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Interactions between commensal bacteria and the gut-associated immune system of the chicken

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Abstract

The chicken gut-associated lymphoid tissue is made up of a number of tissues and cells that are responsible for generating mucosal immune responses and maintaining intestinal homeostasis. The normal chicken microbiota also contributes to this via the ability to activate both innate defense mechanisms and adaptive immune responses. If left uncontrolled, immune activation in response to the normal microbiota would pose a risk of excessive inflammation and intestinal damage. Therefore, it is important that immune responses to the normal microbiota be under strict regulatory control. Through studies of mammals, it has been established that the mucosal immune system has specialized regulatory and anti-inflammatory mechanisms for eliminating or tolerating the normal microbiota. The mechanisms that exist in the chicken to control host responses to the normal microbiota, although assumed to be similar to that of mammals, have not yet been fully described. This review summarizes what is currently known about the host response to the intestinal microbiota, particularly in the chicken.

Introduction

In the chicken, colonization of the gastrointestinal (GI) tract commences immediately after hatch with the composition of the microbiota changing over time. The succession of intestinal colonization by various bacteria may be influenced by several factors including age, diet and the use of antibiotics and/or probiotics until the normal microbiota is established. Given the close association of the microbiota with the various cells and tissues of the GI tract, it is important that the host response to commensal microbes of the intestine be strictly controlled in order to avoid unnecessary inflammation. This dynamic interaction is highly complex and has evolved in such a way that the immune system benefits from the presence of the microbiota. This is demonstrated by the fact that germ-free animals have a higher susceptibility to intestinal infections (O'Hara and Shanahan, 2006). Additionally, Rhee *et al.* (2004) demonstrated that the gut-associated lymphoid tissue (GALT) is underdeveloped in germ-free rabbits and that reestablishing the microbiota quickly restores the antibody-mediated immune response. The cell-mediated immune response is also affected by the microflora as CD4+ and CD8+ cells of germ-free animals have a naïve phenotype, but following intestinal colonization, these cells acquire a more typical activated phenotype (Cebra, 1999). It has also been shown that the microflora has some influence on T cell repertoires in mammals, and that the microflora can have an effect on cytokine profiles (O'Hara and Shanahan, 2006). Therefore, the composition of the microbiota is very important for the health, growth and maturation of the host and any changes to the microbiota can have profound effects on the immune system, from the generation of the primary antibody repertoire to the modulation of T-helper (Th)-cell type 1 or type 2 cytokine profiles (O'Hara and Shanahan, 2006). Studies in mammals have identified a number of host mechanisms that are used in order to maintain intestinal homeostasis, including cytokine polarization in the intestine. Intestinal homeostasis in the chicken, although assumed to be similar to that of mammals, has not yet been fully described. For example, the ability of chickens to mount a typical Th1 or Th2 response was only recently described (Degen *et al.*, 2005) and the role of this

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polarized response in the chicken in maintaining intestinal homeostasis in currently unknown. Therefore, the focus of this review is to provide an overview of the chicken GALT and the interactions between cells and molecules of the GALT and commensal microbes present in the chicken intestinal microbiota.

The chicken GALT

The GALT, a component of the mucosa-associated lymphoid tissue (MALT), consists of organized tissues with single and/or multiple lymphoid follicles, as well as freely dispersed lamina propria lymphocytes (Liebler-Tenorio and Pabst, 2006). The organized lymphoid tissues are made up of cecal tonsils (CT), Peyer's patches (PP), the bursa of Fabricius, Meckel's diverticulum and various lymphoid aggregates located at several locations along the digestive tract (Befus *et al.*, 1980).

CT, large lymphoid aggregates that reside at the caecorectal junction, consist of a central crypt, diffuse lymphoid tissues and germinal centers (Del Moral *et al.*, 1998). Given their similar cellular and morphological features to typical mammalian PP, a role in antigen sampling similar to that of mammalian PP has been proposed (Befus *et al.*, 1980; Yasuda *et al.*, 2002). Although, not as numerous and prominent as in mammals, chickens also possess more typical PP located along the small intestine (Befus *et al.*, 1980; Vaughn *et al.*, 2006).

