2001). Although the mechanism of oral or mucosal tolerance needs to be clarified, it is now accepted that it may involve either deletion and anergy (i.e. the state of unresponsiveness of T lymphocytes to antigenic stimulation), or induction of regulatory CD4⁺ T cells that produce anti-inflammatory cytokines such as IL-10 and/or TGF- β (Mowat *et al.*, 2004).

Bacterial colonization acquired during the early postnatal period contributes to the induction and maintenance of immunological tolerance not only to themselves but also to other luminal (e.g. nutritional) antigens (Moreau and Corthier, 1988; Sudo *et al.*, 1997). For example, oral tolerance induction to ovalbumin was incomplete in germ-free mice, but could be restored after introduction of a single component of the intestinal microbiota (*Bifidobacterium infantis*) (Sudo *et al.*, 1997). However, introduction of *B. infantis* was only efficient when inoculated into neonate mice, but not at an older age. These findings suggested that exposure to intestinal microbiota at the neonatal stage is important for being fully susceptible to the induction of oral tolerance.

Furthermore, oral tolerance to bovine β -lactoglobulin was better induced and maintained in mice colonized with a complete and diversified microbiota (conventional mice), as compared to mono-associated or germ-free mice (Prioult *et al.*, 2003). Also, mono-association was strain-dependent, i.e. colonization with *Lactobacillus*

paracasei led to better suppression of immune response than mono-association with *Bifidobacterium lactis* or *L. johnsonii*.

Probiotics

In recent years, there has been interest in the use of living microorganisms (probiotics) as therapeutic agents. Bacteria so employed are usually members of the human intestinal microbiota, including *Lactobacillus* and *Bifidobacterium* (Alvarez-Olmos and Oberhelman, 2001). Within animal nutrition, they mostly belong to lactic acid bacteria naturally occurring in the GIT, or to the genus *Bacillus* with soil as its natural habitat (Simon *et al.*, 2003). Of the possible mechanisms by which probiotics could beneficially influence intestinal health, one is the promotion of a non-immunologic defence barrier of the GIT, which is characterized by stabilization of the endogenous GIT microbiota (Salminen *et al.*, 1998). However, another possible mechanism of probiotic therapy is improvement of the intestine's immunologic barrier. For example, beneficial effects of probiotics may be related to the production of antimicrobial substances, or to an enhanced tight junction of the intestinal barriers to prevent intercellular bacterial invasion (Bourlioux *et al.*, 2003; Forchielli and Walker, 2005). Several *Lactobacillus* strains have been reported to display stimulatory properties on cells of the innate immune system, thereby aiding in immune elimination. For example, increased macrophage phagocytic activity after oral administration of lactobacilli has been reported in mice (Perdigon *et al.*, 1986), and in humans (Schiffirin *et al.*, 1995). Also, increased IgA responses have been associated with the use of probiotic bacteria. For example, several lactic acid bacteria have been found to increase the number of IgA producing cells in the lamina propria (Vitini *et al.*, 2000). Gnotobiotic rats colonized with *E. coli* and *L. plantarum* were shown to have increased serum IgA concentration in comparison to rats colonized with *E. coli* alone (Herias *et al.*, 1999).

Most studies investigating the effects of probiotics on the immune system have been performed with rodents. However, there are also some studies reported for livestock and companion animals. For example, probiotic treatment using *Bifidobacterium lactis* reduced weaning diarrhoea associated with rotavirus and *E. coli* infection in a piglet model (Shu *et al.*, 2001). In this study, the protective effect of probiotic treatment was associated with higher blood leukocyte phagocytic and T lymphocyte proliferative responses, and higher intestinal pathogen-specific antibody titres. A protective effect was also shown following dietary supplementation with *L. sobrius* to weaning piglets experimentally infected with enterotoxigenic *E. coli* K88 (ETEC) (Konstantinov, 2005). In this study, the presence of *L. sobrius* was accompanied by a significant reduction of ETEC prevalence in the porcine ileum, as well as an increase in body weight gain, when compared to piglets receiving a control diet. Furthermore, the piglets administered *L. sobrius* showed significantly higher saliva sIgA levels one week after the ETEC challenge, indicating a positive effect on the piglets' immunity. However, administration of a probiotic *Enterococcus faecium* strain to pregnant sows and

their piglets showed no immuno-stimulatory effect, as measured by CD4⁺ and CD8⁺ T cell populations in the PP (Scharek *et al.*, 2005).

