

# The bursal secretory dendritic cell (BSDC) and the enigmatic chB6<sup>+</sup> macrophage-like cell (Mal)

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**ABSTRACT** The bursal secretory dendritic cell (BSDC) was discovered more than 40 yr ago. It is a highly polarized, granulated cell, locating in the medulla of bursal follicle. The cytoplasmic granules either discharge or fuse together forming large, irregular-shaped, dense bodies. Formation of the dense bodies could be the first sign of BSDC transformation to macrophage-like cell (Mal) which is the result of terminal maturation of BSDC. The BSDC is non-phagocytic, unlike Mal. The discharged substance may be attached to the cell membrane (membrane-bound form) and after detaching, appears as a flocculated substance in the extracellular space of medulla. Movat pentachrome staining shows, that this substance is a glycoprotein (gp), which may be contributed to the microenvironment of the medulla.

Medullary lymphocytes are floating in the gp. Precursors of the BSDC locate in the corticomedullary epithelial arches, which operate under the effect of Notch/Serrate signaling. The Notch signaling determines the fate of lymphoblast-like precursor cells and inhibits the appearance of immunoglobulin heavy chain. In the arches, the precursor cells proliferate and entering the medulla differentiate. The dense bodies pack the virus particles, which prevents the granular discharge, resulting in disappearance of extracellular gp, but gp emerges inside the virus containing Mal. In infected birds, the Mal contains either apoptotic cells or virus particles. If vaccination or infectious bursal disease virus (IBDV) infection use up the BSDC precursors, the recovery of follicle is critical.

**Key words:** bursa of Fabricius, bursal secretory dendritic cell, IBDV infection and glycoprotein, corticomedullary epithelial arch, BSDC precursor

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## HISTORIC DEVELOPMENT OF BSDC'S SEMANTICS AND INTRODUCTION

During recovery of the bursa of Fabricius after cyclophosphamide (Cy) treatment, large amounts of an extracellular substance appeared in the medullary area of several bursal follicle. Both light- and the transmission electron microscopic examinations revealed a novel granular cell embedded in electron dense, extracellular substance (Figures 1 A and 1B) Cy treatment eliminated the lymphocytes, but not the granular cell (Figure 1B). These findings were published more than 40 yr ago (Oláh and Glick, 1978; Oláh et al., 1979). At the same time, we have recognized another granular cell in caecal tonsil germinal centers (GC), which showed nuclear size and heterochromatic pattern similar to

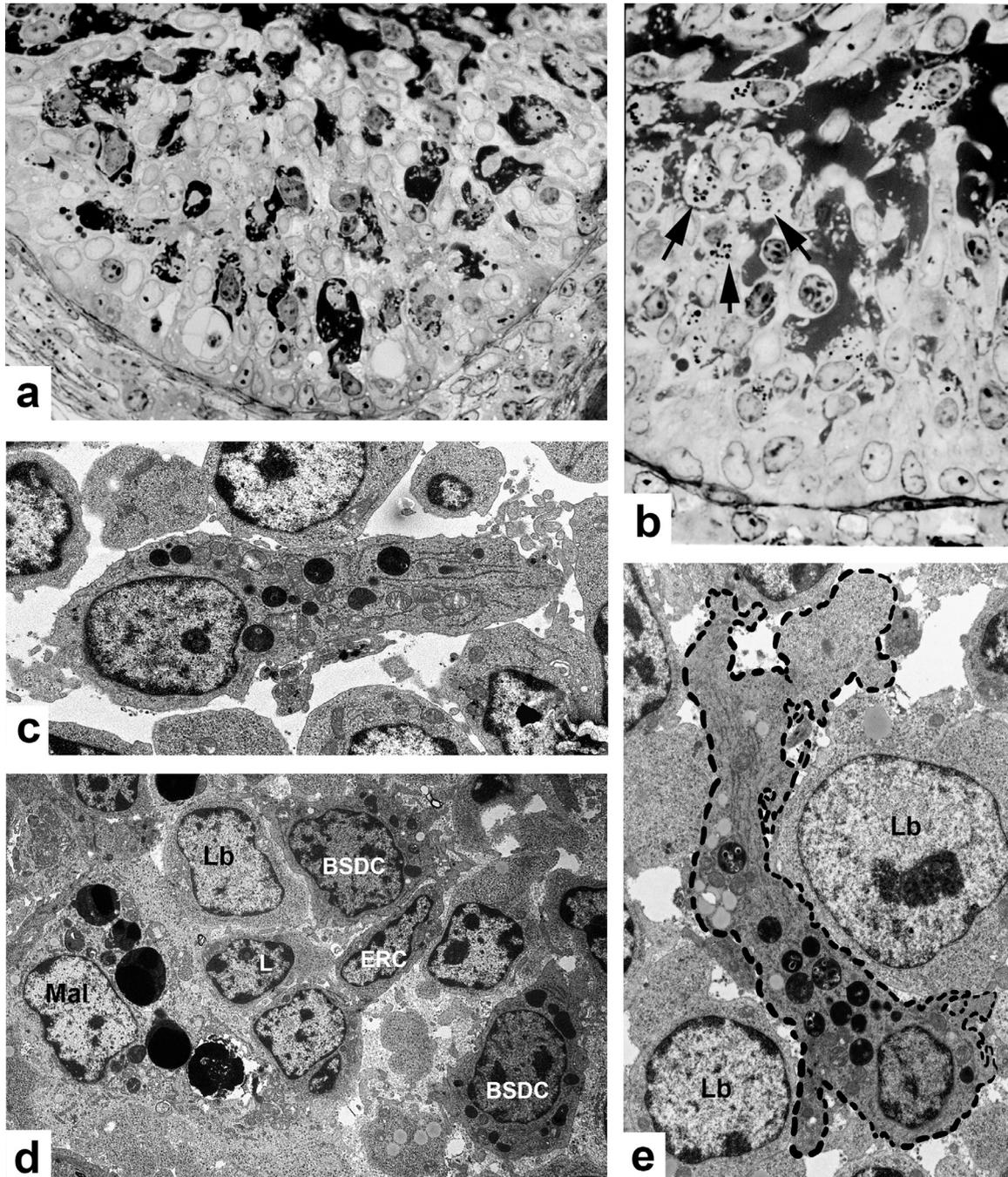
those of small lymphocytes (Oláh and Glick, 1979). Neither bursal nor GC granular cells are phagocytic but both of them produce well-developed cellular extensions. The extracellular substance in the medulla of bursal follicle and on the surface of GC granular cells suggested that both cells have secretory properties. Besides the secretory nature of these cells, we assumed that the granular cells in the GC are chicken's antigen-retaining cells (Oláh and Glick 1979), which were earlier reported in mammals (Nossal et al., 1968; Szakál and Hanna, 1968; Sordat et al., 1970). Both cells were named a secretory cell because the light and transmission electron microscopic structures of both novel, granular cells are largely similar. This occurred only 5 yr after Steinman and Cohn's (1973) discovery of lymphoid dendritic cell in mammals. After a detailed morphological analysis of chicken granular cells, we concluded that the secretory cells could be homologous cells to the mammalian lymphoid dendritic cell. In the bursa, the secretory feature of the novel cell as well as the dendritic attribute has been proved. In this paper, the authors discuss, how the term of bursal secretory dendritic cell (BSDC) gradually developed, and how further studies revealed that

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**Figure 1.** Light- and transmission electron microscopy of BSDC. (A) Cy-treatment resulted in large amounts of extracellular substance in several follicular medulla. The dark extracellular substance surrounds the secretory cells. (B) The secretory cells have a pale-stained, elongated cytoplasm with a few granules (arrow). (C) The BSDC cytoplasmic granules assemble in one of the cell processes, which clearly shows that the cell is not a granular lymphocyte. (D) Survey picture of follicular medulla shows two young BSDC(s) and a Mal. The size and heterochromatic pattern of the BSDC nucleus are remarkably similar to those of small lymphocyte. The cytoplasmic granules scatter around the nucleus. These young BSDC(s) can give the impression being of granular lymphocyte. (E) In mature BSDC, the nucleus locates eccentrically, which lend a highly polarized appearance to the BSDC. The cell (outlined) produces cytoplasmic “buds” among the lymphocytes. Abbreviation: BSDC, bursal secretory dendritic cell.

the secretory cell in GC is identical with the follicular dendritic cell (**FDC**).