The bursa of Fabricius and Meckel's diverticulum are unique to avian species. The bursa of Fabricius is located dorsal to the cloaca and is the site of primary B cell development in the chicken (Reynaud *et al.*, 1991; Lillehoj and Trout, 1996; Yasuda *et al.*, 2002). Along with its role as a primary lymphoid organ, the bursa is also thought to function as a secondary lymphoid organ as the mucosal and submucosal regions of the bursal canal contain multiple lymphoid follicles (Muir *et al.*, 2000). Furthermore, it has been demonstrated that antigens derived from the intestine can gain access to the bursa of Fabricius and may be involved in B cell development (Ratcliffe, 2006). Meckel's diverticulum, a remnant of the yolk sac, is present on the small intestine where it generally persists for the life of the bird. Although its exact function is unclear, the observed presence of germinal centers has lead to the suggestion that it also functions as an inductive lymphoid organ (Lillehoj and Trout, 1996; Muir *et al.*, 2000).

Lymphoid aggregates, which are generally scattered along the epithelium and the lamina propria throughout the chicken GALT, are thought to be similar to the more organized lymphoid tissues of the chicken as they possess a morphologically distinct epithelial layer known as follicle-associated epithelium (FAE). The FAE contains M cells, which are specialized in sampling antigens and transporting them from the lumen to follicular environments.

Cells of the chicken GALT

The chicken intestinal epithelium, similar to that of mammals, is comprised of enterocytes, goblet cells, Paneth cells and M cells along with intra-epithelial lymphocytes (IEL) that are dispersed throughout the intestinal epithelium. IEL comprise a diverse population of lymphocytes that includes natural killer (NK) cells, T cells and B cells (Göbel *et al.*, 2001). The lamina propria, a thin vascular layer just beneath the epithelium, contains a mixture of all immune system cell types including plasma cells, effector and memory lymphocytes, macrophages and granulocytes. There are only a few αβ T cells in the lamina propria as γδ T cells predominate. The major population of lamina propria γδ T cells is CD8+ although the exact proportion of cell types depends on the age of the animal (Lillehoj and Chung, 1992; Liebler-Tenorio and Pabst, 2006).

Throughout the chicken GALT, multiple lymphoid follicles exist. The lymphoid follicles are made up of B cells embedded in a network of follicular dendritic cells (DC), with small numbers of CD4+ T cells and macrophages. The T cell-rich interfollicular areas consist predominately of CD4+ and CD8+ T cells (Lillehoj and Trout, 1996; Yasuda *et al.*, 2002; Liebler-Tenorio and Pabst, 2006). Within the more organized lymphoid structures, such as the CT and PP, αβ CD4+ T cells and B cells are present (Lillehoj and Trout, 1996; Liebler-Tenorio and Pabst, 2006), whereas in the more dispersed areas, such as the epithelium and lamina propria, γδ T cells predominate (Lillehoj and Chung, 1992). Within the bursa of Fabricius, there are much fewer CD4+ and CD8+ T cells as the bursa is the primary source of IgM+ B cells (Liebler-Tenorio and Pabst, 2006).

The chicken microbiota

The chicken GI tract is highly adapted to the presence of commensal bacteria and has a bacterial population in the small intestine within 24 h of hatching (Shapiro and Sarles, 1949). Generally, the main genera of bacteria within the chicken small intestine are *Lactobacillus*, *Enterococcus* and *Clostridium*, with some bacteria from the family *Enterobacteriaceae* (Salanitro *et al.*, 1978; Amit-Romach *et al.*, 2004; Bjerrum *et al.*, 2006; Gong *et al.*, 2007). The ceca contain a more diverse community of bacteria, including genera of *Bacteroides*, *Bifidobacterium*, *Clostridium, Enterococcus*, *Escherichia*, *Fusobacterium*, *Lactobacillus*, *Streptococcus* and *Campylobacter* (Salanitro *et al.*, 1978; Gong *et al.*, 2002; Amit-Romach *et al.*, 2004; Bjerrum *et al.*, 2006; Gong *et al.*, 2007). The microbiota that exists throughout the chicken GI tract increases in density and diversity in the more distal regions of the intestine, although a recent study by Gong *et al.* (2007) reported an unexpectedly diverse community