Koenen *et al.* (2004a) investigated the effects of different probiotic *Lactobacillus* strains in meat-type and layer-type chicken. They found differences in serum IgG levels due to the different strains used, and due to administration dose. For broiler chickens, a stimulating effect of lactobacilli on humoral and cellular responses was found up to three weeks of age, and for high and continuously administered doses. For layers, a lower dose was effective, as well as temporary administration (5 days) of the probiotic. This suggests that host factors such as age may also have an influence on probiotic effects.

In a study of Benyacoub *et al.* (2003), young dogs were administered with the probiotic *Enterococcus faecium* from weaning to one year of age. Fecal IgA tended to be higher for the probiotic group, and plasma IgA was significantly higher for the probiotic group. Also, the probiotic group showed higher levels of vaccine-specific (canine-distemper virus) IgG and IgA. There were no differences in the percentages of CD4⁺ and CD8⁺ T cells between the groups, but the proportion of mature B cells was higher in the probiotic group.

Furthermore, many probiotic effects are mediated through regulation of cytokine production, i.e. by control of the balance of pro-inflammatory and anti-inflammatory cytokines, which may lead to alleviation of intestinal inflammatory responses (Isolauri *et al.*, 2001). A special focus has been placed on the skewing of the Th1/Th2-balance of the immune system. Th1 cells are a functional subset of helper T cells that secretes a particular set of cytokines, such as IL-2 and IFN- γ . They are involved in inducing a cell-mediated immune response aimed at protection against intracellular pathogens. Th2 cells are believed to emphasize protection against extra-cellular pathogens such as multi-cellular parasites. They secrete cytokines such as IL-4, IL-5, IL-10 and IL-13, and induce strong antibody-mediated immune responses. Their principal functions are to stimulate IgE production, and downregulate Th1 responses. On the negative side, the Th1 pathway is often portrayed as being the more aggressive of the two, and apparently, when it is over-reactive, can generate organ-specific autoimmune disease. The Th2 pathway is seen as underlying allergy and related IgE-based disease, and predisposing to systemic autoimmune disease (Singh *et al.*, 1999; Kidd, 2003).

However, differences have been reported with regard to the immuno-modulatory effects of different probiotic bacteria (Table 4). For example, different lactic acid bacteria may induce distinct mucosal cytokine profiles suggesting their distinct effects on the Th1/Th2 balance (Perdigon *et al.*, 2002). This emphasizes the importance of strain selection for immuno-therapeutic purposes. Adult-type bifidobacteria (*B. adolescentis* and *B. longum*) have been reported to induce more pro-inflammatory cytokines (IL-12 and TNF- α) *in vitro* in a murine macrophage-like cell line, than did infant-type bifidobacteria such as *B. bifidum*, *B. breve* or *B. infantis*. Furthermore, *B. adolescentis* did not stimulate the production of anti-inflammatory IL-10 as did the other bacteria tested in this

Table 4. Selected studies investigating effects of probiotics on cytokine profile.			
Probiotic strain	Species Cells/organ	Immuno-modulatory effect	Reference
<i>In vitro</i>			
<i>Streptococcus thermophilus</i> , <i>Lactobacillus bulgaricus</i> , <i>Bifidobacterium</i> sp.	Mice macrophages	- Increase in pro-inflammatory IL-6 and TNF- α production	Marin <i>et al.</i> , 1998
<i>Lactobacillus</i> strains	Human peripheral blood mono- nuclear cells	- Enhanced production of pro-inflammatory IL-6 and TNF- α	Miettinen <i>et al.</i> , 1996
<i>L. rhamnosus</i> E509 <i>L. rhamnosus</i> GG E522 <i>L. bulgaricus</i> E585	Human peripheral blood mono- nuclear cells	- Induction of pro-inflammatory IL-1 β , IL-6 and TNF- α mRNA expression and protein production; - Induction of IL-18 and IL-12 (only <i>L. rhamnosus</i> strains) and IFN- γ production	Miettinen <i>et al.</i> , 1998
<i>In vivo</i>			
<i>L. reuteri</i> <i>L. brevis</i> Oral administration	Mice small intestine	- Induction of pro-inflammatory TNF- α , IL-2, and IL-1 β (only <i>L. reuteri</i>) expression; - No effect on anti-inflammatory cytokines IL-10 and IL-4;	Maassen <i>et al.</i> , 2000
<i>L. casei</i> <i>L. plantarum</i> Oral administration		- Induction of pro-inflammatory TNF- α	
<i>L. delbrueckii</i> or <i>L. casei</i> Oral administration	Mice small intestine	- Increase in IL-10 and IL-4;	Perdigon <i>et al.</i> , 2002
<i>L. acidophilus</i> Oral administration		- Induction of IL-2 and IL-12	
IFN: interferon; IgA: immunoglobulin A; IL: interleukin; PP: Peyer's patches; TNF: tumor necrosis factor.			

