



Review

Dynamic Control of B Lymphocyte Development in the Bursa of Fabricius

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Abstract. The chicken is a foundational model for immunology research and continues to be a valuable animal for insights into immune function. In particular, the bursa of Fabricius can provide a useful experimental model of the development of B lymphocytes. Furthermore, an understanding of avian immunity has direct practical application since chickens are a vital food source. Recent work has revealed some of the molecular interactions necessary to allow proper repertoire diversification in the bursa while enforcing quality control of the lymphocytes produced, ensuring that functional cells without self-reactive immunoglobulin receptors populate the peripheral immune organs. Our laboratory has focused on the function of chB6, a novel molecule capable of inducing rapid apoptosis in bursal B cells. Our recent work on chB6 will be presented and placed in the context of other recent studies of B cell development in the bursa.

Key words: bursa of Fabricius; B lymphocytes; apoptosis; intracellular signaling.

Introduction

The immune system is charged with defending the body against a wide and constantly changing array of potential pathogens. To counter this, vertebrate immune systems create a correspondingly vast array of cells, each bearing a unique receptor for a particular antigen. Generation of unique receptors from limited genetic material entails mechanisms of gene rearrangement and, in some cases, gene conversion. The chicken has served as a foundational model of our current understanding of the immune system. The fundamental distinction of T and B cells in immune function was first elucidated in the chicken. Although the vast majority of current immunologic studies focus on mouse or hu-

mans systems, the chicken remains a viable and interesting model to understand immunity. The chicken is an excellent model to use in studying B lymphocyte development because it has an organ, the bursa of Fabricius, devoted specifically to B cell maturation and differentiation. Avian species are unique in that they possess this primary lymphoid organ that is required for the diversification of immunoglobulin (Ig) genes and whose major purpose is the differentiation of B cells. The bursa's accessibility and relatively large size make it easy to study at various developmental stages and these stages have been well defined^{32, 40}. In particular, the development of avian B cells in a unique organ that is predominantly devoted to producing B cells provides key insights into both repertoire expansion

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and the mechanisms enforcing central tolerance in B cells.

Bursal Ontogeny

The bursa begins as an epithelial infolding of the cloacal wall, visible by embryonic day 5^{13, 16, 36, 61}. The bursa subsequently extends away from the cloaca as it is colonized by hemopoietic cells, developing its characteristic sac-like appearance. The bursa remains connected to the gut by the bursal duct. A wave of approximately 10⁵ committed B cell precursors migrate from the embryonic spleen to the bursa beginning at embryonic day 8²¹. This migration ends by embryonic day 15⁵⁴. These precursors are committed to the B lineage, having already rearranged Ig while in the splenic anlage and expressing the B cell marker *chB6*^{20, 22–24, 47, 51}. These immigrant *chB6*⁺ cells are capable of reconstituting humoral immunity in birds whose bursa has been depleted by irradiation. Only cells with a productive Ig rearrangement are thought to effectively seed the bursa; however, this does not appear to be dependent on V(D)J-encoded determinants of Ig^{43, 58, 59}. Immigrant cells enter the bursa by virtue of their expression of the sialyl Lewis x (*sLe^x*) carbohydrate, suggesting a selectin-mediated migration from the circulation^{40–42}. Furthermore, these carbohydrates mark cells capable of homing to the bursa and reconstituting the B cell compartment of irradiated recipients, cells often referred to as bursal stem cells. As these precursors leave the circulation they migrate toward the epithelial layer along the cloacal wall. Once in contact with the epithelium the lymphocytes begin to proliferate, forming lymphoid follicles. Roughly 2 to 4 precursors initiate the formation of each follicle⁵⁴. During embryonic development the follicles are seen as densely packed masses of cells; distinct cortical and medullary areas are not apparent until post-hatch.

As the cells begin proliferating they also begin a series of gene conversion events that are necessary contributors to a diverse antibody repertoire. Numerous excellent reviews are available concerning gene conversion⁵⁶. Coincident with the initiation of gene conversion is the gradual loss of expression of *sLe^x* and an increase in Lewis x (*Le^x*) carbohydrate⁴¹. This change in carbohydrate moieties also coincides with a loss of the ability to home back to the bursa. Near hatching time, B cells begin to emigrate to the periphery to respond to antigen^{22, 40, 41, 51}. Gene conversion and proliferation continue until the bursa involutes at about 16 weeks after hatch. These processes allow the bursa to

continually send naive B cells to the periphery. Gene conversion is also found during active immune responses in the germinal centers of the spleen^{1, 3}.

