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Review article

The sheep and cattle Peyer's patch as a site of B-cell development

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Abstract – In sheep and cattle, the ileal Peyer's patch (PP), which extends one-two meters along the terminal small intestine, is a primary lymphoid organ of B-cell development. B-cell diversity in the ileal PP is thought to develop by combinatorial mechanisms, gene conversion and/or point mutation. These species also have jejunal PP that function more like secondary lymphoid tissues concerned with mucosal immune reactions. These two types of PP differ significantly in their histology, ontogeny and the extent of lymphocyte traffic. The prenatal development of follicles in the PP begins first in the jejunum during the middle of gestation and then in the ileum during late gestation. B-cells proliferate rapidly in the ileal PP follicle; up to five percent of these cells survive while the majority dies by apoptosis, perhaps driven by the influence of environmental antigen and/or self-antigen. The surviving cells migrate from the ileal PP and populate the peripheral B-cell compartment. By adolescence, the ileal PP has involuted but the function of jejunal PP, compatible with a role as secondary lymphoid organ, continues throughout life. In this review, we focus on the development of PP as a site of B-cell repertoire generation, positive and negative B-cell selection, and the differences between ileal PP and jejunal PP.

B-cell development / apoptosis / antibody diversity / selection / Peyer's patch

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1. INTRODUCTION

Although in the rodent, B-cell development and primary B-cell repertoire diversification occurs in the bone marrow, the major site where these processes occur in sheep and cattle is the ileal Peyer's patch (PP). The ileal PP has the characteristics of a mammalian equivalent of the avian bursa of Fabricius. The original report of PP by Johann Conrad Peyer in 1677 included a description of the "intestinal glands" in sheep [87]. In 1956, Glick showed that the bursa of Fabricius of chickens contributes to antibody production whereas the thymus contributes to graft rejection [36]. This seminal observation was perhaps the start of the modern era of immunology. After the description of the role of the bursa of Fabricius in the immune function of the chicken many investigators hunted for the mammalian homologue. At first, the rabbit appendix received much attention but there was no clear evidence at that time to show that the appendix played a crucial role in B-cell development [6, 20, 21]. Later the ileal PP of ruminants was proposed to be a bursal equivalent [55, 102]. At about the same time, studies in mice reported that early phases of the B-cell lineage occur in both the liver and the bone marrow [5, 80]. The bone marrow also was found to be an important site of B-cell lymphopoiesis in postnatal mice [77–79]. In addition, it was shown that B-cell production in the rudimentary PP of neonatal mice could not account for the rapid postnatal rise in the number of B-cells in the circulation [32]. Furthermore, the rabbit PP was found to be the source of precursors for IgA plasma cells that distributed along the gastrointestinal tract [23]. After these studies, the central role of the bone marrow in B-cell lymphopoiesis and the role of PP in mucosal immunity became an accepted part of the framework of modern immunology.

Our goal is to summarize and reassess the role of PP as a site where B-cell diversity is generated by post-rearrangement diversification mechanisms and both positive and negative selection. We also include comparisons of ileal PP and jejunal PP in ruminants and the avian bursa of Fabricius.

2. MOLECULAR MECHANISMS OF B-CELL DIVERSITY AND Ig ISOTYPES

In mice, there are over 100 VH germline gene segments whereas in the human there are around 100 genes and pseudogenes; these are grouped into 7 families in human and 14 families in mice [19, 54, 85]. The IgH chains of cattle and sheep are very similar at both the nucleotide and protein levels. In sheep, nine germ-line VH gene segments, including three pseudogenes, have been detected and these VH genes belong to only one family, closely related to the human VH4 family (70% nucleotide identity) [24]. Similarly, the cattle Ig heavy chain repertoire is dominated by a single VH gene family that is homologous with human VH2 [109]. Cattle VH genes also contain long CDR3 regions averaging 21 amino acids in length [12]. This is larger than the CDR3's in other mammals. In adults this region appears to be extensively hypermutated [12]. Sheep also have six JH gene segments, of which two are functional and four are pseudogenes [25]. Recently, hypermutation of sheep VH was well studied by Gontier et al. [37]. In sheep VH, it seems that rearrangements continue to occur for several months after birth. It was shown that the bovine IgH could be generated from segments at two distinct genomic locations [49]. The presence of several JH and other gene segments, such as VH and DH, plus flexibility at the junctions between these segments, as well as templated and non-templated nucleotide additions, each contribute to the generation of IgH diversity.

Igλ is the major light chain isotype in sheep and cattle, Igκ light chains being expressed by only 9 to 25% of B-cells in these and other farm animals [10, 39]. In the original studies of the sheep it was concluded that there were 60–90 Vλ genes distributed

in at least six families. It was also concluded that preferential rearrangement occurred such that only about 20 Vλ are found in rearranged DNA. These studies of the ovine Ig repertoire further indicated a major role for somatic hypermutation and that this process is antigen-independent [96–98]. This conclusion has now been modified in light of our recent studies in which we detected many unique nucleotide patterns within Vλ gene segments and these patterns led to the proposition of 64 new gene segments [50]. Therefore, combinatorial rearrangement is likely to make a much larger contribution and somatic hypermutation a much smaller contribution to the Igλ diversity in sheep. However, it cannot be ruled out that gene conversion may also contribute to the generation of diversity in sheep. In addition, studies of the diversification of the bovine Igλ light chain have provided evidence that gene conversion occurs in this species [63, 84]. Gene conversion is well known to be a dominant mechanism that diversifies the B-cell repertoire in the chicken [95]. Recently, it was shown that the bursal B-cell line DT40 undergoes Ig gene conversion and that activation-induced cytidine deaminase (AID) is required for the gene conversion events [8]. AID also has been shown to mediate somatic hypermutation and Ig isotype switch recombination in mice and humans [71, 106]. We have screened for AID gene expression in sheep ileal and jejunal PP single follicles (manuscript in preparation) and found it to be present at a number of developmental stages. Sheep and calf might have very similar molecular machinery driving the generation of Ig diversity but this clearly needs to be investigated further.

The constant regions of bovine IgD, IgM, IgG1, IgG2, IgG3, IgE and IgA have been well characterized [16, 52, 69, 70, 94, 111, 121]. The constant regions identified in sheep are IgD, IgM (possibly two IgM allotypes), IgG1, IgG2, IgE and IgA [18, 24, 31, 45, 86, 114, 121].