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of bacteria present within the duodenum. The proximal small intestine contains approximately 10^3–10^5 bacterial cells per gram of digesta, the distal small intestine harbors 10^8–10^9 bacterial cells per gram of digesta, while the density of bacterial cells in the ceca can reach 10^{12} per gram of digesta (Gong *et al*., 2002). The establishment of the stable microbiota is a complex process that is influenced by a number of factors including the animals' age, diet, and the use of antibiotics and probiotics (Patterson and Burkholder 2003; Xu *et al*., 2003; Gong *et al*., 2008).

The effect of antibiotics on the chicken microbiota is important given the fact that in-feed antibiotic growth promotants are commonly used in poultry production. The benefits of antibiotics, such as the increased animal growth and efficiency of feed conversion, are thought to be due to the induced changes in the intestinal microbiota (Singer and Hofacre, 2006). In general, antibiotics reduce the microbial load in the intestinal tract leading to more nutrient availability for the host. The total number of bacterial genotypes is not always altered by antibiotic treatment; rather antibiotics have been shown to alter the types of bacterial genotypes that are present in the intestinal bacterial community (Knarreborg *et al*., 2002; Dumonceaux *et al*., 2006; Pedrosa *et al*., 2006; Wise and Siragusa 2007; Gong *et al*., 2008). For example, studies performed in our laboratory and by others have demonstrated that chickens fed a diet containing antibiotics had lower numbers of *Lactobacillus salivarius* while the overall numbers of *Lactobacillus* sp. remained unchanged (Engberg *et al*., 2000; Knarreborg *et al*., 2002; Zhou *et al*., 2007). Therefore, it seems possible that antibiotics may not only inhibit bacterial growth, but may also alter the microbiota by selecting for bacteria that are able to confer a health benefit to the host, inhibit bacteria that are pathogenic, or have a negative correlation with the health and well-being of the animal.

The term commensal bacteria has come to refer to the host's normal or indigenous microbiota, while probiotics are currently defined as microbial dietary supplements that contain live beneficial bacteria that confer a health benefit on the host. *Bifidobacterium*, *Lactobacillus* and *Lactococcus*, commonly referred to as lactic acid bacteria (LAB), are the most common microbes used as probiotics. The most effective probiotics are those that contain bacteria that are native to the host (Dogi and Perdigon, 2006), and therefore, can be considered a type of commensal bacteria. Administration of probiotics has long been known to have an effect on the microbiota and in the poultry industry, probiotics have been used to alter the microbiota in order to improve weight gain and feed utilization and to decrease mortality through the ability to decrease the capacity of enteric pathogens to attach and colonize the chicken intestine (Nahashon *et al*., 1994; England *et al*., 1996; Mohan *et al*., 1996; Jin *et al*., 1998; Schneitz *et al*., 1998; Pascual *et al*., 1999; Zulkifli *et al*., 2000; Angel *et al*., 2005; Timmerman *et al*., 2006; Medellin-Peña *et al*., 2007). Probiotics are thought to alter the intestinal microbiota through the inhibition of growth of pathogenic micro-organisms due to the release of antimicrobial substances (Vandenbergh, 1993; Mack *et al*., 1999; Mack *et al*., 2003; Smirnov *et al*., 2005), reduced pH caused by the production of lactic acid, modulation of the host immune system by enhancing cytokine production (Niers *et al*., 2005), interference with transcription of pathogen genes involved in colonization (Jessica *et al*., 2007), and competition with pathogens for available nutrients and growth factors (Rolfe, 2000).

Host-commensal interactions

The relationship between the intestinal microbiota and the host must be tightly regulated, since host responses to members of the mucosal microbiota may pose the risk of inflammatory responses in mucosal tissues. Although, the mechanisms that maintain intestinal homeostasis are just now becoming clear, evidence particularly from studies of rodents and humans has enabled the unraveling of the finely tuned balance that exists between the host and its microbiota (for recent reviews, see Blaser and Kirschner, 2007 and Pamer, 2007).

