

Optimising immunity in broiler production – part one

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While its functions for feed digestion and for the absorption of nutrients are well known the intestinal tract is also the site where the body is in contact with the majority of exogenous challenges including microbes and soluble antigens.

Even while the epithelial cell layer is protected by mucus, the contact of these cells with intestinal microorganisms, their metabolites or with products of their degradation is very intense. Fortunately, the intestinal wall also contains the gut associated lymphoid tissue (GALT), an ingenious system of local immunity.

However, it takes about two weeks post-hatch for the GALT of the chicken to fully mature.

This can bring the young broiler to a vulnerable position, especially in intensive production where stress conditions can lead to immunosuppression. The maturation of the GALT is enhanced by contact with the intestinal flora or with immunomodulating compounds in the feed.

The use of these immunomodulating compounds can thus be valid in starter diets for poultry.

However, they should be used in a strategic way since immune stimulation may release pro-inflammatory cytokines that can block signal transfer of growth hormones and thus can reduce growth.

In this article we will discuss the implications of immune stimulation in young birds and how this can be part of total health management in modern poultry production.

The intestinal epithelium

Like the skin, the intestine is a critical interface between the internal milieu of the body and the external environment, the intestinal lumen. Unlike the skin, the intestines have to support digestion and absorption of nutrients, electrolytes and water.

This is the responsibility of the epithelial enterocytes. Since the intestinal contents may contain

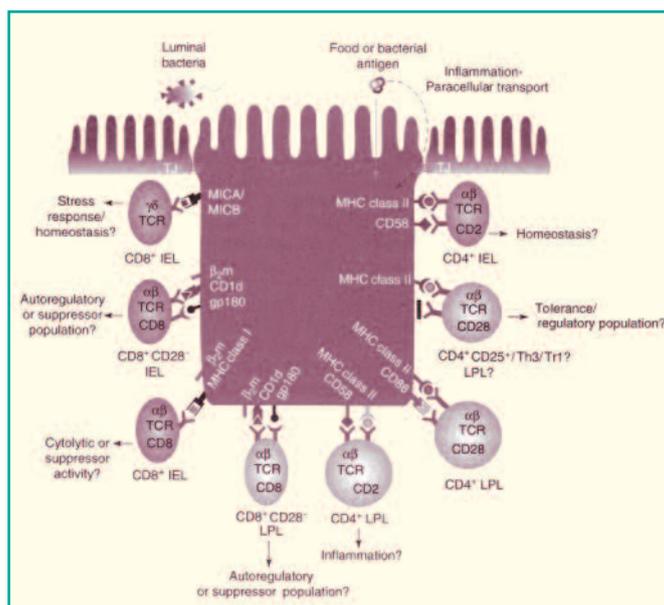


Fig. 1. The interaction of intestinal epithelial cells with T cells (I. Dotan and L. Mayer (2003) in *Microbial pathogenesis and the intestinal epithelial cell, American Society of Microbiology: 43-59*).

pathogens the intestinal permeability must be highly regulated and selective and an ingenious system of mucosal immunity is present in the intestinal wall: the Gut Associated Lymphoid Tissue or GALT.

The different cell populations in the mucosal barrier are equipped to sense and respond to the molecular contents in the lumen and to translate this molecular information into signals that can reach local or distant sites within the body. The general functions of the GALT are:

- 1 Processing and presentation of antigens.
- 1 Production of intestinal antibodies.
- 1 Activation of cell mediated immunity.

In the GALT non-immune, innate and adaptive immune responses all contribute to local defence and immunity. The intestine is the largest immunological organ in the body. It contains 70-80% of all Ig-producing cells and produces more secretory IgA (50-100mg/kg/day) than the total production of IgG (30mg/kg/day).

Table 1. Innate and adaptive immunity in the gut.

Physicochemical	Innate immunity		Adaptive immunity
	Cellular		
Mucus	NK cells (some IEL)		IEL
Tight junctions	Macrophages		LPL
Epithelial membranes	Polymorphonuclear leukocytes		Regulatory cytokines
Luminal/brush border enzymes	PRRs (toll-like receptors)		SlgA
Bile salts	Epithelial cells		PP
pH ranges			Epithelial cells; antigen presentation
Somatostatin			
Trefoil factors			

day).

In the intestinal epithelium we can find many different cell types. The intestinal epithelial cells (IEC) absorb essential nutrients and undergo a continuous cycle of cell death and regeneration.

They compose a major part of the first line defence and display a number of different receptors on the apical and basal surface. They use these receptors as sentinels to relay danger signals from the lumen to the network of mucosal lymphoid cells, like B and T cells (Fig. 1).

Intraepithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL) are specific for the GALT and are different from systemic lymphocytes with regard to phenotype and activation requirements and they differ from one another by phenotype and function. IEL are a subpopulation of T cells that resides between the epithelial cells, influence them via autocrine and paracrine mechanisms and possess the capacity of secreting a wide variety of cytokines and chemokines.

IEL are capable of processing soluble protein antigens into immunogenic peptides but are categorised as non-professional antigen presenting cells since they do not constitutively express class II MHC or costimulatory molecules for T cell activation.

Most IEL can mediate specific cellular immune functions without the requirement for antigen processing and can recognise invading pathogens or damaged cells directly.

As in mammals IEL in chicken are sparse at hatching and increase in number after exposure to environmental antigens.

The structure of the Peyer's patches is outlined in Fig. 2. The intestinal epithelium here contains a high density of M cells. M cells are specialised for the bulk endocytic sampling of luminal contents and have a highly developed vesicular transport system that provides a short and rapid pathway across the epithelial barrier. The M cells deliver viable micro-organisms in the intraepithelial pocket where dendritic cells (DC), B cells and T cells

Continued on page 13

Continued from page 11

are present.

These micro-organisms are then phagocytised by dendritic cells who are the most potent professional antigen presenting cells (APC) known.

They process the antigen and present it to the T and B cells. The activated antigen specific T and B cells then migrate to effector sites like the lamina propria where B cells will produce IgA. Secretory IgA (sIgA) is then transported through the intestinal epithelium into the lumen. sIgA is more resistant to proteolysis by secretory enzymes than other immunoglobulin isotypes.

Depending on their specificity they can mask surface receptors of enteropathogenic bacteria or neutralise bacterial enterotoxins and enterotropic viruses.

Their agglutinating action leads to increased elimination by intestinal peristalsis. Daily, more than 3g of IgA reach the mucosal secretions, blocking the massive entry of pathogens in the body.

Innate immunity

Also in the GALT we can make a distinction between innate and specific or adaptive immunity. As illustrated in Table 1 innate immunity comprises both physicochemical and cellular defence systems. Adaptive immunity involves the specific reaction of the animal's body to a challenge by an antigen.

It is activated after the pathogen has evaded the innate response and entered the body or when the pathogen is presented by APCs like dendritic cells or macrophages to T and B cells. In contrast to the adaptive immune system that responds to antigens with exquisite specificity, the cells of the innate immune system sense more generic molecular signatures or pathogen associated molecular patterns (PAMP).

Initial recognition of pathogens by the innate immune system happens through pathogen pattern recognition receptors.

Toll like receptors (TLRs) are an important group of these pattern recognition receptors and can be classified based on their presence on different cells as ubiquitous (TLR1), restricted (TLR2, TLR4 and TLR5 in myelomonocytic cells) and specific (TLR3 in dendritic cells).

TLRs bind to the following ligands: TLR2 senses lipoproteins (G+ bacteria) like peptidoglycan, yeast extracts and a variety of lipopeptides.

TLR3 senses double stranded DNA.

TLR4 is a signal transducer for LPS (G- bacteria).

TLR5 recognises flagellin.

TLR9 recognises bacterial CpG DNA.