3. PRE-PP PHASE OF B-CELL DEVELOPMENT

B-cell development during the pre-PP phase of development in sheep is summarized in Figure 1. In sheep, hemopoiesis occurs in the yolk sac between 19 and 27 days of gestation (sheep reach term at about 150 days) [1]. The first IgM^+ cells appear in the spleen at 45–48 days gestation [90]; the numbers increase thereafter with proliferating B-cells evident at 51 days gestation and clusters of B-cell lymphopoiesis detected by 60–80 days gestation [43, 90]. Expression of the Ig Vλ gene is found earlier in the spleen, at 48 days gestation, than in any other tissue (liver, intestine, blood and bone marrow) [51]. Also in the fetal lamb, evidence of diversity of the Ig Vλ gene is seen earlier, and at higher levels, in the spleen (61 days gestation) than in other tissues using anchored PCR method [46]. Ig Vλ gene diversity is also found in fetal cattle spleen but not in the liver, ileum, or blood during the pre-PP phase of development [63]. It has been shown in the 56 days old fetal lamb that a single injection of anti-IgM antibody will cause the failure of development of lymphoid follicles in PP; however, splenectomy at the same fetal age did not prevent PP formation [91, 93]. These studies provided evidence that B-cells do not continually enter the rudimentary PP follicles. Also notable was that these studies provided evidence that the fetal spleen can not be solely responsible for the development of PP. In the chicken the B-cell precursors seed various organs but only expand in the bursa of Fabricius, a site of primary B-cell development. The evidence for this comes from studies involving bursectomy before hatching which caused both the depletion of B-cells in the periphery and the elimination of IgG synthesis [22, 36, 116]. It is during the first trimester that both hemopoiesis and lymphopoiesis begin. Apparently at some later stage the predominant site of B-cell development switches from the spleen to the intestinal tract. The events regulating this switch are not understood.

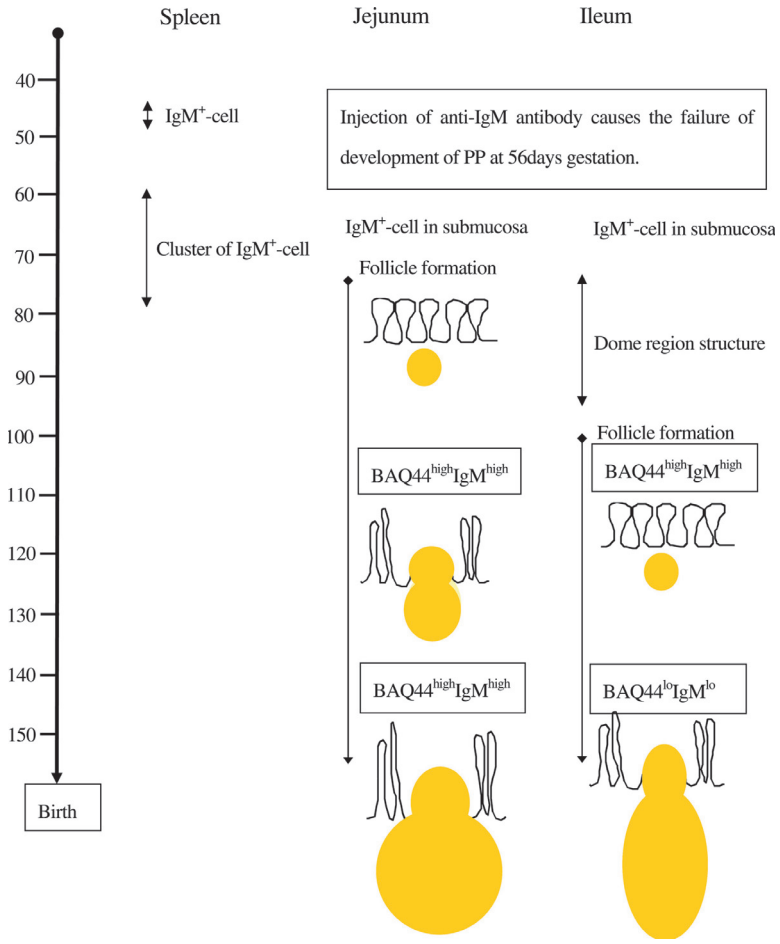

Figure 1. B-cell development in the fetal spleen, jejunum and ileum.

4. PRENATAL DEVELOPMENT OF ILEAL AND JEJUNAL PP

Construction of the structure of the PP starts first in the jejunum and then later in the ileum (Fig. 1). This prenatal development proceeds independently of external antigen. The earliest IgM^+ cells are detected in the jejunal submucosa at 65 days gestation [3]. Primordia of jejunal PP can be found at 60 days gestation and lymphoid follicles are present by 75 days [102]. IgM^+ cells first appear in the terminal ileum at 68–70 days gestation [3, 90]. Primordia of ileal PP are seen with the appearance of a dome structure at 70–95 days gestation and primordial lymphoid follicles appear at 97 days [75]. Lymphopoiesis has been shown by ^3H-thymidine uptake and autoradiography to be well established in the jejunal PP by 100 days gestation [102]. These proliferating cells are found in the follicular region but not in the dome region. At day 132 of gestation the extent of lymphopoiesis in the

follicle is obvious. The PP follicles have a much higher proportion of proliferating cells than is seen in other lymphoid tissues including the thymus. Immunohistochemical analysis of the cells in rudimentary ileal PP at 103 days gestation has shown that their phenotype is similar to that of B-cells in the spleen and peripheral blood of the fetus and this is similar to the mature B-cells in the postnatal lambs [40]. The cells in the rudimentary ileal and jejunal PP have a surface phenotype that is IgM^{high} and $BAQ44A^{high}$. BAQ44A is a cell surface differentiation marker that is present on B-cells in peripheral lymphoid tissues [47]. During the later phases of fetal development there is a decline in the number of $BAQ44A^+$ cells in both the ileal and the jejunal PP. By day 142 of gestation, the surface phenotype of the B-cells in the follicles has changed to become IgM^{lo} and $BAQ44A^{lo}$. During prenatal development the expression of BAQ44A and sIgM are quite similar on B-cells in the ileal and jejunal PP, however, in the postnatal lamb no BAQ44A is detected on the surface of the B-cells in the ileal PP follicles [40]. Therefore, during prenatal development, the phenotype of cells in both types of PP follicles seems similar but the reasons for the earlier development of follicles in the jejunum than in the ileum is unknown.

The total number of PP and their constituent follicles has fully developed before birth with 25–40 discrete PP in the jejunum and one single continuous PP in the terminal ileum [102]. The development of PP in the fetal calf is similar to that in fetal lambs in that the jejunal PP are the first to appear at mid gestation [120]. The lymphoid follicles in both types of PP in the fetal calf also consist of mostly IgM^+ cells, with very few $CD3^+$ and IgG^+ cells found.

B-cells in these ileal PP follicles develop oligoclonally [76, 96]. The oligoclonal development of individual bursal follicles has also been reported in the chicken [7, 88]. In contrast, the clonality of jejunal PP follicles is not yet clear and it needs further analysis. The jejunal PP is considered to be a peripheral lymphoid organ, but as they also develop before birth, in the absence of exogenous antigen, their function is likely to be more complex.

5. POSTNATAL CHANGES IN ILEAL AND JEJUNAL PP PRIOR TO ADOLESCENCE

The postnatal changes in ileal and jejunal PP are summarized in Figure 2. After birth no BAQ44A is detected on the surface of the B-cells in the ileal PP follicles [40]. Instead, this marker is restricted to the region where the dome merges with the follicle. These cells are destined to survive and emigrate to the periphery via adjacent lymphatics. These $BAQ44A^+IgM^+$ cells account for only 5–18% of all B-cells in the ileal PP compared with over 70% of B-cells in ileal mesenteric lymph node and jejunal PP. At 6–12 weeks of age $CD4^+$ T-cells account for about 25% of the cells in jejunal PP but only about 1% of cells in the ileal PP. In calves at around one month of age many $CD4^+$ cells have migrated into jejunal PP follicles but not into ileal PP follicles. Also of note at this stage is that IgG and IgA mRNA can both be detected in the jejunal PP follicles, but not in the ileal PP follicle [120]. Similarly, in the chicken, IgG^+ cells are found in the bursal follicle, as well as in germinal centers, but IgG mRNA expression is only detected in germinal centers [117, 119, 120]. This may be because $CD4^+$ cells do not enter the medulla of the bursal follicle. One interpretation of these results is that Ig class switching does not occur in the follicles of the ileal PP or follicular medulla of the bursa because they do not contain the helper T-cells that are needed for cognate interaction with B-cells.

