The Gut Associated Lymphoid System in the Post-Hatch Chick: Dynamics of Maternal IgA

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Abstract

Adaptive immunity is immature at birth in mammals and birds. Several measures have evolved to protect the neonate or hatchling during this critical period, one of which is totally dependent upon the adaptive immune response of the maternal parent – maternal antibodies. Maternal antibodies have protecting roles both systemically (via the blood system) and locally in the gut. The protective role of maternal antibodies in the chicken gut, and particularly that of maternal IgA, is of particular interest due to the precocial nature of chicken development.Consequent to the rapid colonization of the chick gut by commensal bacteria, as well as the possible entry of pathogenic bacteria, a parallel rapid development of gut associated lymphoid tissue (GALT) is expected. However, the gut-specific adaptive immune response matures within two weeks post hatch. Hence, local protection of the developing gut appears to be dependent upon the presence and activity of maternal antibodies, similar to that described in the mammal. These maternal antibodies are provided during the process of egg formation, and continue to function in the hatchling until its own immune response can take over. Here we briefly review these protective mechanisms and provide several new insights to the protection of maternal IgA and its extended activity in the gut of the post-hatch chick.

Key words: Maternal antibody, maternal protection, chick immune response, gut associated lymphoid tissue, IgA.

INTRODUCTION

The digestive tract's structure and function reflect the feeding habits of animals. Thus, the gut of predators is structured differently than that of herbivores and the digestive tracts of herbivores differ structurally, depending on the site of cellulose digestion (1, 2). Another cause for difference in function between digestive systems relates to aspects determining the development of digestive function in the gut. For example, the rate of gut development, both anatomically and functionally, is different between species that immediately forage an adult type diet (precocial) and those that are fed processed foods or milk by parents (altricial) (1). Several bird species, including *Gallus*, proceed to forage immediately at hatch (3). This intake of an omnivorous diet requires the rapid adaptation of the digestive tract to accommodate breakdown and absorption of complex food stuffs (1, 4, 5). Concomitant with the exposure to an adult-type diet, the intestinal tract of these birds immediately becomes inhabited by microflora (6-10). Interestingly, in *Gallus* sp., the major site for bacterial colonization is the large intestine, particularly the two cecal horns (2, 7, 9), and colonization occurs by rectal as well as by oral routes (7, 9, 10). Consequent to the rapid colonization of the gut by commensal bacteria, as well as the possible entry of pathogenic bacteria, a parallel rapid development of gut associated lymphoid tissue (GALT) is expected. Nevertheless, as we have shown in previous studies, the avian GALT is far from being mature during the rapid period of gut development following hatch (11-15). Thus, while the gut-specific

adaptive immune response matures within two weeks post hatch (13), several innate processes, both antigen dependent and antigen independent, appear to be functional at hatch or immediately thereafter (11). In mammals, protection during critical periods of premature immunity is provided by maternal protection in the form of maternal antibodies continuously provided by milk. In the bird, however, such ongoing protection is unavailable. Hence, if maternal protection is to confer a certain degree of protection on the developing chick then these means need to be provided during the process of egg formation, and more importantly, continue to function in the hatchling until its own immune response can take over. The objective of this manuscript is to briefly review these protective mechanisms and to provide several new insights derived from our own research.

MECHANICAL AND CHEMICAL PROTECTION OF THE DIGESTIVE MUCOSA

The gut mucosal surface (from the pylorus) is covered by microvillus simple columnar epithelium (enterocyte) that functions as a physical barrier as well as in digestion and absorption (16). The paracellular route is blocked by intercellular tight junctions, and enterocytes express apical membrane receptors specific for bacterial antigens, the activation of which leads to local immunity (16). Three other cell types contribute to local immunity: goblet cells, microfold (M) cells (Bockman& Cooper identified unique follicular associated epithelial [FAE] cells in the cloacal bursa, and these, based similarity to the rabbit and mouse, were later designated as M cells (17, 18)) and Paneth cells; of these, only goblet cells have been unequivocally demonstrated in the digestive tract of birds. Goblet cells are columnar epithelial cells that are generated from the same progenitors as the enterocytes, and function as mucin-secretors (19, 20). Mucins, a group of heavily glycosylated proteins, have a central role in the protection of the mucosa against various pathogens (21-23). Secreted mucin adheres to the enterocyte surface in two layers – internal firm and external loose adherent layers (22). These layers impede the penetration capacity of microbes. Furthermore, mucins mimic cell ligands for bacterial receptors thus blocking the binding capacity of bacteria to the cell surface prior to penetration (19). Further protection of the mucosa is sustained by dimeric IgA. This form of IgA is linked by an invariable J chain, and is protected against proteolysis by a peptide, secretory component (SC), a remnant fragment of the polymeric Ig receptor, responsible for transcytosis of dimeric IgA from the lamina propria to the gut lumen (16). Similar to mucin, dimeric IgA blocks adherence of bacteria to the cell surface. IgA is antigen specific and is the consequence of previous exposure of the lamina propria to gut-derived antigens (16).