TLR family signaling can induce a

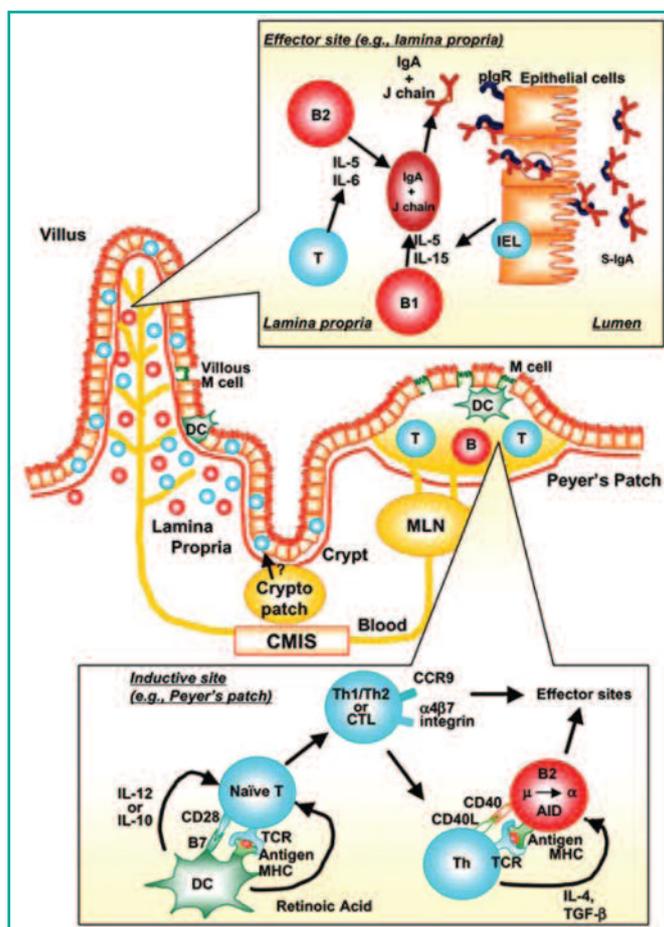


Fig. 2. Interactions between dendritic cells, B and T cells in the Peyer's patches lead to a mucosal immune response in the lamina propria (Kunisawa, J., Fukuyama, S. and Kiyono, H. (2005) *Current Molecular Medicine* 5, 557-572).

variety of adjuvant effects on various cells or tissues including macrophages, dendritic cells and epithelial cells. Mucosal epithelial cells secrete IL6 following contact with bacteria.

Bacterial LPS also activates neutrophils, monocytes and macrophages to produce IL6.

This illustrates the importance of the specific environment in the GALT as IL6 enhances the terminal maturation of mucosal B lymphocytes into IgA producing plasma cells.

These B cells migrate to the effector sites in the lamina propria where they secrete their IgA that is transported through the epithelium and released in the intestinal lumen to neutralise residing pathogens.

Mucosal immunity

The avian GALT contains unique lymphoid structures such as the bursa of Fabricius, caecal tonsils (CT) and Meckel's diverticulum (remnants of the yolk sac) as well as Peyer's patches (PP), intraepithelial lymphocytes (IEL) and scattered immune cells residing in the intestinal lamina propria. As chickens do not have other peripheral encapsulated lymph nodes, GALT lymphoid structures serve as major secondary

lymphoid organs.

Like in pigs the T lymphocytes of the chicken mature in the thymus. Whereas, B lymphocytes of the pig mature in the Peyer's patches at intestinal level, this occurs in the Bursa of Fabricius of the chicken.

Young chicks have approximately six PP; however, following age-associated involution only one PP, the ileal lymphoid aggregate, is evident. The structure of the avian PP is similar to mammalian PP. The caecal tonsils are a relatively immunologically mature lymphoid organ similar to the PP.

Caecal tonsils are rudimentary at hatch and contain a majority of CD4+ T cells. By six weeks of age the number of B cells is significant and few CD8+ T cells are present.

Caecal tonsils may become more important after the involution of the Bursa. Caecal tonsils and the Bursa are the inductive site of IgA+ B cells that migrate from there to the lamina propria.

The infrequency of antigen sampling cells, like M cells, in avian GALT may be compensated for through the increased frequency of lymphocyte infiltration into both epithelial cells and M cells and an increased ability of epithelial cells to sample antigen.

The normal development and

immunological function of the avian GALT in the immediate post-hatch period is critical for the chick's survival after hatch since the chick is immediately exposed to environmental antigens (including pathogens), in the absence of additional post-hatch maternal immunity such as that provided by colostrum and milk in the mammal.

GALT maturation occurs in two stages or waves: the primary wave occurs during the first week post-hatch and a second wave during the second week. Bar-Shira et al. suggest that T cells migrate to the GALT during the first three days post-hatch, followed by an acclimation period before activation by gut borne antigens like environmental antigens and feed.

It has previously been described how activation can occur via pattern recognition receptors.

The arrival of new T lymphocytes commencing day three post-hatch indicates the beginning of a second maturational stage during which the lamina propria compartment matures.

Full activation of NK cells is probably dependent on maturation of T cells, and most of the development is accounted for by responsive T cells.

GALT maturation also involves changes in the B cell compartment. Bar-Shira et al. demonstrated a substantial colonisation of B cells in the small intestine on day four post-hatch, while an increase of a similar magnitude was observed in the hind gut only two days later.

As avian B lymphocytes, like their mammalian counterparts, migrate in response to chemokines, they propose that the differential distribution and early colonisation of B cells in the proximal end of the intestine could be due to chemokines secreted by epithelial cells in that region.

In that case the differential homing pattern of B cells, during the immediate post-hatch period, would be associated with the maturational status of epithelial cells lining the different regions of the intestine.

As the functional maturation of epithelial cells, as expressed by brush border enzyme activities, varies along the intestine, regional differences in secretion of epithelial chemokines might also occur.

Lack of antibody responses during the first week post-hatch might be attributed to immaturity of T lymphocytes residing in the lamina propria or caecal tonsils and in terms of their inability to provide help necessary for the induction of antibody responses. ■

The second part of this article will appear in the next issue of International Poultry Production