6. STROMAL CELLS IN THE ILEAL AND JEJUNAL PP

Putative early stromal cells in the jejunal PP of fetal sheep were first identified by

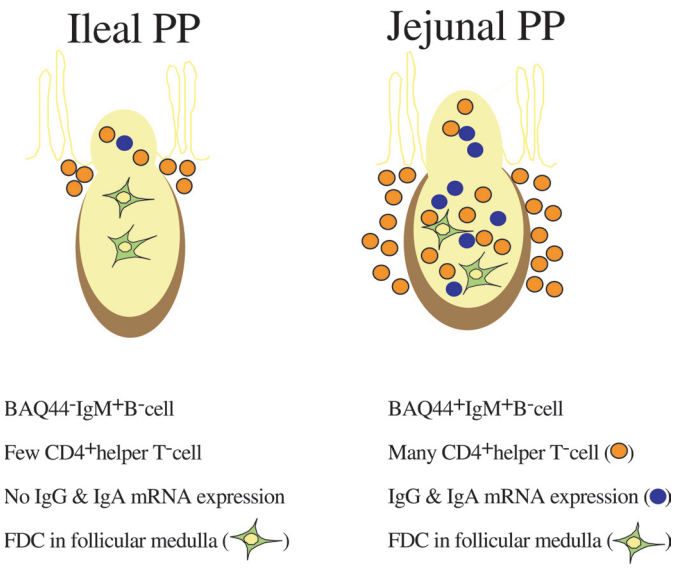

Figure 2. Characteristics and comparison between postnatal ileal and jejunal PP.

histology [102]. By 97 days gestation lymphocytes start to accumulate and primitive mesenchymal cells without extracellular fibers are detected in the ileal PP primordia [75]. By 110 days gestation, the stromal cells in the dome region and in the follicle primordia have differentiated into reticular fibroblasts with processes that are seen to surround clusters of lymphocytes. In fetal lambs during the last month of gestation the ileal PP follicles contain an extensive network of stromal cells that react with 5' nucleotidase [75]. Follicular dendritic cells that are reactive with 5' nucleotidase and Mg^{2+} dependent adenosine triphosphatase are also seen in the ileal PP as well as in other primary lymphoid follicles in peripheral lymph nodes [43, 75]. Depletion of B-cells from fetal lambs by anti IgM antibody injection generated PP follicles composed of clusters of FDC's [60, 92]. Ileal PP follicles have also been used to generate a number of clones of mesenchymally-derived stromal cells [38, 39]. Interestingly, co-culture with one stromal cell line inhibited the B-cell proliferative response, whereas, co-culture with another cell line enhanced B-cell proliferation. Both responses were independent of T-cells and extrinsic antigen but contact between the B-cell and stromal cell line was necessary. Thus, in the ileal PP follicle there may be stromal cells that can have either a positive or a negative impact on B-cell growth.

7. FOLLICLE-ASSOCIATED EPITHELIUM AND OTHER ACCESSORY CELLS OF ILEAL AND JEJUNAL PP

Follicle-associated epithelium (FAE) is found in the epithelium that covers the dome region of the jejunal and ileal PP. Scattered among the absorptive epithelial cells of the FAE are membranous cells (M-cells) with short microvilli or folds and cytoplasmic vesicles similar to those in other species. The FAE of ileal PP, however, has some unique characteristics. For example, they contain fifty nanometer membrane-bound particles that contain carbonic anhydrase; these are found in the ileal PP of both calves and lambs, but not in FAE of the jejunal PP

[56, 58]. In vitro studies of ileal epithelial cells from fetal lambs suggest that epithelial differentiation does not occur in the absence of lymphocytes [2]. A similar in vitro co-culture system has been established in which murine PP lymphocytes convert human intestinal epithelium cells (CaCo2) into functional M-cells [53]. The early differentiation of epithelial cells into FAE might be dependent on the early presence of B-cells [89]. However, the FAE in ileal PP of B-cell depleted fetal lambs showed a normal differentiation [60]. FAE and M-cells are very important for phagocytosis and transcytosis of external antigens [57]. Large particles and bacteria induce phagocytosis that is associated with rearrangement of the actin cytoskeleton and this permits active formation of pseudopod-like structures [15, 33]. Viruses and adherent particles are taken up by endocytosis, but non-adherent materials are internalized by fluid phase endocytosis [14, 74, 81]. We are a long way from understanding the origin, differentiation, and fate of FAE in ileal PP.

The analysis of other accessory cells found in the ileal PP follicles has involved the use of the B-cell depleted fetal lambs described above [42, 60, 89, 92]. The rudimentary follicles of ileal PP in these animals show strong reactivity for 5' nucleotidase, indicating the presence of follicular dendritic cells. Mg-ATPase$^+$ dendritic cells are present at an early developmental stage [89] as are macrophages that stain with non-specific esterase and acid phosphatase [42, 92]. The sheep ileal PP follicle also contains many tingible-body macrophages whose role it is to rapidly eliminate apoptotic cells [13].

8. APOPTOSIS OF B-CELL IN ILEAL AND JEJUNAL PP

The ileal PP in lambs is a major site of B-cell lymphopoiesis, generating approximately 3.6×10^9 cells/hour (mitotic rate is 5.2%/hour in fetal ileal PP) [83, 99, 100]. Although the thymus is known as a major site of T-cell proliferation and selection, the mitotic rate of the thymus is 15–20 fold less than for the ileal PP. However, very few of the B-cells (up to 5%) produced in the ileal PP and in the chicken bursa of Fabricius differentiate and emigrate, instead the vast majority of the cells soon die by apoptosis [66, 99]. Apoptosis is characterized morphologically by chromatin condensation and fragmentation into multiple nucleosome-sized units [17, 66, 67]. Due to the extensive cell death occurring in the ileal PP the rapid clearance of these apoptotic cells is essential for tissue homeostasis. It is reported that macrophages resident in the ileal PP follicle mediate the phagocytosis and removal of discarded cells [13]. Using the DNA laddering assay, low molecular weight DNA is found in the BAQ44A$^-$ cells in the ileal PP but not detected in the BAQ44A$^+$ cells which are isolated from ileal PP [65]. Therefore, BAQ44A$^+$ cells might be positively selected within the ileal PP and then migrate to the periphery. The specific mechanisms leading to the induction of apoptosis in the ileal PP are not well characterized. Some studies have implicated increased Ig sequence diversity in the induction of apoptosis [65]. This proposal was based on the observation of (1) significantly greater replacement to silent mutation ratios in the complementarity determining regions, (2) a more random distribution of mutations, and (3) the lack of mutational specificity compared with the mutational bias favoring transitions and purines in B-cells about to migrate. It is important to consider that these observations were made prior to the proposition of more than 60 new germline Vλ gene segments and as such may not reflect the situation in the ileal PP. Other studies have reported the induction of apoptosis in ileal PP B-cells following the cross-linking of surface Ig [70]. This B-cell receptor mediated apoptosis is likely to play an important role in the elimination of autoreactive B-cells generated during B-cell maturation and/or after antigenic challenge in ileal and jejunal PP. The cross-linking of surface immunoglobulin also induces apoptosis in cell lines with an immature B-cell