Distinct changes in bursal architecture occur around the time of hatch. Each follicle begins to form distinct cortical and medullary areas delineated by a follicle-associated epithelial (FAE) layer^{13, 16}. B cells near the FAE express Notch-1 while the FAE cells express the Notch ligand *Serrate-1*⁴⁵. Although the significance of Notch expression in the bursa remains unclear, Notch signals have been implicated in suppressing Ig expression and in various steps of B cell development in mice³⁸. Cells from the medullary layer migrate across the FAE to form the cortical lymphocytes⁵⁰. While ultrastructurally indistinguishable from the medullary lymphocytes¹³, the cortical lymphocytes are noted to be densely packed, more mitotically active, and have lowered expression of the LT2 antigen⁵⁰. Cortical cells also differ functionally from medullary cells in that they emerge from the bursa to form a short-lived population of peripheral B cells. Medullary cells remain in the bursa longer and form a longer-lived population of B cells in the periphery^{48, 49}. Although medullary cells migrate to form the cortical lymphocytes, the developmental mechanism leading to these functionally distinct populations remains enigmatic. B cells within the medulla are enmeshed in a reticular network of epithelial cell processes, whereas epithelial cells are comparatively rare in the cortex¹³. Macrophages are common in both cortical and medullary areas. The epithelial network within the medulla is connected via desmosomes and includes a variety of antigenically distinct types of cells^{6, 72, 73}. No evidence of distinctive gene conversion events or the extent of gene conversion between cortical and medullary B cells has been presented. In all, this suggests a more complex microenvironment within the medulla, yet the nature of the signals influencing development of the medullary B cells has not been elucidated. Apoptotic B cells become apparent in the medulla around the time of hatch⁵⁹.

The bursa provides a microenvironment essential for proper B cell diversification and maturation. Through several studies it became clear that interactions between immature B cells and the bursal epithelium were required for B cell differentiation, although the underlying mechanism was not well understood. Birds that were surgically bursectomized at 60 h of incubation had only very limited mature B cell diversity, indicating that the bursa provides an environment that is crucial for the differentiation of B cells^{18, 26, 39, 64, 69}. This is needed to generate a complete and diverse antibody repertoire for the adult bird. The bursectomized

animals are deficient in that they are unable to produce specific antibodies even after repeated immunization and have an oligoclonal B cell repertoire. Therefore the bursa is required for the initiation of gene conversion, for proliferation of B cells, and to ensure that the B cells produced are competent to participate in immune reactions.

Involvement of External Antigens

The bursa remains connected to the gut via the bursal duct and gut antigens may enter the bursa^{7, 10, 63}. Furthermore, reverse peristaltic contractions can introduce exogenous antigens and blood-borne antigens have also been found in the bursa⁵⁹. These antigens are sequestered predominantly in the medullary follicles and may persist there for some time, possibly as immune complexes with hen-derived IgG⁹. Animals with either a bursal duct ligation or raised in a germ-free environment still develop a diverse repertoire by gene conversion, but there are lowered numbers of B cells compared with normal animals and a lowered proliferation of medullary B cells^{8, 34}. It does appear that selective expansion of cells with productive VJ joints requires the presence of external antigen, consistent with the loss of cells lacking V(D)J-encoded determinants². The role of antigen within the medullary follicles is unclear, but its presence certainly suggests an important, if not absolutely required, function. Certainly antigen presence is important in the maturation of other vertebrate immune systems. For instance, bacterial colonization of the rabbit intestine is required for the initiation of gene conversion in the rabbit appendix^{30, 31}.

Molecular Control of Development

As noted, B cell precursors rearrange Ig genes in the embryonic spleen prior to migration to the bursa. Chickens have a limited number of functional V, D, and J segments and consequently generate little diversity by rearrangement⁵⁶. Most of the diversity is generated by gene conversion of the rearranged Ig V exons. As B cell precursors enter the bursa and begin proliferation, cells with functional Ig rearrangements are selectively expanded⁴³. This has led to the hypothesis that Ig-dependent signals are required to initiate both proliferation and gene conversion^{59, 65}. Indeed, the Ig present on bursal lymphocytes is coupled to a functional, signal competent B cell receptor (BCR)^{17, 55, 59}. According to a model proposed by THOMPSON⁶⁵, the undiversi-

fied Ig engages a self-antigen expressed by the bursal epithelium, initiating proliferation and gene conversion. After sufficient gene conversion such that the surface Ig no longer binds self antigen, the absence of BCR signals would lead to the cessation of proliferation and exit to the periphery.

Recent data from the Ratcliffe laboratory highlights the importance of BCR-derived signals in the colonization of the bursa and subsequent lymphocyte expansion. Using a retroviral vector, cells expressing a truncated IgM heavy chain that lacks a variable domain were competent to seed the embryonic bursa, initiate follicle formation, and begin gene conversion^{58, 59}. This argues against a distinct interaction via the variable domain of the Ig molecule. However, after hatching, these cells exhibit a proliferative defect and increased apoptosis in medullary follicles⁶⁰. This argues for a distinct post-hatch event in selecting cells expressing a bonafide Ig.