One of the major benefits to the host from commensal bacteria is largely imparted by the ability of normal microbiota bacteria to competitively exclude pathogens from colonizing the intestine. This is achieved by forming biofilms and by binding to the intestinal epithelium effectively blocking the sites from pathogens (Baranov and Hammarström, 2004; Granato *et al*., 2004). It has been clearly demonstrated that commensal bacteria also have the ability to directly affect the innate and adaptive immune systems and importantly, the resident microbiota are recognized to suppress unnecessary inflammatory responses, thereby helping to maintain immune homeostasis (Moal and Servin, 2006).

Innate defenses

The innate defense system is made up of many germline-encoded molecules, which have the capacity of limiting both commensal and pathogenic bacteria. Intestinal epithelial cells (IEC) provide a physical barrier against invasion by various micro-organisms. They are also intimately involved in host–bacterial interactions through the secretion of mucins. Mucins protect and lubricate the epithelial surfaces and play a role in epithelial growth and renewal (Moal and Servin, 2006). In the chicken, as in mammals, it has been demonstrated that the mucous secretions are not only a source of nutrients for the resident microbiota, but are also a mechanism that the host microbiota may use to inhibit other bacteria (Smirnov *et al*., 2005). In spite of their similar function, chicken mucins differ in structure, folding, glycosylation and

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charge compared to human mucins (Verma *et al.*, 1994; Smirnov *et al.*, 2005). Additionally, when compared to human mucus, chicken intestinal mucus was able to attenuate *Campylobacter jejuni* virulence which is of interest given the role of *Campylobacter* as a food-borne pathogen (Byrne *et al.*, 2007).

Antimicrobial proteins are present at the intestinal epithelial surface and serve as another innate defense mechanism. These molecules are effective at killing a wide variety of bacteria, fungi, protozoa and viruses (Moal and Servin, 2006). One category of antimicrobial peptides named defensins are highly conserved evolutionarily and are present in mammals, birds, invertebrates and plants (Ganz, 2003). Defensins are cationic proteins that function by permeabilizing the cell membrane thereby causing cell lysis (Jenssen *et al.*, 2006). Three subfamilies of defensins exist, α-, β- and θ-defensins. To date, 13 avian β-defensins, also called gallinacins or Gal have been described (Zhao *et al.*, 2001; Sugiarto and Yu, 2004; Xiao *et al.*, 2004). Avian macrophages, epithelial cells and heterophils have all been shown to be capable of producing gallinacins (Brockas *et al.*, 1998; Harmon, 1998). Gallinacins are important innate defense proteins in the chicken GALT with potent activity against intestinal pathogens (Hasenstein *et al.*, 2006; Milona *et al.*, 2007; van Dijk *et al.*, 2007;). Our group has observed an increase in the expression of several gallinacins (including Gal 1, 2, 6 and 7) following *Salmonella* infection in chickens. Importantly, administration of probiotics prior to inoculation with *Salmonella* resulted in a decrease in the expression of the genes that encode certain gallinacins (unpublished results). Cathelicidins, another family of antimicrobial peptides with broad-spectrum antimicrobial activity, have also been identified in the chicken. Xiao *et al.* (2006) identified three cathelicidins in the chicken which they termed fowlicidins (1–3). Others have also identified cathelicidin-like proteins in chickens (van Dijk *et al.*, 2005).