phenotype such as WEHI-231 cells [11, 44]. However, using a human follicular lymphoma cell line and a Burkitt lymphoma cell line as an in vitro model, apoptotic signaling events occurring before mitochondrial dysfunction have been demonstrated to be totally reversed by anti-apoptotic signaling, such as CD40 stimulation [26, 64]. The cognate interaction between CD40 expressed on B-cell and CD40 ligand (CD154) expressed on helper T-cell induces the expression of the proto-oncogene Bcl-2 that acts as a repressor of apoptosis [61]. In lambs, the expression of Bcl-2 is detected in the spleen, mesenteric lymph nodes and thymus but not in ileal PP [68]. However, under cell culture conditions, the expression of Bcl-2 can be markedly induced when ileal PP B-cells are cultured with phorbol ester and Ca^{2+} ionophore, a procedure that has been demonstrated to rescue these cells from apoptosis. In other studies, co-culture of B-cells that had been isolated from sheep PP follicles with murine CD40 ligand induced B-cell responsiveness to several cytokines, whereas the removal of murine CD40 ligand resulted in the induction of cell death. A similar response is observed with both ileal PP B-cells and jejunal PP B-cells [41]. In chickens, B-cells derived from splenic germinal centers express Bcl-2 mRNA, but not those from the bursa [30, 118]. Helper T-cells are distributed in the light zone of chicken germinal centers but not in the bursal follicular medulla, a region that is similar to a germinal center light zone [117]. Similar results are reported in the calf where many T-cells are found in jejunal but not in ileal PP follicle [47, 117, 120]. Therefore, cognate T-cell interaction might not occur within ileal PP follicles and or the chicken bursal follicular medulla. Despite this absence of T-cells, further study on the kinetics and distribution of other accessory cells such as dendritic cells, macrophage and follicular dendritic cells within both the ileal and jejunal PP is needed particularly because these accessory cells also have many co-stimulatory molecules on their surface. Some of these co-stimulatory molecules may rescue B-cells from apoptosis whereas others may induce B-cell death by apoptosis.

9. ILEAL AND JEJUNAL PP AS THE SITE OF B-CELL SELECTION AND THE EFFECT OF ANTIGEN

In fetal lambs lymphopoiesis in ileal and jejunal PP is not dependent on antigens. Newborn animals, however, are exposed to many extrinsic antigens that come into the gut. The effect of these antigens on the development of ileal and jejunal PP in sheep has been examined using intestinal loops isolated from the rest of the gut during the last month of gestation [35, 73, 103]. After birth these ileal PP loops, have no contact with antigen yet, they grow at normal rates before birth and for the first two weeks after birth. After this time, however, the follicles of the isolated ileal PP undergo an accelerated involution such that by 3–4 months of age they have almost completely disappeared. The follicles of normal ileal PP do not involute until around 15 months of age. From these experiments it appears that extrinsic antigen is very important for the development of sheep PP after birth. In other studies the immune reactivity of the ileal and jejunal PP were compared using intestinal loops [72]. Following antigen injection into jejunal and ileal PP loops, specific antibody forming cells and antibody titer of the jejunal PP loops were both much higher than those of ileal PP loops. In addition, after oral injection of rotavirus/iscom vaccines, specific antibody was detected in jejunal PP of lambs [112]. Hence, the jejunal PP seems to be an efficient site for the induction of a mucosal immune response. Similar approaches have also been used to study the chicken bursa of Fabricius as it can be isolated from external antigens by bursal duct ligation [27–29]. Isolation from gut-derived antigens causes suppression of each of bursal development, splenic germinal center formation, and the development of

serum natural agglutinins to bacteria or heteroerythrocytes. Studies involving an analysis of Ig light chain diversity in single follicles derived from the chicken bursa, have shown that the pre-immune repertoire is generated by gene conversion during antigen independent B-cell proliferation. After birth, antigen stimulation via bursal epithelium plays a role in the selection of B-cell clones [9]. Using retroviral gene transfer techniques which induce truncated IgM as B-cell receptor, it is clearly demonstrated that the regulation of chicken bursal B-cell development after hatching differs from that in the embryo [107, 108]. Therefore further studies are required to determine the effect of external antigens on the selection of B-cells within follicles of both types of PP follicles as well as the functional difference between the ileal and jejunal PP.

10. CELL MIGRATION FROM ILEAL AND JEJUNAL PP

Evidence that the ileal PP supplies B-cells to the periphery has come from several studies. Surgical removal of the ileal PP from neonates caused a prolonged, widespread B-cell deficiency [34]. Although the ileal PP is a site of vigorous B-cell production, approximately 95% die soon after they are generated. In spite of the significant excess of ileal PP B-cells production over emigration, the total number leaving ileal PP is still very large, approximately $5–10 \times 10^7$ cells/hour [99]. Ileal PP emigrants compose 10–15% of ileal mesenteric lymph node, 1–2% of jejunal mesenteric lymph node, jejunal PP, prescapular lymph node and 3–4% of spleen cells [82, 83, 105]. It is also reported that the number of cells emigrating from ileal PP is 10 times greater than from jejunal PP. All cells that emigrate from the PP must pass through the mesenteric lymph node on their way to the circulation. During this passage there may be an additional opportunity for B-cell regulation/selection [104, 105]. The B-cells that emigrate from the ileal PP have higher levels of surface IgM^+ than most follicular B-cells and they also express BAQ44A, a marker of more mature B-cells.

11. INVOLUTION OF ILEAL PP

At about 12 weeks after birth, the sheep ileal PP begins to involute and only a few PP follicles remain in this region of the intestine at 18 months of age [102]. It has also been demonstrated that the life-span of both types of PP is not related to their specific location in the small intestine [101]. Recently it was reported that jejunal PP type tissue is seen in the ileal region during involution of ileal PP and even four year-old sheep still have some PP-like follicles [59].

12. FUTURE PERSPECTIVES ON ILEAL AND JEJUNAL PP

The ileal PP of young sheep and cattle are not unique lymphoid tissues, at least from the perspective that many species of veterinary relevance (sheep, cattle, swine, horse, rabbit, and chicken) generate their B-cell repertoires and populate their peripheral B-cell compartment using gut-associated lymphoid follicles (Tab. I). The PP in rodents does not function in the same fashion. Therefore, results obtained from studies of PP in the mouse do not necessarily apply to species in which B-cell development depends on gut-associated B-cell follicles. The jejunal PP in sheep and cattle may be more functionally equivalent to the PP in rodents, playing a role as a secondary lymphoid organ and focusing on mucosal immune responses. In the jejunal PP, various aspects of B-cell differentiation such as memory B-cell induction, Ig isotype switching, and plasma cell induction will likely require the influence of helper T-cell. The lack of T cells in the ileal PP begs the question of how a process like proliferation might be regulated? Do the jejunal PP begin life as a primary lymphoid organ but then evolve after birth into a secondary organ

Table I. Interspecies comparison of the gut-associated lymphoid tissues.