DEVELOPMENT OF IMMUNITY IN THE AVIAN DIGESTIVE TRACT

This topic is briefly described here and readers are referred to our previous extensive reviews (12, 15). Essentially, the adaptive immune response in the avian gut is immature at hatch (13). While small lymphocyte numbers are present at hatch, they acquire full functionality within the first 10 days post-hatch. During this period, specific protection is made available via maternal antibodies active both in serum and gut (Elad and Friedman, in preparation; Cohen and Friedman, in preparation). Even though B cell development during this period is very active (24), gut specific secretory IgA (sIgA) is still undetectable (25). Interestingly, the poly Ig receptor (pIgR) develops in parallel with the IgA secretory system (Cohen and Friedman, in preparation). Innate immunity appears to be fully functional at hatch as expressed by numerous parameters, i.e. granulocytes, knockout (NK) cells, defensins and more (11).

MATERNAL PROTECTION: COLOSTRUM AND ITS FUNCTION IN MAMMALS

Adaptive immunity is immature at birth in mammals and birds. This leaves the neonate or hatchling exposed to numerous infective agents. Several measures evolved to protect the neonate during this critical period, one of which is totally dependent upon the adaptive immune response of the maternal parent – maternal antibodies (26, 27). These antibodies reflect the adaptive immunity experience of the mother, and are transferred to the developing offspring. In mammals, there are two mechanisms of antibody transfer – via the placenta and via milk, particularly colostrum (28). Placental transport of antibodies is possible in mammals with a hemochorial placenta, such as rodents (29) and mammals (30). This transport allows selective transport of IgG via a unique Fc receptor (FcRn) (31, 32) and occurs during embryonic development. In mammals with different placental structure, i.e. ruminants,

dogs, cats, horses or pigs, placental transfer of antibodies is very limited to non-existent (33).

In all mammals, plasma cells migrate to the mammary gland lamina propria during pregnancy (34); this directed migration of plasma cells appears to be controlled by pregnancy hormones – progesterone, prolactin and estradiol (35, 36). These hormones also regulate the expression of the pIgR in mammary gland secretory epithelium (37). Thus, pregnancy leads to increase of antibody secretion into mammary alveoli (38). Interestingly, the main sources of the migrating plasma cells are mucosal lamina propriae a fact that guarantees protection based on active maternal immune experience (16). Colostrum-rich antibodies and milk-enriched antibodies are provided to the suckling neonate during the immediate postpartum period (39, 40). As digestive proteolytic processes are immature in the under-developed gut, whole proteins – including antibodies – are easily absorbed (40). Moreover, colostrum trypsin inhibitors as well as high absorption capacity of enterocytes, further promote absorption of intact proteins (40). High intestinal wall permeability is drastically reduced within 24-48 post parturition due to commensal bacterial colonization and gut maturation (33, 39). The cardinal importance of colostrum/milk derived antibodies for neonate protection is demonstrated by uniform immune failure leading to morbidity and mortality consequent to early infection (41, 42). The gut continues to be passively protected by maternal dimeric IgA (and IgG in several species) that is continuously provided by milk (43, 44). The milk-derived antibodies neutralize enteral pathogens, thus denying contact between pathogen and gut wall (immune exclusion) (44).