It is thought that in chickens, similar to the case in mammals, when either commensal or pathogenic bacteria breach the IEC barrier, whether by host-mediated or bacterial-mediated mechanisms, they are dealt with by cells, such as macrophages, DC, NK cells, heterophils and γδ T cells. These cells are all capable of recognizing members of the microbiota by binding to large groups of conserved pathogen-associated molecular patterns (PAMPs) or the more recently used term, microbe-associated molecular patterns (MAMP) via pathogen recognition receptors (PRR), such as toll-like receptors (TLR), nucleotide-binding oligomerization domain (NOD)-like proteins (NLR) and RNA helicases, such as retinoic-acid-inducible gene I (RIGI) and melanoma-differentiation-associated gene 5 (MDA5) (Meylan *et al.*, 2006). These PRR are designed to initiate host responses to invading pathogens (Takeda *et al.*, 2003). In chickens, a number of TLR have been found, including TLR1 (types 1 and 2), TLR2 (types 1 and 2), TLR3, TLR4, TLR5, TLR7, TLR15, TLR16 and TLR21 (Fukui *et al.*, 2001; Leveque *et al.*, 2003; Iqbal *et al.*, 2005; Philbin *et al.*, 2005; Yilmaz *et al.*, 2005; Higgs *et al.*, 2006; Keestra *et al.*, 2007; Higuchi *et al.*, 2008). Chicken TLR1 (types 1 and 2) and chicken TLR2 (types 1 and 2) were shown in various combinations to respond to diacylated and triacylated lipoproteins and peptidoglycan (Higuchi *et al.*, 2008). Similar to their mammalian orthologs, TLR3, TLR4, TLR5 and TLR7 recognize double stranded RNA, lipopolysaccharide, flagellin and single stranded RNA, respectively (Kogut *et al.*, 2005; Philbin *et al.*, 2005; Schwarz *et al.*, 2007). TLR16 is functionally similar to human TLR1 and TLR6, and in combination with TLR2 responds to diacylated and triacylated lipoproteins (Keestra *et al.*, 2007). TLR15 and TLR21 have no known function at this time. Although chickens respond to CpG DNA, the ortholog of mammalian TLR9, which recognizes CpG DNA, has not been identified (He *et al.*, 2006). Therefore, it seems that the chicken TLR repertoire, although very similar to the mammalian system, has a number of unique features. No known chicken ortholog of the mammalian NOD2 protein has been described. Although the sequences for chicken RNA helicases, RIG-I and MDA5, along with NOD1 have been described, their function in this species is unknown.

Intestinal homeostasis requires that a pro-inflammatory response to the normal microbiota is either not generated or is rapidly controlled. In mammals, it is generally accepted that the recognition of commensal bacteria by PRR is partially regulated by the expression pattern of these receptors. TLRs are expressed intra- and extracellularly, while NLR and the RNA helicases are predominately intracellular. It has been demonstrated that TLR are not typically expressed on the apical surfaces of IEC in mammals, but are expressed intracellularly and/or basolaterally (Iwasaki and Medzhitov, 2004; Rakoff-Nahoum *et al.*, 2004). This represents an intentional down-regulation of response to micro-organisms that typically reside within the lumen, such as the normal microbiota. This is not absolute, however, as recent studies have demonstrated apical expression of TLR4 on IEC (Lotz *et al.*, 2006; Stokes and Waly, 2006). In chickens, the expression of the various TLRs have been observed throughout the various regions of the intestine (Iqbal *et al.*, 2005), however, the expression has not been examined on a cell type-specific basis and, therefore, it is unknown if similar methods of control exist in chickens.

Additionally, intestinal macrophages have also been shown to be hyporesponsive to TLR ligands while remaining highly phagocytic, therefore, helping to ensure that any bacteria that cross the epithelium are rapidly killed without causing unnecessary inflammation (Smythies *et al.*, 2005). In chickens, intestinal macrophages have been described, but their role in immune homeostasis has not been described (Higgins *et al.*, 2007). Another mechanism described in mammals for

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regulating the response of TLR-mediated pro-inflammatory signals is cytokines, such as transforming growth factor (TGF)-β (Monteleone *et al.*, 2005) and interleukin (IL) -10 (Steidler *et al.*, 2000), both expressed by a number of cells in the intestine and thought to maintain homeostasis. A similar mechanism is assumed to exist in chickens given the fact that functional studies on chicken cytokines have shown them to perform similar properties as in their mammalian counterparts. For example, chicken TGF-β4 shares sequence homology with mammalian TGF-β1 and has a similar anti-inflammatory function (Withanage *et al.*, 2005).