Species	Primary B-cell repertoire		References
	Organ	Molecular mechanisms	
Sheep	**Ileal PP**[a]	rearrangement and/or SH[b]	[50, 97, 98, 102]
Calf	**Ileal PP**[a]	GC[c] and SH	[63, 84, 117, 120]
Swine	**Ileal PP**[a]	GC[d] and SH	[4, 110]
Horse	**Ileal PP**[a]	GC[d] and SH	[48, 62]
Rabbit	**Appendix**[a]	GC and SH	[113, 115]
Chicken	**The bursa**[a]	GC and SH	[36, 95, 117]
Mice	Bone marrow	V (D) J recombination	

[a] Bold shows gut-associated organ.
[b] SH: somatic hypermutation.
[c] GC: Gene conversion.
[d] Antibody repertoire in these animals forms from a relatively small number of variable genes of one or several families. Therefore gene conversion events are expected.

with a focus on mucosal immunity? Why does this not happen in the ileal PP? Is it due to the lack of T-cells in the ileal PP? The location of the primary gut-associated B-cell follicles adjacent to the intestinal lumen is likely to be an important reason that we do not yet understand. These and many other questions can be most effectively addressed using models based on sheep and calf PP.

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REFERENCES

[1] Al Salami M., Simpson-Morgan M.W., Morris B., Haemopoiesis and the development of immunological reactivity in the sheep foetus, in: Morris B., Miyasaka M. (Eds.), Immunology of the Sheep, Editions Roche, Basel, 1985, pp. 19–36.

[2] Alitheen N., McClure S., McCullagh P., Segregation of B lymphocytes into stationary apoptotic and migratory proliferating subpopulations in agglomerate cultures with ileal epithelium, Eur. J. Immunol. 31 (2001) 2558–2565.

[3] Alitheen N., McClure S., McCullagh P., Development of B cells in the gut-associated lymphoid tissue of mid-gestational fetal lambs, Dev. Comp. Immunol. 27 (2003) 639–646.

[4] Andersen J.K., Takamatsu H., Oura C.A.L., Brookes S.M., Pullen L., Parkhouse R.E.M., Systemic characterization of porcine ileal Peyer's patch. I. Apoptosis-sensitive immature B cells are the predominant cell type, Immunology 98 (1999) 612–621.

[5] Andrew T.A., Owen J.J.T., Studies on the earliest sites of B cell differentiation in the mouse embryo, Dev. Comp. Immunol. 2 (1978) 339-346.

[6] Archer O.K., Sutherland D.E.R., Good R.A., Appendix of the rabbit: a homologue of the bursa in the chicken? Nature 200 (1963) 337–339.

[7] Arakawa H., Kuma K., Yasuda M., Furusawa S., Ekino S., Yamagishi H., Oligoclonal development of B cells bearing discrete Ig chains in chicken single germinal centers, J. Immunol. 160 (1998) 4231–4241.

[8] Arakawa H., Hauschild J., Buerstedde J.M., Requirement of the activation-induced deaminase (AID) gene for immunoglobulin gene conversion, Science 295 (2002) 1301–1306.

[9] Arakawa H., Kuma K., Yasuda M., Furusawa S., Ekino S., Shimizu A., Yamagishi H., Effect of environmental antigens on the Ig diversification and the selection of productive V-J joints in the bursa, J. Immunol. 169 (2002) 818–828.

[10] Arun S.S., Breuer W., Hermanns W., Immunohistochemical examination of light-chain expression (lambda/kappa ratio) in canine, feline, equine, bovine and porcine plasma cells, Zentralbl. Veterinarmed. A 43 (1996) 573–576.

[11] Benhamou L.E., Cazenave P.A., Sarthou P., Anti-immunoglobulins induce death by apoptosis in WEHI-231 B lymphoma cells, Eur. J. Immunol. 20 (1990) 1405–1407.

[12] Berens S.J., Wylie D.E., Lopez O.J., Use of a single VH family and long CDR3s in the variable region of cattle Ig heavy chains, Int. Immunol. 9 (1997) 189–199.

[13] Bhogal H.S., Kennedy L.J., Babic K., Reynolds J.D., The role of macrophages in the removal of apoptotic B-cells in the sheep ileal Peyer's patch, Dev. Comp. Immunol. 28 (2004) 843–853.

[14] Bockman D.E., Cooper M.D., Pinocytosis by epithelium associated with lymphoid follicles in the bursa of Fabricius, appendix, and Peyer's patches. An electron microscopic study, Am. J. Anat. 136 (1973) 455–477.

[15] Borghesi C., Regoli M., Bertelli E., Nicoletti C., Modifications of the follicle-associated epithelium by short-term exposure to a non-intestinal bacterium, J. Pathol. 180 (1996) 326–332.

[16] Brown W.R., Rabbani H., Butler J.E., Hammarstrom L., Characterization of bovine C α gene, Immunology 91 (1997) 1–6.

[17] Buja L.M., Eigenbrodt M.L., Eigenbrodt E.H., Apoptosis and necrosis. Basic types and mechanisms of the cell death, Arch. Pathol. Lab. Med. 117 (1993) 1208–1214.

[18] Clarkson C.A., Beale D., Coadwell J.W., Symons D.B., Sequence of ovine Ig gamma 2 constant region heavy chain cDNA and molecular modelling of ruminant IgG isotypes, Mol. Immunol. 30 (1993) 1195–1204.

[19] Cook G.P., Tomlinson I.M., The human immunoglobulin VH repertoire, Immunol. Today 16 (1995) 237–242.

[20] Cooper M.D., Perey D.Y., McKneally M.F., Gabrielsen S.E., Sutherland D.E.R., Good R.A., A mammalian equivalent of the avian bursa of Fabricius, Lancet (1966) 1388–1391.

[21] Cooper M.D., Perey D.Y., Gabrielsen A.E., Sutherland D.E.R., McKneally M.F., Good R.A., Production of an antibody efficiency syndrome in rabbits by neonatal removal of organized intestinal lymphoid tissues, Int. Arch. Allergy Appl. Immunol. 33 (1968) 65–88.

[22] Cooper M.D., Cain W.A., Van Alten P.J., Good R.A., Development and function of the immunoglobulin producing system. 1. Effects of bursectomy at different stages of development on germinal centers, plasma cells, immunoglobulin and antibody production, Int. Arch. Allergy 35 (1969) 242–252.

[23] Craig S.W., Cebra J.J., Peyer's patches: a enriched source of precursors for IgA producing immunocytes in the rabbit, J. Exp. Med. 134 (1971) 188–200.

[24] Defour V., Malinge S., Nau F., The sheep Ig variable region repertoire consists of a single VH family, J. Immunol. 156 (1996) 2163–2170.

[25] Defour V., Nau F., Genomic organization of the sheep immunoglobulin JH segments and their contribution to heavy chain variable region gene diversity, Immunogenetics 46 (1997) 283–292.