study. This suggests that adult-type bifidobacteria may be less able to down-regulate inflammatory responses (He *et al.*, 2002). Differences in the effects of orally administered *Lactobacillus* strains seem also to be related to the growth phase, i.e. the stationary or log phase, of the bacteria used (Maassen *et al.*, 2003).

Probiotic bacteria are interesting candidates in the treatment of inflammatory diseases such as allergy (Isolauri *et al.*, 2001). It has been shown that specific species of lactobacilli, e.g. *L. reuteri* and *L. casei*, induced the development of regulatory T cells by modulating DCs *in vitro* (Smits *et al.*, 2005). These regulatory T cells then produced increased levels of anti-inflammatory IL-10. Regulatory T cells are known to be involved in promotion of mucosal tolerance by inhibiting the proliferation and cytokine production of other immune cells (Mason and Powrie, 1998). Therefore, the beneficial effects of some probiotic bacteria, might, at least in part, be explained by the priming of DC for regulatory T cell development (Smits *et al.*, 2005).

Recently, a novel way has been described by which lactic acid bacteria may affect the production of inflammation-related cytokines and protect the host from intestinal disorders (Grangette *et al.*, 2005). The authors showed that the composition of lipoteichoic acid (LTA), specifically the D-alanine content modulates the immune response. In this study, a mutant of *L. plantarum* incorporated much less D-alanine in its LTA than the wild-type strain. Lipoteichoic acid is one of the main immunostimulatory components of the pathogenic Gram-positive bacterial cell wall. It is recognized by pattern recognition factors such as TLR, which signal the presence of specific microorganisms to the host (Barton and Medzhitov, 2002). In the study of Grangette *et al.* (2005), the mutant induced reduced secretion of pro-inflammatory

cytokines by peripheral blood mononuclear cells and monocytes *in vitro*, as compared with the wild-type strain. Concomitantly, an increase in anti-inflammatory IL-10 production was stimulated by the mutant in comparison with the wild-type strain. In a murine colitis model, the mutant was also more protective than the wild-type strain, with less severe lesions and reduced weight loss. This indicates that the LTA composition of lactic acid bacteria can modulate the inflammatory response and change it to an anti-inflammatory equilibrium. Thus, the use of mutant lactic acid bacteria might offer a treatment of intestinal disorders (De Vos, 2005; Grangette *et al.*, 2005).

However, results regarding the effects of probiotics are contradictory, as indicated by several studies. For example, Spanhaak *et al.* (1998) failed to show any effects of probiotic *L. casei* on the immune system of healthy volunteers with regard to NK cell activity, phagocytosis, or cytokine production in a placebo-controlled trial. Similarly, no difference was found in relative percentages in macrophages and NK cells from intestine in pigs after oral administration of *L. rhamnosus* GG (Solano-Aguilar *et al.*, 2003). Finally, no effects could be detected on the phagocytotic activity of immune cells from blood and spleen, nor on sIgA production in the ileum and cecum in the rat after oral administration of *L. rhamnosus* GG/*Bifidobacterium lactis* Bb12 (Roller *et al.*, 2004).

Conclusions

The GIT of mammals is colonized by a complex and dynamic microbiota. Molecular methods assist to gain deeper insights into its structural and functional complexity. This indigenous intestinal microbial community plays an important role in terms of nutrient processing, as well as in supporting the host against colonization with potential pathogens. Moreover, recent evidence has demonstrated

that commensal bacteria also regulate intestinal development and function (Hooper *et al.*, 2002). By use of gnotobiotic and germ-free animals, it has been shown that this complex microbial community exerts major influences on the maturation of the gut, e.g., in terms of regulating angiogenesis or production of microbicidal proteins. Furthermore, the indigenous microbiota influences the development of the GALT, mucosal immunity and induction of oral tolerance.