Chicken heterophils, another important cellular component of innate defenses, may play a role in maintaining intestinal homeostasis through interactions with the intestinal microbiota. Heterophils have been shown to express cytokines and chemokines in response to peptidoglycan and CpG from normal mucosal bacteria (He *et al.* 2005; Kogut *et al.*, 2006). In a recent study, it was demonstrated that heterophils recognize peptidoglycan from staphylococci, which are a normal bacterial resident in the intestine within the first few days of life, via TLR2 (Kogut *et al.*, 2005). This recognition results in the activation of innate defenses. In another recent study, Farnell *et al.* (2006) showed that manipulation of the intestinal microbiota immediately after hatch by probiotic bacteria could significantly increase oxidative burst and degranulation activities of heterophils.

DC, the most efficient antigen presenting cell, are an important link between the innate and adaptive immune systems and are thought to be an important factor in immune homeostasis (Reis e Sousa, 2004). The cytokine microenvironment provided by DC and other cells of the GALT influences the type of immune response that is generated. In mammals, a number of DC populations have been described, and together they are responsible for making the decision to respond with a regulatory or an effector response (Coombes *et al.*, 2007). Regardless of the type, the intestinal DC carrying commensal bacteria do not penetrate beyond mesenteric lymph nodes and are thought to play a key role in inducing protective local IgA responses (Macpherson and Uhr, 2004). However, it is difficult to explain such a mechanism in chickens as they lack organized, encapsulated lymph nodes and the DC populations in this species are ill-defined. Only recently have markers for chicken DC been identified (Hansell *et al.*, 2007) and, therefore, these cells have not been fully characterized.

It is conceivable that a number of innate mechanisms, similar to those described in mammals, exist in chickens to limit the response to members of the normal microbiota. In spite of the similarities that exist between avian and mammalian species, a number of differences also exist and, therefore, more research is needed to fully understand intestinal homeostasis in the chicken and other avian species.

Adaptive immune response

Innate defense components can initiate downstream adaptive responses to both pathogenic and commensal bacteria. The cytokine microenvironment induced by the innate defense cells through the interactions between PRR expressed by these cells and their microbial ligands influences the type of immune response generated. However, in chickens, the cross talk between innate and adaptive system is less clear, because the full repertoire of PRR and cytokines has not been fully identified and characterized. It has, however, been demonstrated that in chickens, bacterial members of the microbiota have the ability to modulate cytokine and chemokine gene expression (Dalloul *et al.*, 2005; Brisbin *et al.*, 2008; Chichlowski *et al.*, 2007; Haghighi *et al.*, 2008), thereby influencing the type of immune response that is generated within the GALT. Immunomodulation is thought to occur through the initiation of a series of reactions that culminate in enhanced antibody- and cell-mediated immune responses (Dalloul *et al.*, 2003; Haghighi *et al.*, 2005).

As determined from studies on mice and humans, the important difference between responses to pathogenic and commensal bacteria is T cell help. T-dependent responses to pathogens elicit antibody-mediated immune responses of high affinity and specificity (Bachmann *et al.*, 1997), whereas responses to commensal bacteria consist of a large T-independent portion with antibodies of broader specificity and lower affinity (Macpherson *et al.*, 2000). IgA is considered the primary antibody isotype in the secretions of both mammals and birds (Lillehoj and Trout, 1996; Snoeck *et al.*, 2006). IgA prevents the entry of commensal bacteria into subepithelial areas by coating them to prevent their adherence to IEC or by returning those bacteria that have penetrated to the basolateral space to the lumen (Macpherson *et al.*, 2001).

In mammals, intestinal IgA production is shared by B1 and B2 (conventional) B cells, with approximately half of the IgA and the majority of the T cell-independent IgA being B1-derived (Macpherson *et al.*, 2000). It seems that the role of B1 cells in control of the immune response to intestinal commensal microbes is achieved by producing large amounts of T-independent IgA with broader specificity and lower affinity that is capable of binding to diverse members of the normal microbiota with multiple redundant surface epitopes (Macpherson *et al.*, 2000). However, B1 cell contribution to intestinal IgA synthesis in species other than mice and humans is not known. In chickens, it is difficult to explain the T-independent mechanisms since all chicken B cells possess the typical B1 cell surface markers (CD5 and IgM) (Koskinen *et al.*, 1998; Ratcliffe, 2006).