[26] Eeva J., Postila V., Mättö M., Nuutinen U., Ropponen A., Eray M., Pelkonen J., Kinetics and signaling requirements of CD40-mediated protection from B cell receptor-induced apoptosis, Eur. J. Immunol. 33 (2003) 2783–2791.

[27] Ekino S., Nawa Y., Tanaka K., Matsuno K., Fujii H., Kotani M., Suppression of immune response by isolation of the bursa of Fabricius from environmental stimuli, Aust. J. Exp. Med. Sci. 58 (1980) 289–296.

[28] Ekino S., Suginohara K., Urano T., Fujii H., Matsuno K., Kotani M., The bursa of Fabricius: a trapping site for environmental antigens, Immunology 55 (1985) 405–410.

[29] Ekino S., Riwar B., Krose F.G.M., Schwander E.H., Koch G., Nieuwenhuis P., Role of environmental antigen in the development of $sIgG^+$ cells in the bursa of Fabricius, J. Immunol. 155 (1995) 4551–4558.

[30] Eguchi Y., Ewert D.L., Tsujimoto Y., Isolation and characterization of the chicken bcl-2 gene: expression in variety of tissues including lymphoid and neuronal organs in adults and embryo, Nucleic Acids Res. 20 (1992) 4187–4192.

[31] Engwerda C.R., Sandeman R.A., Stuart S.J., Sandeman R.M., Isolation and sequence of sheep immunoglobulin E heavy-chain complementary DNA, Vet. Immunol. Immunopathol. 34 (1992) 115–126.

[32] Friedberg S.H., Weissman I.L., Lymphoid tissue architecture. II. Ontogeny of peripheral T and B cells in mice: evidence against Peyer's patches as the site of generation of B cells, J. Immunol. 113 (1974) 1477–1492.

[33] Fujimura Y., Functional morphology of microfold cells (M cells) in Peyer's patches–phagocytosis and transport of BCG by M cells into rabbit Peyer's patches, Gastroenterol. Jpn. 21 (1986) 325–335.

[34] Gerber H.A., Morris B., Trevella W., The role of gut-associated lymphoid tissues in the generation of immunoglobulin-bearing lymphocytes in sheep, Aust. J. Exp. Med. Sci. 64 (1986) 201–213.

[35] Gerdts V., Uwiera R.R.E., Mutwiri G.K., Wilson D.J., Bowersock T., Kidane A., Babiuk L.A., Griebel P.J., Multiple intestinal "loops" provide and in vivo model to analyse multiple mucosal immune responses, J. Immunol. Methods 256 (2001) 19–33.

[36] Glick B., Chang T.S., Jaap R.G.A., The bursa of Fabricius and antibody production on the domestic fowl, Poult. Sci. 35 (1956) 224–225.

[37] Gontier E., Ayrault O., Godet I., Nau F., Ladevèze V., Developmental progression of immunoglobulin heavy chain diversity in sheep, Vet. Immunol. Immunopathol. 103 (2005) 31–51.

[38] Griebel P.J., Hein W.R., Dudler L., Ferrari G., Phenotype and function of stromal Cells cloned from the ileal Peyer's patch of sheep, Stem Cells 11 (1983) 130–143.

[39] Griebel P.J., Ferrari G., Evidence for a stromal cell-dependent, self-renewing B cell population in lymphoid follicles of the ileal Peyer's patch of sheep, Eur. J. Immunol. 24 (1994) 401–409.

[40] Griebel P.J., Kennedy L., Graham T., Davis W.C., Reynolds J.D., Characterization of B-cell phenotypic changes during ileal and jejunal Peyer's patch developmental sheep, Immunology 77 (1992) 564–570.

[41] Griebel P.J., Beskorwayne T., Van den Broeke A., Ferrari G., CD40 signaling induces B cell responsiveness to multiple members of the gamma chain-common cytokine family, Int. Immunol. 11 (1999) 1139–1147.

[42] Halleraker M., Landsverk T., Nicander L., Organization of ruminant Peyer's patches as seen with enzyme histochemical markers of stromal and accessory cells, Vet. Immunol. Immunopathol. 26 (1990) 93–104.

[43] Halleraker M., Press C.M., Landsverk T., Development and cell phenotype in primary follicles of foetal sheep lymph nodes, Cell Tissue Res. 275 (1994) 51–62.

[44] Hasbold J., Klaus G.G., Anti-immunoglobulin antibodies induce apoptosis in immature B cell lymphomas, Eur. J. Immunol. 20 (1990) 1685–1690.

[45] Hein W.R., Dudler L., Nucleotide sequence of the membrane form of sheep IgM and identification of two C mu allotypes, Mol. Immunol. 30 (1993) 783–784.

[46] Hein W.R., Dudler L., Diversity of Ig light chain variable region gene expression in fetal lambs, Int. Immunol. 10 (1998) 1251–1259.

[47] Hein W.R., Dudler L., Mackay C.R., Surface expression of differentiation antigens on lymphocytes in the ileal and jejunal Peyer's patches of lambs, Immunology 68 (1989) 365-370.

[48] Home W., Ford J., Gibson D., L chain isotype regulation in horse. I. Characterization of Ig λ genes, J. Immunol. 149 (1992) 3927–3936.

[49] Hosseini A., Campbell G., Prorocic M., Aitken R., Duplicated copies of the bovine JH locus contribute to the Ig repertoire, Int. Immunol. 16 (2004) 843–852.

[50] Jenne C.N., Kennedy L.J., McCullagh P., Reynolds J.D., A new model of sheep Ig diversification: shifting the emphasis toward combinatorial mechanisms and away from hypermutation, J. Immunol. 170 (2003) 3739–3750.

[51] Jeong Y., Osborne B.A., Goldsby R.A., Early V lambda diversification in sheep, Immunology 103 (2001) 26–34.

[52] Kacskovics I., Butler J.E., The heterogeneity of bovine IgG2-VIII. The complete cDNA sequence of bovine IgG2a (A2) and an IgG1, Mol. Immunol. 33 (1996) 189–195.

[53] Kernéis S., Bogdanova A., Kraehenbuhl J.-P., Pringault E., Conversion by Peyer's patch lymphocytes of human enterocytes into M cells that transport bacteria, Science 277 (1997) 949–952.

[54] Kofler R., Geley S., Kofler H., Helmberg A., Mouse variable-region gene families: complexity, polymorphism and use in non-autoimmune responses, Immunol. Rev. 128 (1992) 5–21.

[55] Landsverk T., Is the ileo-caecal Peyer's patch in ruminants a mammalian "bursa-equivalent"? Acta Pathol. Microbiol. Immunol. Scand. A 92 (1984) 77–79.

[56] Landsverk T., The follicle-associated epithelium of the ileal Peyer's patch in ruminants is distinguished by its shedding of 50 nm particles, Immunol. Cell Biol. 65 (1987) 251–261.

[57] Landsverk T., Phagocytosis and transcytosis by the follicle-associated epithelium of the ileal Peyer's patch in calves, Immunol. Cell Biol. 66 (1988) 261–268.