Precursors of B lymphocytes arise from pluripotent hemopoietic stem cells (**HSC**), which enter the bursa of Fabricius (**BF**), and differentiate to immunological mature cells under the influence of bursa specific stromal microenvironment (**ME**). Structurally and functionally, the bursal follicle consists of 3 distinct regions: cortex, medulla, and follicle-associated epithelium (**FAE**,

[Oláh and Glick, 1992](#)). Supporting stromal cells of the cortex and medulla are mesenchymal reticular cell (**MRC**) of mesodermal origin and epithelial reticular cell (**ERC**) of ectodermal origin ([Nagy and Oláh, 2010](#)), respectively. In addition to the MRC, the monocyte-macrophage and endothelial cell also contribute to the ME of follicular cortex. In the medulla besides the ERC ([Houssaint et al., 1986](#)), the terminal maturation of BSDC results in a macrophage-like cell (**Mal**), which

makes the medullary ME histologically complete. The FAE compartment is distinct, devoid of lymphocyte (Oláh and Glick, 1992) but contains Mal (Houssaint and Hallet, 1986). The BSDC locates exclusively in the medulla and may be secreting glycoprotein (gp) into the extracellular space (Felföldi et al., 2021). Classical extracellular matrix (ECM), which consists of fibers: collagen, reticular, elastic, fibrillin, and ground substance, is absent in the medulla, whereas the cortex produces ECM, but not gp.

Gilmour et al. (1977) discovered the Bu-1 or chB6 membrane alloantigens; transmembrane glycoproteins by using immunization between inbred lines of chickens. There are several monoclonal antibodies (mAb) directed against chB6 alloantigens, which are: L22; Bu-1a (Pink and Rynbeek, 1983); 11G2, Bu-1b (Veromaa et al., 1988); AV-20, Bu-1b (Rothwell et al., 1996) HIS-C1 (Jeurissen and Janse, 1998), and BoA1, Bu-1a and b (Igyártó et al., 2008). The Bu-1 alloantigen is not restricted to B cell lineage. It appears on a subset of Mal (Houssaint et al., 1989), which are glass-adherent, nonspecific esterase and MHC class II positive, and non-phagocytic cells (Houssaint et al., 1987). The chB6 antigen is expressed by microglia intraganglionic macrophages (Dora et al., 2020). However, there is no lineage relationship between chB6<sup>(+)</sup> B cell and Mal because in irradiated recipients, bone marrow cells from young chickens can repopulate chB6 positive<sup>(+)</sup> Mal, but not chB6<sup>(+)</sup> B lymphocyte. The chB6<sup>(+)</sup> Mal(s) appear exclusively in the follicular medulla, express MHC class II antigen, are nonspecific esterase positive, glass adherent, and non-phagocytic (Houssaint et al., 1987). These features are characteristics for BSDC.

Any changes in the ME influence B cell development. Testosterone propionate (TP), Cy, and IBDV infection all deplete bursal B cells. However, embryonic TP treatment results in loss of BF, while Cy causes only temporary changes in stroma of ME, because follicles can be reconstituted with lymphocytes (Houssaint et al., 1989; Ratcliffe, 1989; Wilson and Boyd, 1990a). After IBDV infection, bursal follicles may be newly repopulated by pre-B cells (Withers et al., 2006). The topographical pattern of the IBDV infected IgM<sup>(+)</sup> expressing cells are identical with that of BSDC and BSDC transformed Mal (Felföldi et al., 2021).

Early works proposed differences between cortical and medullary B lymphocytes: cortical cells show prolonged proliferation, and medullary B cells are immunologically fully competent cells (Grossi et al., 1974). On the basis of life-span, Paramithiotis and Ratcliffe (1994a) reported 3 populations of blood B cells with about 60 and 5% of B cells are short-lived (2–3 d) bursal emigrants and progeny of post bursal B cells, respectively. The third blood B cell population has a life-span more than 2 wk. According to Paramithiotis and Ratcliffe (1994b) the B cells leave the bursa “directly from follicular cortex”. A few years earlier, Lassilla (1989) reported that the cells emigrating from the bursa were surface IgM<sup>(+)</sup> cells. In mature follicle, only the medullary lymphocytes express IgM (Grossi et al., 1977; Boyd et al., 1987; Olah and

Glick, 1992) the cortical B cells are IgM<sup>(-)</sup>. This contradiction can be explained by the finding, that bursa produces 2 different B cell populations: a cortical IgM<sup>(-)</sup> and a medullary IgM<sup>(+)</sup> B cells, both of which express chB6 alloantigen. It is possible that both cortical and medullary lymphocytes can emigrate from the bursa. Recently, Dora et al. (2020) reported, that cortical chB6<sup>(+)</sup> B cells express high level of CXCR4, while medullary cells, located near the cortical capillaries, had lower CXCR4 expression in the adult BF.

Petkov et al. (2009) also reported 2 IgM<sup>(+)</sup> B cell subpopulations “based on cell size and granularity”, as well as IBDV susceptibility. Subpopulation A is resistant to IBDV infection, and postinfection the number of cells is “constant”, unlike subpopulation B, in which the cells are IBDV susceptible, and after infection their number decreases.

During embryogenesis, the EIV-EI2<sup>(+)</sup> dark cells are the first cells that enter the BF surface epithelium (Pharr et al., 1995) to bring about a transitional, dendroepithelial tissue (Oláh et al., 1988), which is colonized by prebursal B cells. Wilson and Boyd (1990b) studied the ontogeny of bursal stromal cells, and concluded that the BSDC could be of mesenchymal rather than hemopoietic origin. This was our original proposal for BSDC precursors, because we have observed transitional cells between mesenchymal and dark cells (Oláh et al., 1986). The origin of BSDC precursors: mesenchymal, HSC, or yolk sac remains unclear.

The bursal ME is still poorly understood. A short summary can be read in the paper of Houssaint et al. (1986). Cortical and medullary ME can be distinguished, in which possibly 2 different B cell subpopulations are produced, released, and survive after bursal involution. The current study describes the physiological turnover of BSDC. Furthermore, the work attempts to elucidate the “operation” of BSDC and its terminal maturation chB6<sup>(+)</sup> Mal during IBDV infection. Finally, this paper summarizes the morphological stromal cell differences between follicular cortex and medulla, which are responsible for the ME and development of cortical and medullary B cells.

## MATERIALS AND METHODS

### Animals

New Hampshire chickens selected for small bursa size between 2 and 72 d old (Glick and Dreesen, 1967) were used in the Cy experiments and immunocytochemical studies. For transmission electron microscopy (EM) and Movat pentachrome staining, White Leghorn layer-type SPF chickens (Charles River) without vaccination were used.