Similar to the work on mammals, the antibody-mediated response of chickens after treatment with

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various probiotic species results in increased production of specific and natural antibodies. Koenen *et al.* (2004a and 2004b) demonstrated the ability of LAB to improve systemic antibody response to soluble antigens, such as trinitrophenyl-keyhole limpet hemocyanin (KLH) and KLH alone. Oral administration of a probiotic product containing *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Streptococcus faecalis* also induced significantly more systemic antibodies in chickens following immunization with sheep red blood cells (Haghighi *et al.* 2005). Administration of the same probiotic product enhanced significantly natural antibodies in serum and in intestinal contents (Haghighi *et al.*, 2006). Yurong *et al.* (2005) also reported increased amounts of IgA in the intestinal fluid as well as an increase in IgG- and IgM-producing cells in the PP. This was associated with an increase in the density of microvilli, and an increase in the length of cecal tonsils. Altogether, this data demonstrates a role for probiotics, and most likely commensal microbiota in enhancing both the chicken intestinal mucosal immune response as well as systemic immune responses.

Along with antibody-mediated immune responses, increases in cell-mediated immune responses in the chicken following probiotic treatment have also been demonstrated. Dalloul *et al.* (2003) showed significant proliferation of IEL expressing CD3, CD4, CD8 and αβ TCR in chickens that received a *Lactobacillus*-based probiotic. The same group also demonstrated a significant increase in IFN-γ and IL-2 expression in the intestine following challenge with oocysts of *Eimeria acervulina* in probiotic-fed chickens (Dalloul *et al.*, 2005). Our group has also demonstrated that when components of *L. acidophilus* are co-cultured with CT mononuclear cells and splenocytes, the expression of a number of genes including chemokine and chemokine receptors, cytokine and cytokine receptors, adhesion molecules, surface molecules, and immunoglobulins and T-cell receptors as well as genes involved in antigen processing, apoptosis, transcription and signal transduction showed differential spatial and temporal expression profiles (Brisbin *et al.*, 2008).

In mammals, CD4+ regulatory T-cells (Tregs) have been shown to be important for maintaining intestinal homeostasis. Three populations of CD4+ Tregs have been described, the naturally occurring CD4+CD25+ Treg cells, the induced Tr1 cells that secrete IL-10, and the induced Th3 cells that secrete TGF-β (Izcue *et al.*, 2006). Although chicken CD25 has recently been described and the constitutive expression of this molecule in a population of CD4+ T cells has been shown (Teng *et al.*, 2006), the regulatory function of CD4+CD25+ T cells in the chicken has remained to be addressed. Moreover, it remains to be seen whether CD4+CD25+ T cells or other regulatory T cell populations exist in the chicken intestine or have a role in intestinal homeostasis in this species.

Conclusion

Overall, although not completely described in the chicken, the response to commensal bacteria is initiated by various cells within the induction sites of the GALT. This is followed by stimulation of T cells and regulatory T cells that collectively contribute to a non-inflammatory phenotype characterized by TGF-β and IL-10. IgA, another important adaptive mechanism used to limit bacteria to the intestinal lumen, is thought to arise in part from a T-independent manner. Future research on host-commensal interactions in the chicken needs to focus on further characterization of interactions between commensal bacteria and the immune system, including cells and molecules involved, and the location where these interactions take place given the anatomical differences between the chicken and mammalian species. Additionally, characterization of the various chicken cell subsets involved in intestinal homeostasis, such as DC, NK cells, B cells and Treg cells and the development of reliable markers for these various subsets would allow for the examination of the role of these cells in host-commensal interactions. An understanding of how the chicken immune system and the normal microbiota interact would allow for a more focused search for a reliable alternative to in feed antibiotics.

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