[58] Landsverk T., Jansson A., Nicander L., Ploen L., Carbonic anhydrase– a marker for particles shed from the epithelium to the lymphoid follicles of the ileal Peyer's patch in goat kids and lambs, Immunol. Cell Biol. 65 (1987) 425–429.

[59] Lie K.-I., Aleksandersen M., Landsverk T., Lymphoid follicles of different phenotype appear in ileum during involution of the sheep ileal Peyer's patch, Dev. Comp. Immunol. 29 (2005) 539–553.

[60] Lie K.-I., Press C.M., McCullagh P., McClure S.J., Landsverk T., Differentiation of the follicle-associated epithelium in ileal Peyer of 50 nm particles are maintained in B-cell-depleted fetal sheep, Cell Tissue Res. 319 (2005) 395–404.

[61] Liu Y.-J., Mason D.Y., Johnson G.D., Abbot S., Gregory C.D., Hardie D.L., Gordon J., MacLennan I.C.M., Germinal center cells express Bcl-2 protein after activation by signals which prevent their entry into apoptosis, Eur. J. Immunol. 21 (1991) 2951–2962.

[62] Lowden S., Heath T., Lymphoid tissues of the ileum in young horses: distribution, structure and epithelium, Anat. Embryol. 192 (1995) 171–179.

[63] Lucier M.R., Thompson R.E., Waire J., Lin A.W., Osborne B.A., Goldsby R.A., Multiple sites of V lambda diversification in cattle, J. Immunol. 161 (1998) 5438–5444.

[64] Mackus W.J.M., Lens S.M.A., Medema R.H., Kwakkenbos M.J., Evers L.M., van Oers M.H.J., van Lier R.A.W., Eldering E., Prevention of B cell antigen receptor-induced apoptosis by ligation of CD40 occurs downstream of cell cycle regulation, Int. Immunol. 14 (2002) 973–982.

[65] Maybaum T.A., Reynolds J.D., B cells selected for apoptosis in the sheep ileal Peyer's patch have enhanced mutational diversity in the Ig Vλ light chain, J. Immunol. 157 (1996) 1474–1484.

[66] Motyka B., Reynolds J.D., Apoptosis is associated with the extensive B cell death in the sheep ileal Peyer's patch and the chicken bursa of Fabricius: a possible role in B cell selection, Eur. J. Immunol. 21 (1991) 1951–1958.

[67] Motyka B., Bhogal H.S., Reynolds J.D., Apoptosis of ileal Peyer's patch B cells is increased by glucocorticoids or anti-immunoglobulin antibodies, Eur. J. Immunol. 25 (1995) 1865–1871.

[68] Motyka B., Reynolds J.D., Rescue of ileal Peyer's patch B cells from apoptosis is associated with the induction of Bcl-2 expression, Immunology 84 (1995) 383–387.

[69] Mousavi M., Rabbani H., Hammarsrom L., Characterization of bovine ε gene, Immunology 92 (1997) 369–373.

[70] Mousavi M., Rabbani H., Pilstrom L., Hammarstrom L., Characterization of the gene for the membrane and secretory form of the IgM heavy-chain constant region gene (Cm) of the cow (*Bos Taurus*), Immunology 93 (1998) 581–588.

[71] Muramatsu M., Kinoshita K., Fagarasan S., Yamada S., Shinkai Y., Honjo T., Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA-editing enzyme, Cell 102 (2000) 553–563.

[72] Mutwiri G., Watts T., Lew L., Beskorwayne T., Papp Z., Baca-Estrada M.E., Griebel P., Ileal and jejunal Peyer's patches play distinct roles in mucosal immunity of sheep, Immunology 97 (1999) 455–461.

[73] Mutwiri G., Bowersock T., Kidane A., Sanchez M., Gerdts V., Babiuk L.A., Griebel P., Induction of mucosal immune responses following enteric immunization with antigen delivered in alginate microspheres, Vet. Immunol. Immunopathol. 87 (2002) 269–276.

[74] Neutra M.R., Phillips T.L., Mayer E.L., Fishkind D.J., Transport of membrane-bound macromolecules by M cells in follicle-associated epithelium of rabbit Peyer's patch, Cell Tissue Res. 247 (1987) 536–546.

[75] Nicander L., Halleraker M., Landsverk T., Ontogeny of reticular cells in the ileal Peyer's patch of sheep and goats, Am. J. Anat. 191 (1991) 237–249.

[76] Niku M., Pessa-Morikawa T., Andersson L.C., Iivanainen A., Oligoclonal Peyer's patch follicles in the terminal small intestine of cattle, Dev. Comp. Immunol. 26 (2002) 689–695.

[77] Osmond D.G., Population dynamics of bone marrow B lymphocytes, Immunol. Rev. 93 (1986) 103–124.

[78] Osmond D.G., Everett N.B., Radioautographic studies of bone marrow lymphocytes in vivo and in diffusion chamber cultures, Blood 23 (1964) 1–17.

[79] Osmond D.G., Nossal G.J.V., Differentiation of lymphocytes in mouse bone marrow. I. Quantitative radioautographic studies of antiglobulin binding by lymphocytes in bone marrow and lymphoid tissues, Cell. Immunol. 13 (1974) 117–131.

[80] Owen J.J., Wright D.E., Habu S., Raff M.C., Cooper M.D., Studies on the generation of B lymphocytes in fetal liver and born marrow, J. Immunol. 118 (1977) 2067–2072.

[81] Owen R.L., Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in the normal unobstructed mouse intestine: an ultra structural study, Gastroenterology 72 (1977) 440–451.

[82] Pabst R., Reynolds J.D., Evidence of extensive lymphocyte death in sheep Peyer's patches. II. The number and fate of newly-formed lymphocytes that emigrate from Peyer's patches, J. Immunol. 136 (1986) 2011–2017.

[83] Pabst R., Reynolds J.D., Peyer's patches export lymphocytes throughout the lymphoid

system in sheep, J. Immunol. 139 (1987) 3981–3985.

[84] Parng C., Hansal S., Goldsby R.A., Osborne B.A., Gene conversion contributes to Ig light chain diversity in cattle, J. Immunol. 157 (1996) 5478–5486.

[85] Pascual V., Capra J.D., Human immunoglobulin heavy-chain variable region genes: organization, polymorphism and expression, Adv. Immunol. 49 (1991) 1–74.

[86] Patri S., Nau F., Isolation and sequence of a cDNA coding for the immunoglobulin mu chain of the sheep, Mol. Immunol. 29 (1992) 829–836.

[87] Peyer J.C., Exercitatio anat. de glandulis intestinorum earumque usu et affectionibus, Schaffausen, Switzerland, 1677.

[88] Pink J.R., Vaino O., Rijinbeek A.M., Clones of B lymphocytes in individual follicles of the bursa of Fabricius, Eur. J. Immunol. 15 (1985) 83–87.

[89] Press C.M., Halleraker M., Landsverk T., Ontogeny of leukocyte populations in the ileal Peyer's patch of sheep, Dev. Comp. Immunol. 16 (1992) 229–241.

[90] Press C.M., Hein W.R., Landsverk T., Ontogeny of Leukocyte populations in the spleen of fetal lambs with emphasis on the early prominence of B cells, Immunology 80 (1993) 598–604.