MATERNAL PROTECTION: THE EGG AS A MATERNAL ANTIBODY RESERVOIR

The protective strategy in egg-laying species is more complicated than that in mammals. While protection of the developing embryo in mammals is made possible via direct anatomical contact, the developing embryo in the egg has no such contact with its hen. Consequently, protective maternal strategy in birds (and other egg laying species) must provide means to protect the egg, the embryo and the hatchling. Strategies to protect the egg and embryo are not within the scope of this review and will be discussed elsewhere (Bar-Shira et al., in preparation). Strategies to protect the hatchling, as in the mammal, rely on maternal antibodies, but must take account of the chick's different life style: the hatchling immediately proceeds to forage adult type food (see above) and at the same time continues to use nutrients present in residual yolk up to 7 days post hatch (45, 46). This lifestyle leads to a rapid rate of intestinal development that has ramifications for protein absorbance (47)

Maternal protection in the form of specific antibodies in *Gallus* is absolutely dependent upon the presence of protective antibodies in the egg. Previous studies have shown that the chicken egg contains all 3 known antibody types: IgY is prevalent in the yolk, while IgM and IgA are predominantly found in the egg white (48-50). The differential placement of the antibody types is a consequence of the different mechanisms responsible for transferring antibodies to the egg (50). IgY, present in the hen's serum is transferred to the developing yolk via transporters in the follicular epithelium, while IgA and IgM are transferred to the developing albumen as it passes down the oviduct. To date, IgY transporters in the chicken have only been described in the yolk sac (51-53), and it is unknown whether follicular transporters are the same or similar. The IgA transporter is probably the avian pIgR that has been described in the gut, with the SC derivative described in both gut and oviduct (54-56). The source of egg white IgM and IgA are mucosal plasma cells present in the lamina propria of the oviduct, adjacent to the epithelial basal lamina (Elad and Friedman, unpublished; (57, 58)). Similar to the mammal, the presence of oviduct plasma cells is under endocrine control, particularly estradiol (59, 60). However, while this control is periodic in the mammal, it is quite constant in the laying period of hens (61).

Thus, the non-fertilized egg contains all three isotypes in different compartments. IgY is mainly present in the yolk (20-25mg/ml yolk). IgA and IgM are only found in the egg white at 0.7 and 0.15 mg/ml respectively (48-50). The distribution of antibody isotypes in the fertilized egg changes significantly with the development of the embryo. At first, antibody distribution is identical to that of the non-fertilized egg (49). By the third day of incubation (E3) the amnion, chorion and allantoic membranes are formed: the former encloses the embryo immersed in the amniotic fluid. The amniotic fluid nourishes the embryo and is a site for excretion. The chorion membrane develops till it coats the entire inner surface of the egg shell, and functions as an oxygen exchanger. The alantoic membrane contains developing blood vessels and is the primary site for hematopoiesis. By the third day of incubation (E9) the allantois converges with the chorion, and the

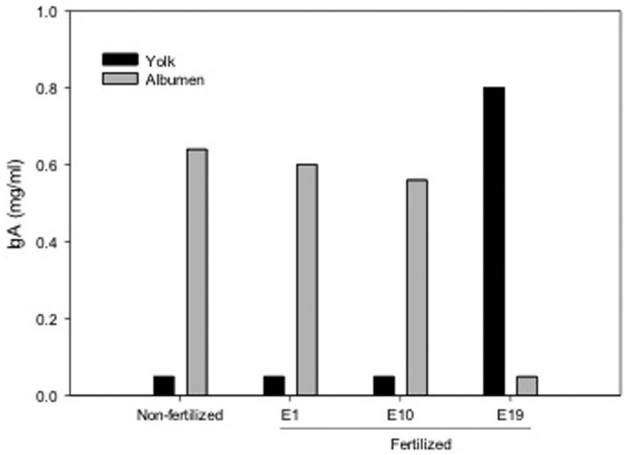

Figure 1: IgA in yolk and albumen of fertilized and non-fertilized eggs at different stages of incubation (*Elad and Friedman*, *unpublished*).