### Infection

Infectious bursal disease virus (IBDV) infection was performed by very virulent field isolate (0407/02/04/TR) and variant Delaware-E strain. The IBDV stains

were characterized by sequence analysis. The virus suspensions were applied via per os at dose of 3.0 IgE ID<sub>50</sub>/chicken (diluted in 0.2 mL sterile PI3S)

### **Cyclophosphamide Treatment and EM**

Seven-wk-old chicken (900–1,100 g body weight) were injected intraperitoneally with Cy on 5 consecutive days. On d 1, they were injected with 50 mg Cy/kg body weight followed by 4 d with 25 mg/kg body weight. The animals were euthanized 4 d after last injection. The bursal tissues were fixed in 4% glutaraldehyde, dehydrated, and then embedded in Polybed/Araldite 6005 mixture (Polysciences, Warrington, PA). The 1- $\mu$ m thin sections were stained with toluidine blue and contrasted with uranyl acetate and lead citrate, respectively.

### **Antibodies and Immunostaining**

The primary antibodies for the immunohistochemistry were anti-vimentin (clone 3B4), anti-cytokeratin (clone Lu5) (Boehringer-Mannheim GmbH, Indianapolis, IN), and anti-chicken IgG (Y specific, clone CG-106, Sigma, St. Louis, MO). Biotinylated anti-mouse IgG and ABC kit were purchased from Vector Laboratories (Burlingame, CA). Tissue samples were frozen in liquid nitrogen and 10- to 12- $\mu$ m cryostat sections were fixed in cold acetone. After rehydration in PBS, the endogenous peroxidase was quenched with 3% H<sub>2</sub>O<sub>2</sub> for 10 min.

### **Movat Pentachrome Staining**

Both reticular fiber and mucin producing goblet cells contain glycoprotein(s) and are stained by Movat pentachrome staining (Bancroft and Gamble, 2002). Reticular fiber consists of collagen fibrillar units and the interfibrillar proteoglycans bind the fibrillar units together to form a three-dimensional reticular network. In the histological lab for medical students the Movat pentachrome staining has been used for identification of intestinal and tracheal goblet cells, which initiated of employing the Movat staining for interfollicular epithelium (IFE) cells. If the IFE cells, which histologically are not goblet cells, produce Movat positive mucin? The apical portion of IFE cells are highly Movat positive. This Movat staining resulted in the discovery of extracellular glycoprotein in the follicular medulla of BF.

## **RESULTS AND DISCUSSION**

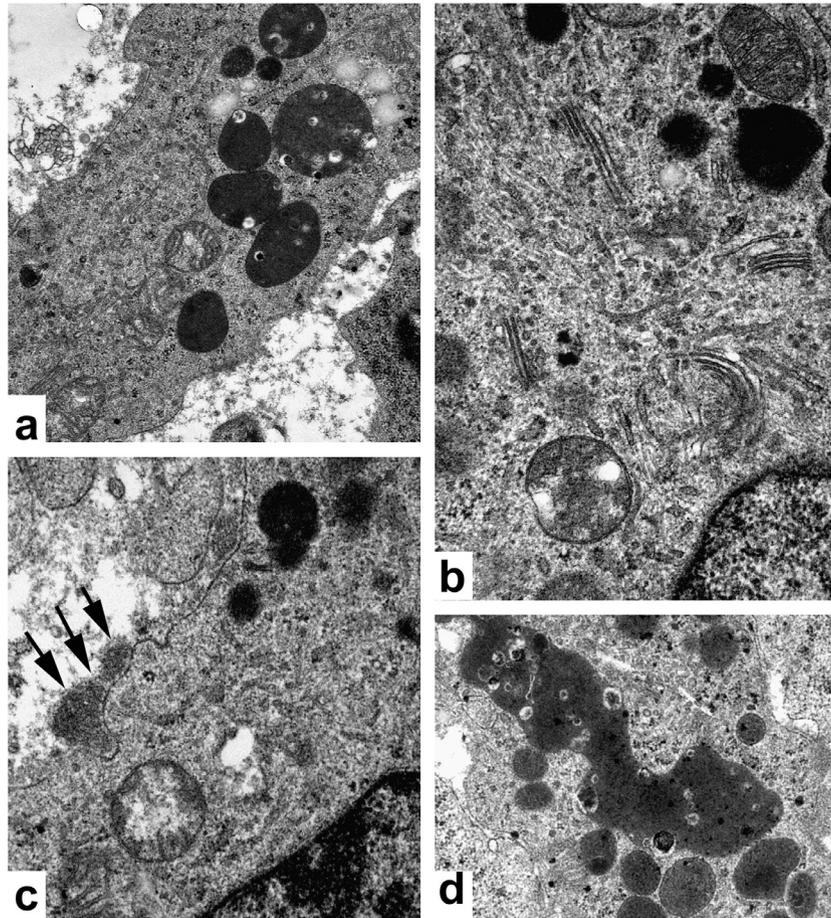
### **Cytology and Surface Markers of BSDC**

The BSDC locates, exclusively, in the medullary region of bursal follicle. The mature cell is highly polarized, which is established by the eccentrically located nucleus and the cytoplasmic granules occupying one of the cell processes (Figures 1 C and 1E). The nuclear size, shape and heterochromatic pattern of BSDC are highly similar to that of small lymphocyte (Figures 1 C and

1D). In young BSDC, the cytoplasmic granules, occasionally, appear around the nucleus, which may be showed a granular lymphocyte (Petkov et al., 2009). These observations suggest a false picture, namely, existence of a granular lymphocyte even at transmission electron microscopic level (Figure 1D). However, in most case the electron density of the nucleus and cytoplasm of young BSDC used to be higher than that of the small lymphocytes (Figures 1D and 1E), which proves that the cell is not lymphocyte. The electron density of the nucleus and cytoplasm decreases during cell maturation, becoming more similar to a small lymphocyte (Figure 1C).

In the BSDC, the shape and size of the electron dense granules are highly variable. The granules frequently show spot-like electron lucent areas (Figure 2A) which may be indicated that the granules are complex and some small amounts of material releases from them. The cytoplasm may be produced protrusions, containing ectoplasm (Figure 1E). Lipid droplets are common feature of the cell (Figure 1E). In young cells, close to the nucleus, a rich Golgi region is found with unusually flat cisternae and numerous small vesicles scattered on both cis- and trans faces of Golgi (Figure 2B). Granules are found around the Golgi complex, and in immature cells may be scattered around the nucleus. Finally, in mature cells, the granules assemble in the elongated cytoplasm and either discharge by exocytosis (Figure 2C) or fuse to form large, irregular-shaped, electron dense bodies (Figure 2D). The appearance of dense bodies may indicate the initiation of BSDC transformation to Mal (Figure 1D). The discharged granules temporarily attached to the BSDC cell membrane (membrane-bound form of granular substance); then after detaching appear as a fine flocculated substance in the extracellular space in the medulla (soluble form of granular substance) (Figures 2A and 2C). This flocculated substance can be shown by Movat pentachrome staining (Bancroft and Gamble, 2002) which recognizes a gp in the cells of interfollicular epithelium (IFE) and the extracellular space of medullary region of follicles. The FAE cells are Movat negative (Figure 3A). The staining intensity of gp varies from follicle to follicle suggesting that the phase of secretory cycle is not synchronized in the follicles (Figure 3A). The fine flocculated, Movat positive gp could be an essential component in the medullary ME.