[91] Press C.M., Reynolds J.D., McClure S.J., Simpson-Morgan N.W., Landsverk T., Fetal lambs are depleted of IgM^+ cells following a single injection of an anti-IgM antibody early in gestation, Immunology 88 (1996) 28–34.

[92] Press C.M., Reynolds J.D., McClure S.J., Landsverk T., Development of accessory cells in B-cell compartments is retarded in B-cell-depleted fetal sheep, Dev. Immunol. 6 (1998) 223–231.

[93] Press C.M., McCullagh P., Landsverk T., Effect of early fetal splenectomy on prenatal B-cell development in sheep, Immunology 102 (2001) 131–136.

[94] Rabbani H., Brown W.R., Butler J.E., Hammarstrom L., Polymorphism of the IGHG3 gene in cattle, Immunogenetics 46 (1997) 326–331.

[95] Reynaud C.-A., Anquez V., Grimal H., Weill J.C., A hyperconversion mechanism generates the chicken light chain preimmune repertoire, Cell 48 (1987) 379–388.

[96] Reynaud C.-A., Mackay C.A., Muller R.G., Weill J.-C., Somatic generation of diversity in a mammalian primary lymphoid organ: The sheep ileal Peyer's patches, Cell 64 (1991) 995–1005.

[97] Reynaud C.-A., Garcia C., Hein W.R., Weill J.-C., Hypermutation generating the sheep immunoglobulin repertoire is an antigen-independent process, Cell 80 (1995) 115–125.

[98] Reynaud C.-A., Dufour V., Weill J.-C., Generation of diversity in mammalian gut-associated lymphoid tissues, J. Immunol. 159 (1997) 3093–3095.

[99] Reynolds J.D., Evidence of extensive lymphocyte death in sheep Peyer's patches. I. A comparison of lymphocyte production and export, J. Immunol. 136 (1986) 2005–2010.

[100] Reynolds J.D., Mitotic rate maturation in the Peyer's patches of fetal sheep and in the bursa of Fabricius of the chick embryo, Eur. J. Immunol. 17 (1987) 503–507.

[101] Reynolds J.D., Kirk D., Two types of sheep Peyer's patches: location along gut does not influence involution, Immunology 66 (1989) 308–311.

[102] Reynolds J.D., Morris B., The evolution and involution of Peyer's patches in fetal and postnatal sheep, Eur. J. Immunol. 13 (1983) 627–635.

[103] Reynolds J.D., Morris B., The effect of antigen on the development of Peyer's patches in sheep, Eur. J. Immunol. 14 (1984) 1–6.

[104] Reynolds J.D., Pabst R., The emigration of lymphocytes from Peyer's patches in sheep, Eur. J. Immunol. 14 (1984) 7–13.

[105] Reynolds J.D., Kennedy L., Peppard J., Pabst R., Ileal Peyer's patch emigrants are predominantly B cells and travel to all lymphoid tissues in sheep, Eur. J. Immunol. 21 (1991) 283–289.

[106] Revy P., Muto T., Levy Y., Geissmann F., Plebani A., Sanal O., Catalan N., Forveille M., Dufourcq-Labelouse R., Gennery A., Tezcan I., Ersoy F., Kayserili H., Ugazio A.G., Brousse N., Muramatsu M., Natarangelo L.D., Kinoshita K., Honjo T., Fischer A., Durandy A., Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the hyper-IgM syndrome (HIGM2), Cell 102 (2000) 565–575.

[107] Sayegh C.E., Demaries S.L., Iacampo S., Ratcliffe M.J.H., Development of B cells expressing surface immunoglobulin molecules that lack V(D)J-encoded determinants in the avian embryo bursa of Fabricius, Proc. Natl. Acad. Sci. USA 96 (1999) 10806–10811.

[108] Sayegh C.E., Ratcliffe M.J.H., Perinatal deletion of B cells expressing surface Ig molecules that lack V(D)J-encoded determinants in the bursa of Fabricius is not due to intrafollicular

competition, J. Immunol. 164 (2000) 5041–5048.

[109] Sinclair M.C., Gilchrist J., Aitken R., Bovine IgG repertoire is dominated by a single diversified VH gene family, J. Immunol. 159 (1997) 3883–3889.

[110] Sun J., Hayward C., Shinde R., Christenson R., Ford S.P., Butler J.E., Antibody repertoire development in fetal and neonatal piglets. I. Four VH genes account for 80 percent of VH usage during 84 days of fetal life, J. Immunol. 161 (1998) 5070–5078.

[111] Symons D.B.A., Clarkson C.A., Beale D., Structure of bovine immunoglobulin constant region heavy chain gamma 1 and gamma 2 genes, Mol. Immunol. 26 (1989) 841–850.

[112] Van Pinxteren L.A.H., Bruce M.G., Campbell I., Wood A., Clarke C.J., Bellman A., Morein B., Snodgrass D.R., Effect of oral rotavirus/iscom vaccines on immune responses in gnotobiotic lambs, Vet. Immunol. Immunopathol. 71 (1999) 53–67.

[113] Waksman B.H., Ozer H., Blythman H.E., Appendix and M-antibody formation IV. The Functional Anatomy of the Rabbit Appendix, Lab. Invest. 28 (1973) 614–626.

[114] White G.P., Roche P., Brandon M.R., Newton S.E., Meeusen E.N., Cloning and characterization of sheep (*Ovis aries*) immunoglobulin alpha chain, Immunogenetics 48 (1998) 359–362.

[115] Winstead C.R., Zhai S.-K., Sethupathi P., Knight K.L., Antigen-induced somatic diversification of rabbit IgH genes: gene conversion and point mutation, J. Immunol. 162 (1999) 6602–6612.

[116] Yasuda M., Furusawa S., Matsuda H., Taura Y., Urano T., Yokomizo Y., Ekino S., Development of maternal IgG-free chick obtained from surgically bursectomized hen, Comp. Immunol. Microbiol. Infect. Dis. 21 (1998) 191–200.

[117] Yasuda M., Tanaka S., Arakawa H., Taura Y., Yokomizo Y., Ekino S., A comparative study of gut-associated lymphoid tissue in calf and chicken, Anat. Rec. 266 (2002) 207–217.

[118] Yasuda M., Horiuchi H., Matsuda H., Furusawa S., Immunobiology of chicken germinal center. II. Accumulation of apoptotic cells within the germinal center, Cell Tissue Res. 314 (2003) 215–221.

[119] Yasuda M., Kajiwara E., Ekino S., Taura Y., Hirota Y., Horiuchi H., Matsuda H., Furusawa S., Immunobiology of chicken germinal center. I. Changes in surface Ig class expression in the chicken splenic germinal center after antigenic stimulation, Dev. Comp. Immunol. 27 (2003) 159–166.

[120] Yasuda M., Fujino M., Nasu T., Murakami T., Histological studies on the ontogeny of bovine gut-associated lymphoid tissue: appearance of T cells and development of IgG^+ and IgA^+ cells in lymphoid follicles, Dev. Comp. Immunol. 28 (2004) 357–369.

[121] Zhao Y., Kacskovics I., Pan Q., Liberles D.A., Geli J., Davis S.K., Rabbani H., Hammarstrom L., Artiodactyl IgD: The Missing Link, J. Immunol. 169 (2002) 4408–4416.