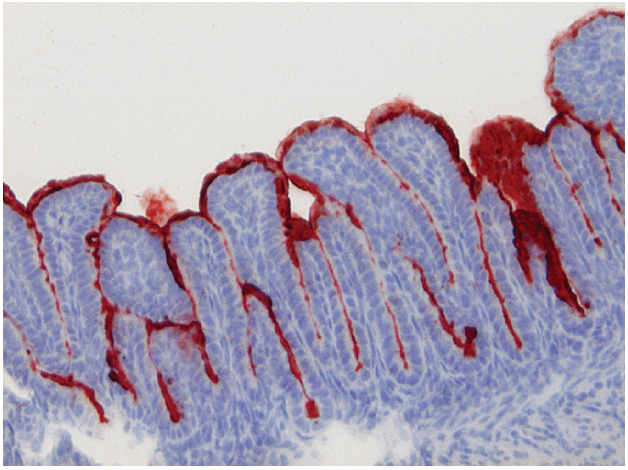

Figure 2: Cross section of E19 duodenum heavily stained to show specific anti-BSA specific IgA. IgA is contained in a paste-like substance coating the external apical face of enterocytes. This substance coats entire villi down to the crypt areas. No IgA is present in lamina propria, mucosa or other subepithelial areas, indicating absence of enterocyte uptake (*Elad and Friedman*, *unpublished*)

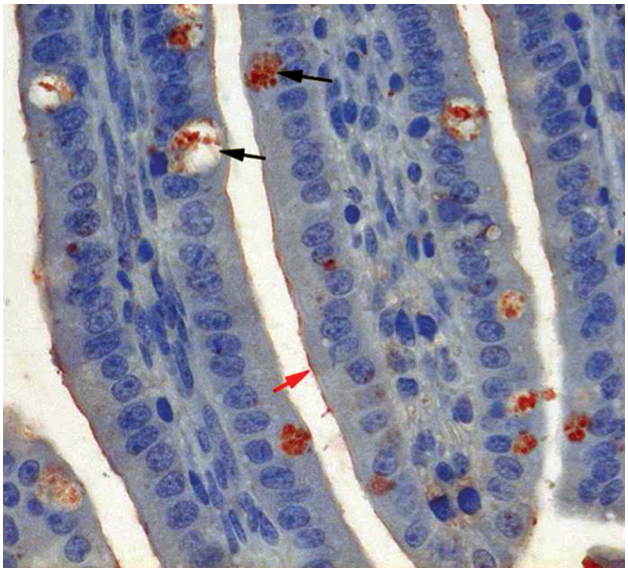

Figure 3: High magnification low-level staining of E19 duodenum for anti-porcine immunoglobulin (Pig) IgA. A thin coating of IgA is apparent on external surfaces of enterocytes (red arrow). Goblet cells (black arrows) contain different quantities of IgA; this content has a droplet appearance (*Elad and Friedman*, *unpublished*).

subsequent chorio-allantoic membrane continues to function in oxygen exchange and water absorption. By E11, following the rupture of the *sero-amniotic raphe*, free exchange of contents (and antibody isotypes) occurs between albumen and yolk (49, 62). Consequently, from E12 IgA is found in the sero-amniotic fluid and yolk (see Fig. 1 and (63)).

MATERNAL IGA IN THE CHICK GUT: EXTENDING ITS LIFE SPAN AND FUNCTIONALITY

The mechanisms of antibody absorption or transfer to the embryo also differ between isotypes. IgY was thought to be absorbed via the hepatic portal system or via the vitelline circulation (50), however the recent discovery of an IgY transporter on the yolk sac (52, 53) might indicate direct IgY uptake from the yolk. IgY is the only maternal isotype present in the embryo's blood system (49). As yolk is delivered to the embryonic digestive tract, all three isotypes are found in the developing gut as well. While they might serve as nutritional proteins, it is possible that they serve to protect the gut lumen as well (49, 63). It is interesting to note that while maternal IgA was located throughout the E21 digestive tract, including the lumen of the cloacal bursa, no maternal IgA was detected in the E21 respiratory airways, thus substantiating the premise that the yolk is the sole source of maternal IgA (Adlershtein-Cohen, Cohen and Friedman, in preparation).

Thus, antibody distribution in the embryo gut and serum provides an elegant means for specific maternal protection. While maternal serum antibodies continue to protect the chick for several weeks (depending on antigen) (64, 65), the effective protective period of gut lumen antibodies is unknown. Furthermore, the mechanism that allows persistence of these antibodies until the emergence of an endogenous source of protective antibodies is also by large unknown. To gain insight into these issues we initially confirmed the absence of

any IgA gene expression during E17 up to PH7. Thus, IgA in the gut was most probably maternal. To further insure this, we immunized hens with protein antigens (bovine serum albumin – BSA and porcine immunoglobulin -PIg), and detected maternal anti-protein specific IgA. The results of these studies have led us to describe a new mechanism for maternal IgA protection in the digestive tract of the post-hatch chick.