During the last 25 yr, the accumulating experimental findings show that glycosaminoglycans like chondroitin sulfate and heparan sulfate play an important role in the homeostasis and function of macrophages and dendritic cells. Immature human dendritic cells express syndecan-1 and glypican-1, while mature dendritic cell produces glypican-3, which is not expressed other type of cells (Wegrowski et al., 2006), but both immature and mature dendritic cells express glypican-5. Moreover, heparin-sulfate contributes to migratory capacity and maturation of dendritic cells (Kodaira et al., 2000; Johnson et al., 2002) and works as a receptor for human immunodeficiency virus on macrophages (Saphire et al., 2001). Chicken medullary B lymphocytes are floating in



**Figure 2.** Cytology of BSDC. (A) The variable-sized granules show one or more translucent dots. Around the cell, there is a fine flocculated substance in the intercellular space of follicular medulla. (B) Beside the nucleus (N), the Golgi cisternae are unusually flat and scattered, small vesicles occur in the central region of the cell. Around the rich, Golgi region cytoplasmic granules of different sized found. (C) Exocytosis of a granule (arrow) shows a medium-dense substance in the cell membrane invagination. A fine flocculated substance seem to be released from the exocytic granules. (D) The cytoplasmic granules fuse together forming large, irregular-shaped dense bodies. Abbreviation: BSDC, bursal secretory dendritic cell.

the extracellular gp and gp attaches to their cell membrane (Chechik et al., 1988). Taken together, the mammalian data with our findings provide evidence that the gp is an essential molecule for B cell maturation and survival or function in avian species. The remarkable differences of stromal cells between cortex and medulla (Table 1) may be created 2 kinds of B cells, like in mammals.

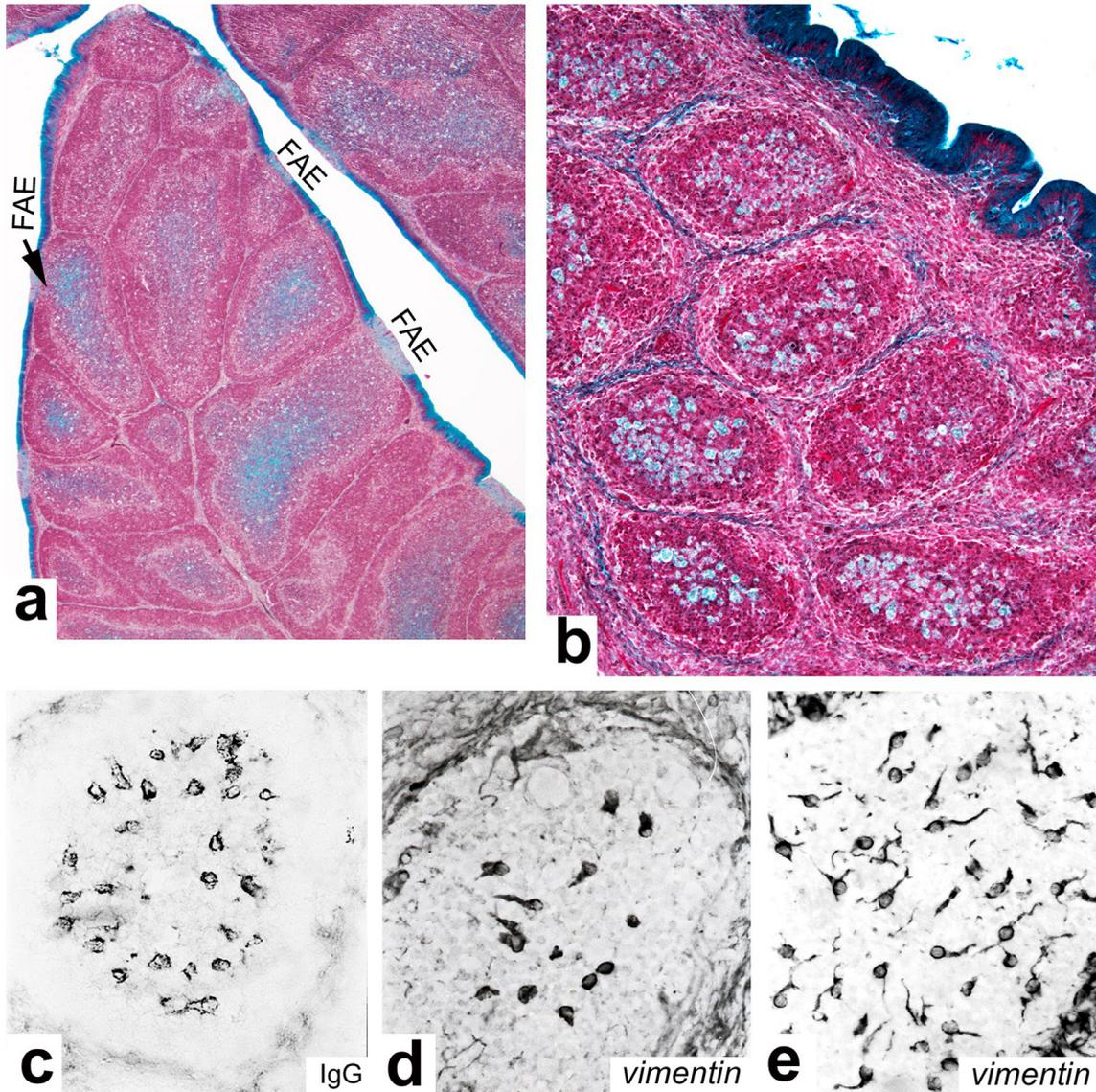
The BSDC expresses MHC class II histocompatibility antigen (Oláh et al., 1992), chB6 alloantigens (Gilmour et al., 1977), an EIV-EI2 surface gp positive antigen (Pharr et al. 1995), which differs from chB6<sup>(+)</sup> transmembrane gp (Nuthalapati et al., 2015) and Fc receptor (Oláh and Glick, 1995). In addition to the listed surface antigens, the BSDC produces colony stimulating factor- 1 receptor (CSF-1R) (Garcia-Morales et al., 2014). During embryogenesis, the vimentin positive BSDC can be detected at d 15 (Oláh et al., 1992) and around hatching, the Fc<sup>(+)</sup> BSDC binds IgY (Figure 3C; Oláh and Glick, 1995), which is of maternal origin (Ekino et al., 2012; Ekino et al., 2015). During bursal growth, the number of BSDC rapidly increases (Figures 3D and 3E). At hatch, the number of vimentin<sup>(+)</sup> and IgY<sup>(+)</sup> BSDC on cryostat sections is 10/follicle and by reaching a steady state increased about

18 times (Oláh et al., 1992). Considering the vimentin and keratin intermediate filaments in BSDC and epithelial reticular cells (ERC), respectively, the cells seem to be identical with mammalian Langerhans cells and epithelial cells in the skin (DeWaal et al., 1984; Rappensberger et al., 1990).

### **Corticomedullary Epithelial Arch and Enclosed Cells**

There are several lines of evidence for the unique behavior of corticomedullary epithelial arches (CMEA) and the enclosed “lymphoid” cells (Figures 4A and 4C). The CMEA-forming cells was first described by Olah and Glick (1987) and their existence was confirmed by anti-cytokeratin immunostaining (Figure 4B). Monoclonal antibody BEP-1 recognizes CM epithelium, but not ERC (Houssaint et al., 1986); and MUI-91 also identify basement membrane-associated epithelium (BMAE; Boyd et al., 1987), confirming the unique feature of CMEA.