Understanding how the gut mucosal immune system generally responds to intestinal microbiota may be an important basis for targeting manipulation of the microbial composition. This might be of special interest at stressful times, such as weaning, when young animals have to cope with major changes in their diet and environment, and simultaneously face a change in the composition of their GIT microbiota. Weaning marks the time at which protective immunity shifts from passive maternal immunity to the active, adaptive immunity of the piglet. For example, in naturally suckled piglets, the lowest serum IgG concentrations are found at four weeks of age, after which, IgG levels rise as a result of *de novo* synthesis of Ig (Klobasa *et al.*, 1986). Also, phenotypic studies suggest that the mucosal immune system remains relatively immature throughout the 'normal' weaning period (Stokes *et al.*, 2004). The changes occurring in the immune system around weaning (e.g. as shown by altered percentages of T cell subsets, or cytokine production) emphasize the importance of weaning time in immune system development and thus potentially as key management factors in pig production systems. Weaning at an older age, and thus at a more advanced level of developmental maturity, might reduce weaning-induced inflammatory responses.

The strong influence of the indigenous microbiota on the GIT immune system might offer a possibility to beneficially steer the development of the GIT immune system, by manipulating microbial composition. This could be reached by the use of probiotics. Apart from their alleged properties to positively influence both development and stability of the GIT microbiota, these agents also may stimulate specific and non-specific components of the immune system. However, results are sometimes contradictory, and differences also exist due to specific bacterial strains. The use and further development of *in vitro* assays for careful pre-selection of possible probiotic strains would be useful. Recently, an *in vitro* assay for probiotic bacteria has been developed and validated by Koenen *et al.* (2004b). This *in vitro* assay is suitable for pre-selection of lactic acid bacteria in poultry, but it might also be useful for other species. However, it has also been shown that, despite similar *in vitro* properties, distinct probiotic strains may colonize the gut differently and generate divergent immune responses (Ibnou-Zekri *et al.*, 2003).

It is also possible that effects on the immune system might not only be mediated by viable, but also by non-viable probiotic bacteria or bacterial DNA. This has been shown in a study of Rachmilewitz *et al.* (2004), who administered bacterial DNA (probiotic or *E. coli*) intra-gastrically to mice prior to induction of experimental colitis. Both DNA (probiotic and *E. coli*) ameliorated the severity

of experimental colitis, as did viable or irradiated non-viable probiotics. This is interesting for safety reasons, as irradiated probiotics could also be used in immunocompromised hosts without the risk of bacteraemia.

It has been suggested that some bacteria species, e.g. *B. thetaiotaomicron*, downregulate the overall pro-inflammatory effect of enterobacteria (Kelly *et al.*, 2004). Since neonatal gut colonization with such symbiotic 'anti-inflammatory' microorganisms depends on the environment and nutrients, it can be tightly controlled by the early diet. Supporting such colonization of the GIT by use of specific dietary components renders a further possibility to beneficially influence the development of the intestinal immune system. Also, dietary modulation of the GIT microbiota can result in an enhancement of colonization resistance against potential pathogens. For example Konstantinov *et al.* (2004a), by use of fermentable carbohydrates in weaning piglet diets, showed a stimulation of lactobacilli, with a concomitant suppression of *Clostridium*-like species. Such stimulation of the beneficial intestinal bacteria may also result from supplemental prebiotics. These are low molecular oligosaccharides, also referred to as non-digestible oligosaccharides (NDO) which are believed to enhance the beneficial activity of specific members of the microbiota, such as lactobacilli or bifidobacteria (Gibson and Roberfroid, 1995). They may be used alone, or alternatively, in combination with probiotics (synbiotic approach).

Until now, there have been few studies investigating the effects of microbial colonization on the immune system in livestock or companion animals. However, valuable information can be drawn from human studies and by the use of rodent models, particularly the gnotobiotic/germ-free model. These might contribute to the development of hypotheses to be validated in livestock and companion animals.

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