By following anti-BSA and anti-PIg specific IgA we were able to confirm the IgA transport pathway described above, namely oviduct – albumen – yolk. Specific immunohistochemical studies revealed a thin paste-like coating of IgA on all apical surfaces of enterocytes. Most interestingly, mature goblet cells were positively stained for BSA or PIg specific IgA (Figures 2 & 3).

IgA was not detected in any sub-epithelial compartment at this time, nor were there any IgA positive plasma cells. Taken together it appears that yolk IgA is immersed within a mucin-like substance that acts to associate IgA with enterocyte surfaces and that this IgA does not undergo uptake. Furthermore, the hitherto non-documented presence of IgA in goblet cells indicates a mechanism for controlled release of IgA and its temporal preservation.

IgA, as a free protein in the intestinal lumen, faces two possible fates, degradation by proteolytic enzymes and flush out with movement of the intestinal content. The latter might be considered the biological objective of antigen-bound IgA, though it would be a waste of a useful resource if the IgA is of limited supply or if it is antigen non-bound. Thus, measures contributing towards the extension of IgA lifetime in the gut would serve to alleviate IgA depletion in the immunologically immature chick. Thus, detecting maternal IgA in a mucin coated surface and in goblet cell cytoplasm both indicate a functional connection. Goblet cells are considered as single cell exocrine glands that are present in numerous epithelial linings (20). The mucus layer coating the gastrointestinal tract is the front line of innate host defence, largely because of the secretory products of intestinal goblet cells. Goblet cells synthesize secretory mucin glycoproteins and bioactive molecules such as epithelial membrane-bound mucins, trefoil factor peptides, resistin-like molecule beta, and Fc-gamma binding protein (22). Colonization by commensal microbes is limited to an outer "loose" mucus layer, and interacts with the diverse oligosaccharides of mucin glycoproteins, whereas an "inner" adherent mucus layer is largely devoid of bacteria (22). The interaction between microbes and the mucin layer has been described in both mammals (21, 22) and the chicken (23). Secretory IgA in the mammal has been reported to form a mesh within the mucin layer, thus allowing IgA to bind adherent enteral pathogens (66). The IgA layer described in our studies conforms to these mucin layers.

The excretion rate of goblet cells is regulated by several regulators (nerve excitation, hormones, lipids and inflammatory cytokines) (19, 20, 22, 23). Thus increased excretion, *compound exocytosis*, leads to the thickening of the mucin layer and increases its protective ability. Steady state secretion, or continuous secretion, occurs under normal non-stressful conditions and constantly replenishes the mucin layers (19). Steady state secretion of mucin-containing IgA would provide a constant source of IgA overtime (until a source of endogenic IgA becomes available – in the chick, 7 d post-hatch). Thus immersing IgA in mucin protects it from degradation and its uptake by goblet cells extends its effective lifetime.

While the presence of IgA in mucin has been demonstrated in mammals (20), a putative mechanism by which goblet cells uptake intestinal substances is hitherto unknown. Use of an analog of the pIgR seems unlikely for several reasons. First, pIgR is a basal and lateral membrane receptor; there are no reports to date describing it as an apical membrane receptor. Second, there is no report to date describing pIgR in goblet cells. Third, maternal IgA is dimeric and contains a SC, thus it is highly unlikely, if not impossible to undergo uptake a second time with the identical receptor.

The observation that maternal IgA might be protected by the mucin secretory system, thus extending its effective functionality till an endogenic source of IgA becomes available is of importance in the development of oral vaccines. Oral vaccines are of major advantage when immunizing large populations. These vaccines need to evoke immunity rather than oral tolerance (67), and they should not block maternal antibodies (68). The observation that maternal antibodies interfere with developing adaptive immune responses following immunization of chicks is well documented (64, 69); furthermore, the presence of maternal antibodies is an important consideration for the timing of commercial vaccination (65, 70). Thus, the presence of antigen specific maternal IgA in the embryonic and post hatch digestive tracts, might render oral immunization during this period to be counterproductive, for the immunizing antigen might be blocked by resident maternal IgA, thus both depleting maternal IgA and limiting the effective priming dose of the vaccine.

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