In the CMEA, histologically lymphoblasts, few small lymphocytes, mitotic cells, and occasionally apoptotic cells can be found (Figures 4 A and 4C).

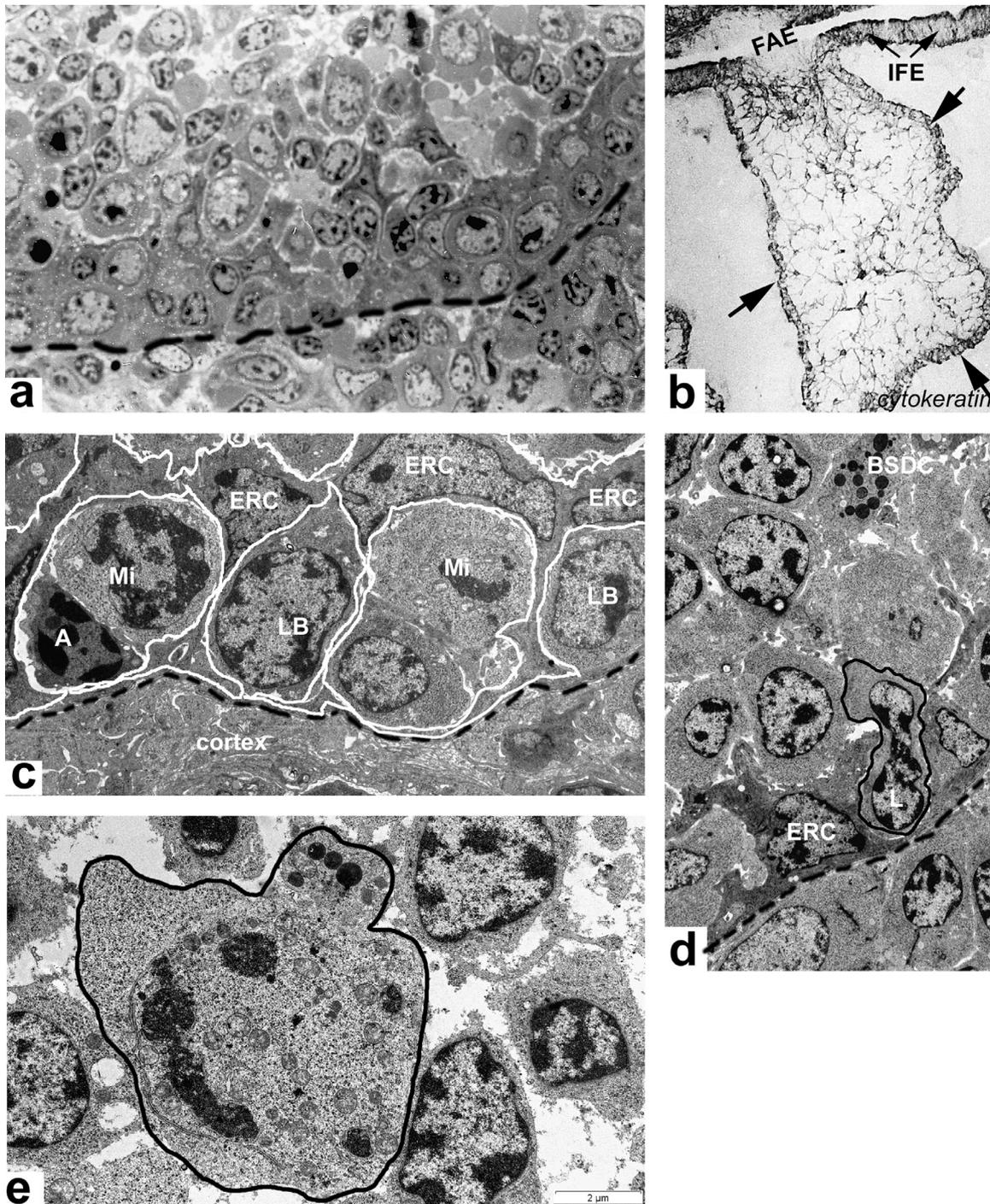


**Figure 3.** Glycoprotein and BSDC (A) Control bird. The medulla shows variable intensity of Movat pentachrome staining. Several follicles are negative or the Movat staining sensitivity is insufficient to identify a very small amount of gp. The FAE cells are negative for gp unlike the cells of IFE. (B) Five-day postinfection, the extracellular gp disappeared from the medulla, but gp “emerged” in the Mal. (C) One-wk-old chicken bursa shows that the BSDC membrane binds IgY. (D) Three-day old chicken bursa, in which the majority of anti-vimentin stained BSDC are of round and ovoid shaped. Two cells are already elongated. (E) By d 72, the BSDC(s) show one or two cell processes filled with vimentin intermediate filaments. Abbreviation: BSDC, bursal secretory dendritic cell.

**Table 1.** Stromal cell differences between cortex and medulla.

The morphological differences between follicular cortex and medulla	
Cortex	Medulla
Development after hatching	Development during embryogenesis
Microenvironment supporting cell	ERC - ectodermal origin
MRC-mesodermal origin	Intermediate filament in ERC: keratin
Intermediate filament in MRC: vimentin and desmin	BSDC → Mal
Macrophage (Ma) monocyte-macrophage markers; positive 68.1 <sup>+</sup> , 68.2 <sup>+</sup> KULO1 <sup>+</sup>	CSF1R <sup>+</sup> , sIgM <sup>+</sup> , sIgG <sup>+</sup> , chB6 <sup>+</sup> , MHC class II <sup>+</sup> Notch/Serrate signaling monocyte-macrophage markers; negative 68.1 <sup>-</sup> , 68.2 <sup>-</sup> , KULO1 <sup>-</sup>
Origin of macrophages	BSDC Mesodermal? Yolk sac origin?
Blood-borne, HSC origin	Terminal maturation of BSDC results in Mal extracellular glycoprotein – product of BSDC
ECM – product of MRC (fibers: collagen + reticular and ground substance)	
B cells are sIgM <sup>-</sup>	B cells are sIgM <sup>+</sup>

Abbreviations: BSDC, bursal secretory dendritic cell; CSF1R, colony stimulating factor 1 receptor; ERC, epithelial reticular cell; HSC, Hemopoietic stem cell; sIgM and sIgG, surface immunoglobulins; MRC, mesenchymal reticular cell; Notch1/Serrate2, cell fate determination receptors and ligand.



**Figure 4.** Corticomedullary epithelial arch (CMEA) (A) Semi-thin section ( $1.5\text{-}\mu$  thick) shows a compact layer in the medulla close to the basal lamina (dashed line). Lymphoblast and small lymphocyte-like cells locate in the arches. (B) The anti-cytokeratin immunostaining confirms the CMEA. (C) The transmission micrograph shows mitotic (Mi) and an apoptotic cells (A) in the CMEA. The epithelial cells of CMEA(s) are outlined. The dashed line shows the basal lamina. LB, lymphoblast-like cell (D) Release of a lymphocyte-like cell from the CMEA (outlined) (E) Mitosis in an immature BSDC (outlined). The cytoplasmic granules occupy an outpocketing of the mitotic cell (36 h after IBDV infection). Abbreviations: BSDC, bursal secretory dendritic cell; IBDV, infectious bursal disease virus.

These lymphoid-like cells do not express chB6 alloantigen, indicating that they are not B cells. The presence of mitosis and the absence of differentiated cells show that the cells are somekind of progenitors, which slowly proliferate (Figure 4C), but not differentiate. These progenitor cells might be identical with the very early, IBDV resistant lymphoblasts (Beug et al., 1981). The “roof” of the arch may be

opened allowing cells to enter the medulla (Figure 4D), where differentiation of the cells begins.

Morimura et al. (2001) reported, that lymphoid associated cells with BMAE (identical with CMEA), express Notch1 transcription factor, while Notch1 ligand, *Serrate2* is expressed exclusively in the BMAE. The downstream target of Notch signaling is another transcription factor, *Hairy1*, which is expressed in “immature B cell

line”, and downregulates the IgM expression. The Notch signaling has a critical role in cell fate determination while maintaining a progenitor pool (Milner and Bigas, 1999). In the CM arch, the undifferentiated cells are BSDC progenitors. The terminal maturation of BSDC is a Mal (Felföldi et al., 2021), which may enter the FAE (Houssaint and Hallet, 1986; Olah and Glick, 1992) or emigrate into the cortex (Felföldi et al., 2021).

During embryogenesis, the epithelial bud-inducing dark mesenchymal cells (Oláh et al., 1986) express an EIV-EI2<sup>(+)</sup> cell surface antigen (Pharr et al., 1995). Cortical and medullary B lymphocytes share this antigen with BSDC and possibly with Mal in the mature follicles. However, in the epithelial arches the progenitor pool of BSDC are agranular, EIV-EI2, chB6, vimentin, IgY, and IgM negative. These findings show that the cells in the CMEA can not be descendants of EIV-EI2<sup>(+)</sup> dark, bud-inducing cells. The fate of dark, EIV-EI2 mAb<sup>(+)</sup> cells is waiting for further elucidation. In addition to the dark cells and pre-bursal B cells, possible a third type of cells also enters the developing follicle, lodges in the posthatch forming CMEA and maintains the BSDC progenitor pool. After IBDV infection, the exhausting progenitor pool in the CMEA is critical for the recovery of follicle; namely, the number of precursor cells may determine the future fate of the individual follicle.

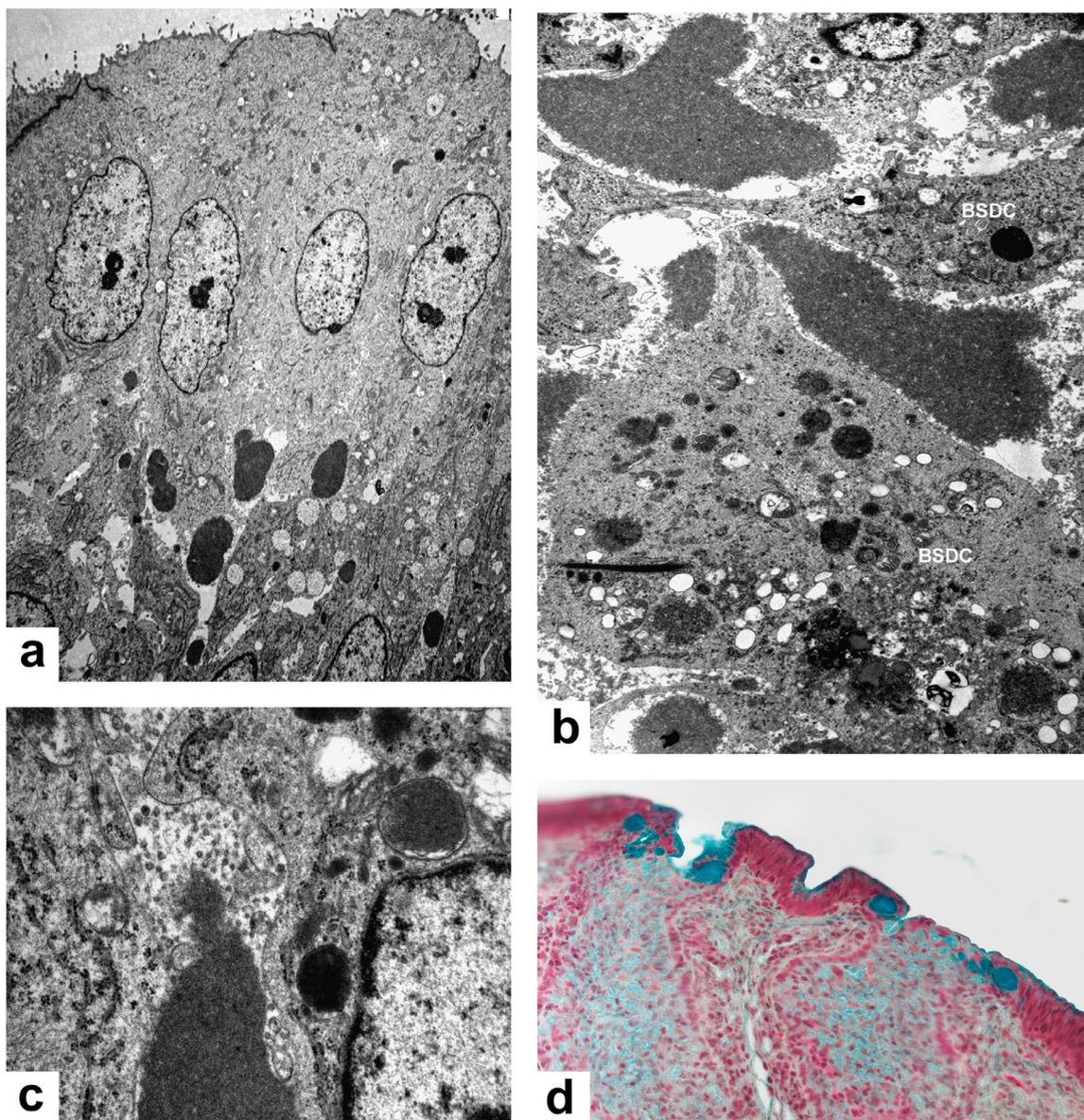
Occasionally, maladaptation of BSDC or a progenitor of Mal can be found in the CMEA (Figure 4C). Around 2 wk of age many Mal are found in the medulla close to the epithelial arch, which may be raised the possibility, that among the precursor cells could be precursors of Mal. Nevertheless, morphological evidence exists for the progenitor -> BSDC -> Mal serial development but data do not exclude that the undifferentiated progenitor cells can directly develop to Mal. The CMEA opens towards the medulla and the BSDC progenitor-cells entering the medulla (Figure 4D) start to differentiate. During differentiation, the cells produce secretory granules (Figure 2B) vimentin intermediate filament (Figures 3D and 3E; Oláh et al., 1992), one or 2 cell processes and binds IgY around hatching (Figure 3C; Kincade and Cooper, 1971).

### **The chB6<sup>(+)</sup> Macrophages in the Follicular Medulla**

As we have mentioned earlier, the name of chB6<sup>(+)</sup> macrophage-like cell was introduced by Houssaint et al. (1987,1989). Weber (2000) reported 2 chB6<sup>(+)</sup> cell populations in the bursa; the majority of chB6<sup>(+)</sup> cells are small, strongly expressing the alloantigen and prone to apoptosis, which can be accelerated either by exposure to anti-chB6<sup>(+)</sup> mAb (Funk and Palmer, 2003) or IBDV infection (Lam, 1997). The other chB6<sup>(+)</sup> cell is “larger, less dense, and weak chB6<sup>(+)</sup> cell often with vacuoles in a more abundant cytoplasm”. This population of surface IgM<sup>(+)</sup> B cells did not develop in unstimulated culture. Weber (2000) suggested that these chB6<sup>(+)</sup> cells represent a chB6<sup>(+)</sup> macrophage subset.

CMTD-2 is an anti-glycoprotein mAb, which recognizes a subpopulation of peritoneal Ma elicited by Sephadex injection (Trembicki et al., 1986). This mAb reacted with about 10% of stimulated peritoneal macrophages, but failed to react with blood monocyte, bone marrow cells or lymphocytes. The appearance of this Ma subpopulation is time-dependent (24–52 h during elicitation), heterogeneous for phagocytosis of sheep erythrocytes and in spleen can be detected in low incidence of chicken suffered from spontaneous autoimmune thyroiditis (Trembicki et al., 1986). There is no information about these cells in healthy birds. There are 2 kinds of Ma in chicken spleen: a resident, ellipsoid-associated cell (EAC) in the white pulp, which is precursor of lymphoid dendritic cells (Oláh and Glick, 1982) and a monocyte-macrophage origin in the red pulp. The EAC is a sentinel cell at the antigen exposed area of the spleen (Oláh et al., 1984), and could be identical with the splenic CMTD-2<sup>(+)</sup> cells. These CMTD-2<sup>(+)</sup> cells have been observed in the medulla of several bursal follicles, and occasionally, scattered in the cortex. The topographical pattern of CMTD-2<sup>(+)</sup> cells in the medulla, their shape, density and the presence of intracellular antigen suggest that the CMTD-2 mAb may be recognizing granular BSDC and possible Mal. The BSDC and Mal are heterogenous as the former is non-phagocytic whereas the latter is phagocytic. After IBDV infection, gp appears in the Mal (Figure 3B), while the extracellular gp is missing (Felföldi et al., 2021). Finally, it seems that the features of CMTD-2 mAb covers some functional morphology of BSDC and Mal, namely: the follicles, which are positive for CMTD2 mAb, may be positive for gp.

Reactivity of BEP2 mAb is intracytoplasmic and shows granular appearance (Houssaint et al., 1986). The indirect immunofluorescence assay clearly shows the BEP2 positive substance is in the extracellular space of medulla. The authors suggested that the ERC(s) secreted BEP2<sup>+</sup> substance into the follicular medulla (Houssaint et al., 1986). The transmission micrograph shows a very poor cytological structure and absence of granules in the ERC, while the BSDC has a well-developed secretory machinery, Golgi-system with secretory granules, which may be recognized by BEP2 mAb. It can be assumed, that BEP2 mAb and Movat pentachrome staining recognize extracellular gp. The BEP2 mAb reacts with goblet cells, which secrete gp-containing mucin. Cy treatment markedly decreased the BEP2 reaction product (Houssaint et al., 1986), like the extracellular gp during IBDV<sup>(+)</sup> infection (Felföldi et al., 2021). Around d 20 pi, and in vaccinated, nonchallenged birds we have observed extracellular substance in solid form during recovery after IBDV infection, (Figures 5A and 5B). The solidified extracellular gp (Figure 5D) creates strong Movat positive, dense bodies among the FAE cells and malformed BSDC or Mal (Figures 5A and 5B). Numerous microvesicles, whose origin and function are unknown, occur around the extracellular dense bodies (Figure 5C). The bursal follicles are polarized, therefore the transformed BSDC to Mal enter the FAE



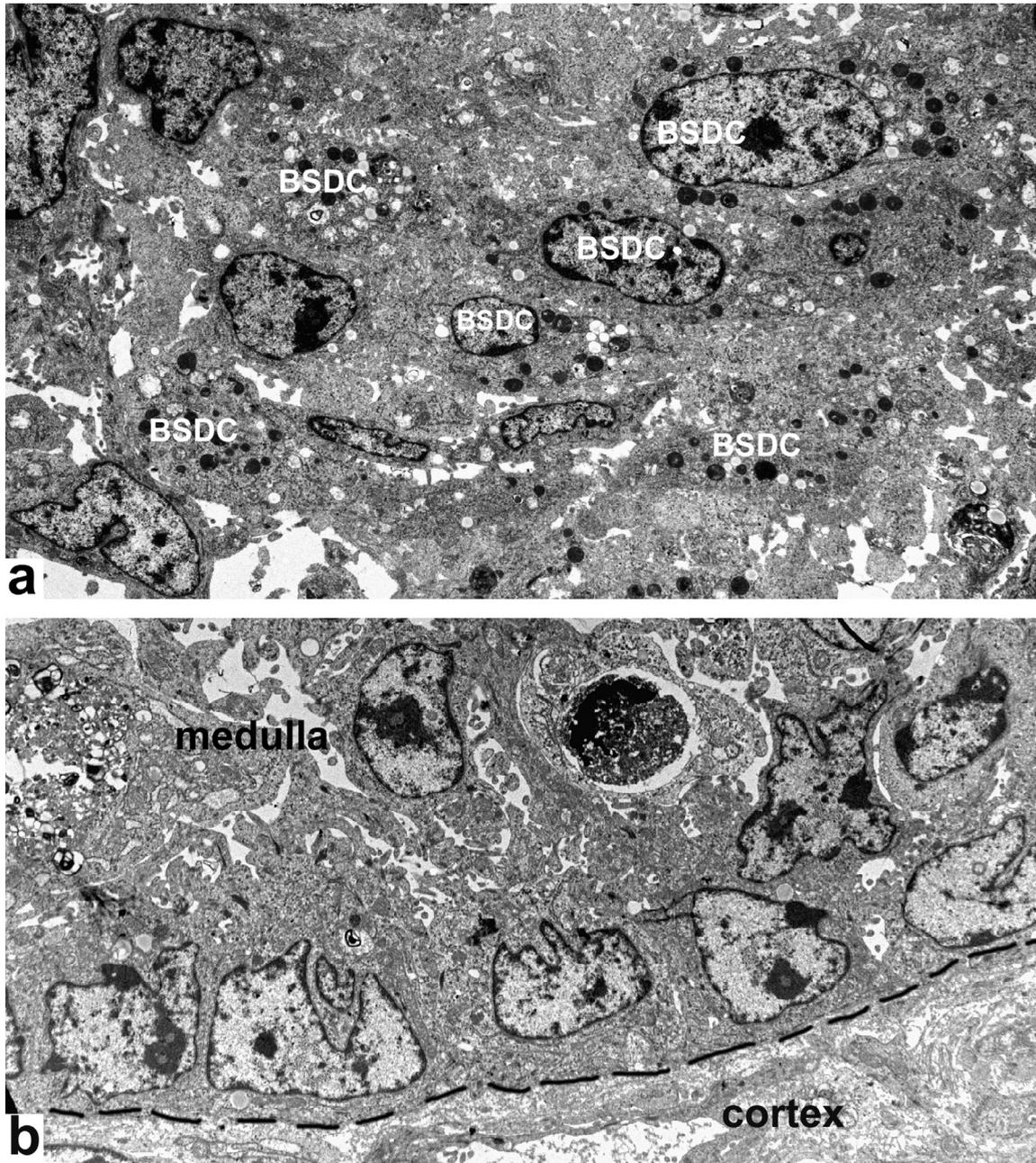
**Figure 5.** (A) In the FAE, the transmission electron micrograph shows solid electron dense extracellular bodies at the recovery stage of IBDV infection (21 d postinfection). (B) Beside the vacuolized BSDC the electron dense, extracellular substance forms irregular-shaped bodies. (C) Numerous microvesicles are found around the dense bodies. (D) Heavily stained Movat positive bodies are associated with FAE. Abbreviations: BSDC, bural secretory dendritic cell; FAE, follicle-associated epithelium; IBDV, infectious bursal disease virus.

(Houssaint and Hallet, 1986), and the rest of extracellular gp may be concentrated in FAE.

The difference in the appearance of gp in control and IBDV infected birds raises the possibility that the cytoplasmic granules of BSDC are complex and their components can be discharged separately. The spot-like electron lucent areas in the granules may support this hypothesis. (The complex granular content and the separate release of components is well known in another cell of innate immunity in mast cells.) Finally, we believe that both CMTD2 and BEP2 mAbs recognize intracellular BSDC granules and they react with one of the granular components. The discovery of extracellular gp in the medulla, may underline the importance of BEP-2 and CMTD-2 mAb(s) in the biomedical function of BF.

In the BF of an IBDV infected bird, the transmission electron microscope shows that the Mal contains either

apoptotic cells or virus particles, but not both. The basic cells of innate immunity (macrophage, dendritic, and mast cell) recognize pathogen-associated molecular patterns (PAMPs) on the infection agents by Toll-like receptors (TLRs). The TLRs are present on the cell membrane or intracellularly on the endosome surface. The latter type of TLR can be activated by double-stranded RNA of viruses or small immunomodulatory molecules (Kawai and Akira, 2010). In the BF of normal birds possible, the cell-surface membrane-associated TLR(s) are activated by apoptotic cells, while the presence of IBDV activates the intracellular TLR(s) and the cell membrane associated TLR(s) may be inhibited. In the bursa, the TIM<sup>hi</sup> and TIM<sup>lo</sup> receptor positive cells could be phagocytic or non-phagocytic (Hu et al., 2019) and may be corresponded to Mal and BSDC, respectively.



**Figure 6.** Effect of IBDV infection on BSDC precursor and CMEA (A) Four-day postinfection, the transmission electron micrograph shows a cluster of immature BSDC. (B) Seven days pi the “lymphoblast-like cells” have disappeared from the epithelial arches, which collapsed. The arch forming epithelial cell transformed to cuboidal shaped cell layer. CM basal lamina (dashed line). Abbreviations: BSDC, bursal secretory dendritic cell; CMEA, corticomedullary epithelial arch; IBDV, infectious bursal disease virus.

### Epithelial Arch and IBDV Infection

In IBDV infected chickens, a progressive loss of MUI-36<sup>+</sup> cells was observed in the bursa (Boyd et al., 1987). However, there was an IBDV resistant cell population (1–3%) in the peripheral blood, which localized at the CM junction of the bursa. It has been suggested that these IBDV resistant cells were macrophage progenitors (Boyd et al., 1987). We believe that these progenitor cells are precursor cells of BSDC (Oláh et al., 1992), lodging in the CM epithelial arches (Olah and Glick, 1987).

The first IBDV infected cells are medullary chB6 and IgM<sup>(+)</sup> cells, which show similar topographical pattern as the vimentin and IgY positive BSDC(s). This finding

suggests that the first infected cells are BSDC(s), which rapidly transform to Mal (Felföldi et al., 2021). In control birds, the number of BSDC and BSDC transformed to Mal are likely balanced, but the proportion of the 2 cells may be varied by developmental, functional or pathological conditions. The transformation of BSDC to Mal diminished the number of BSDC, which by feedback manner, releases the progenitor cells from the CM arches to differentiate to new BSDC. The above-described physiological maturation of BSDC is highly accelerated by IBDV infection, therefore, occasionally mitotic BSDC can be found in the medulla (Figure 4E). The fused cytoplasmic granules of BSDC pack the virus particles (Felföldi et al., 2021) which inhibits the granular

discharge and results in loss of extracellular gp and subsequently B cell apoptosis. This feedback mechanism may be confirmed by the observation that 4-d postinfection immature BSDC(s) accumulated in the medulla (Figure 6A) and by d 7 the epithelial arch collapsed (Figure 6B).

### Origin of BSDC

In the medulla of bursal follicles, the histologically identifiable macrophages are negative for monocyte-macrophage markers (68.1, 68.2, KULO-1), but positive for colony-stimulating factor 1 receptor (CSF1R) (García-Morales et al., 2014). The cortical macrophages are positive for monocyte-macrophage markers, indicating that they originate from blood-borne HSC. These observations raised the question about the origins of BSDC and medullary macrophages (Mal). In mouse, the tissue resident macrophages (epidermis-associated dendritic cell; the Langerhans cell, liver Kupffer cell, in brain the microglia and in the lung the alveolar macrophage) are partially self-maintained (Hashimoto et al., 2013), CSF1R positive cells, which are largely independent from the HSC. They may be derived from the yolk sac (Dóra et al., 2017). Precursors of BSDC locate in the CMEA, where they can proliferate but not differentiate. Occasionally, inside the medulla, the less mature BSDC shows mitosis (Figure 4E), which suggests that these cells and their progenitors form a locally self-maintained population. The terminal maturation of BSDC results in phagocytic Mal (Figure 1D). Some features – self-maintained, local proliferation, CSF1R positivity of BSDC – may be homologous to those found in mammalian tissue resident macrophages, supporting our assumption that the BSDC are of yolk sac origin.

### CONCLUSION AND FURTHER DIRECTION

The BSDC is a highly polarized, granule containing cell locating in the medulla of bursal follicle. Precursor cells of BSDC occupies the corticomedullary epithelial arches, where they slowly proliferates. Opening of the epithelial arch, the precursor cell enter the medulla and begins to differentiate. During differentiation, the precursor cell produces vimentin intermediate filaments, secretory granules and cell surface antigens and receptors: MHC class II antigen, Fc receptor, by which binds maternal IgY around hatching and CSF-1R. The presence of CSF-1R and local proliferation of cell in the BF (self-maintained) may be shown that the BSDC precursors are of yolk sac derived cells, which may settle down in bone marrow.

The differentiated BSDC discharges the secretory granules: a gp, which in membrane-bound form is in high concentration on the BSDC surface. The soluble form of gp may contribute to the medullary microenvironment. Actually, the medullary lymphocytes “float” in the gp, which attaches to their cell membrane, but the gp concentration is much lower than on the BSDC. The

IBDV infection inhibits the glycoprotein release, which results in absence of extracellular glycoprotein and subsequently B cell apoptosis.

During bursal growth, the number of BSDC increases and in steady state condition, the number of BSDC is about 18 times more than at hatching. In normal, physiological condition, the BSDC transforms to Mal, which enters the FAE and eliminated in the bursal lumen. The IBDV infection highly accelerates this transformation process, which results in decrease in the number of BSDC. The decreased number of BSDC facilitates the differentiation of precursor cells in the epithelial arch. Exhaustion of BSDC precursors prevents the recovery of the follicle suggesting that the BSDC and its precursor cell are crucial for bursal physiology. Our recent studies shows that vaccination eliminates remarkable number of BSDC (including the precursors), which is the primary target of IBDV. Thus, the immunity of vaccination against IBDV infection – besides humoral and cellular immune responses – the decreased number of primary target cell is also crucial.

Future studies will focus on: What is the real function of gp in the B cell maturation or survival? How the secretory phase of individual follicle is regulated, or each follicle is a “mini” bursa? How the vaccination influences the BSDC and production of extracellular gp?

What is the contents of BSDC granules?

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### DISCLOSURES

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