Apoptosis within the Bursa

Coincident with the histological changes and the introduction of exogenous antigens at hatching, levels of apoptosis within the bursa rise dramatically⁵⁹. Only about 5% of B cells ever actually leave the bursa, the remainder will die by apoptosis³². This phenomenon may be analogous to events in mammalian bone marrow that cause most B cells to undergo apoptosis. It is presumed that these B cells fail the selection process because they either do not possess a functional Ig molecule, or that their Ig reacts inappropriately with self molecules. In addition, bursal lymphocytes are notably susceptible to transformation, so apoptosis may be a mechanism to detect and control aberrantly proliferative cells.

Many investigators have suggested that gene conversion events may alter the reading frame, resulting in the loss of Ig expression and death. However, in the absence of selection for V(D)J-encoded determinants the vast majority of gene conversion events occur in-frame, at least for VJ joints⁵⁹. This argues against gene conversion as a mechanism leading to the loss of such a large proportion of B cells. Chickens are susceptible to antibody-mediated autoimmune diseases, so it seems logical that some of the cell death in the bursa could be attributed to the censoring of self-reactive clones.

Interactions with epithelial elements of the bursal stroma are essential in the regulation of apoptosis by bursal B cells. Disrupting bursal follicles by gamma radiation or mechanical stress causes B cells developing there to undergo apoptosis⁴⁶. If bursal follicles

remain intact, B cells continue proliferating, highlighting the importance of close bursal contact during development. When that contact is lost, it appears that some sort of signal is also lost and rapid cell death results. Close B cell/epithelial contacts made in the bursa may serve to control the immense proliferative capacity of bursal B cells. Inducing apoptosis soon after these interactions are disrupted appears to be a mechanism to avoid the potentially hazardous consequences of uncontrolled B cell growth. There is good evidence for signaling between the bursa and developing B cells. Activation of protein kinase C (PKC) mimics a B cell stimulation signal and can protect B cells from apoptosis *in vitro*^{4, 70}. It can be inferred that B cells undergoing apoptosis *in vitro* were doing so because they were deprived of some sort of signal that would normally be provided by the bursal contact. The chL12 antigen has been associated with selective survival of bursal cells²⁹. Expression of the 40 kDa chL12 molecule is first noted on bursal cells just prior to exit from the bursa to the peripheral immune tissues⁴⁸. However, the data are correlative and the function of chL12 remains unknown. Expression of chL12 is also noted on hemopoietic precursors and T cells, so its function is not confined to the B lineage²⁴.

The chB6 Alloantigen

A well-known marker of avian B cells is the chB6 (formerly called BU-1) alloantigen¹⁵. chB6 is expressed on the earliest identifiable B cell precursors and continues throughout ontogeny, with the exception of plasma cells in the Harderian gland⁵¹. Aside from B cells, chB6 is expressed at low levels on a subset of macrophages in the bursa, liver, and intestine⁵¹. Recently, three separate alleles of chB6 were cloned, designated chB6.1, chB6.2, and chB6.3⁶⁶. chB6 is a type I transmembrane glycoprotein with an N-terminal extracellular region, a single predicted transmembrane region, and a 105-amino acid-long cytoplasmic tail. The cytoplasmic tail is notably rich in acidic residues and proline. All three cloned alleles of chB6 are equally divergent from one another, and differ by a few single amino acid changes in the extracellular domain. In the cytoplasmic domain the only difference is that chB6.3 has an isoleucine at amino acid 217, whereas chB6.1 and 6.2 have a leucine. The chB6 molecule is expressed on the cell surface with an apparent molecular weight of 70 kDa and is present as a homodimer on both primary B cells and on the DT40 lymphoma cell line (FUNK et al., submitted for publication)^{53, 57}. The protein

encoded by the chB6 cDNA is predicted to be ~35 kDa in size, so it is likely that half of the molecular weight of chB6 is accounted for by carbohydrate modifications. There are 6 potential sites for N-linked glycosylation⁶⁶. Database searches reveal no potential homologues of chB6, suggesting that chB6 may be either a highly conserved molecule or a unique molecule developed during avian evolution. Since chB6 is expressed predominantly on B lineage cells and is present throughout their differentiation, it is likely to be an important molecule in B cell physiology.

chB6 has been used to mark B cells in a number of studies, but a function was never reported. In our early experiments we found that antibodies to chB6 stimulated extremely rapid cell death in primary B cells, a finding that has since been confirmed in other laboratories^{14, 70}. We have found this effect in using the 21-1A4 and FU5-11G2 monoclonal antibodies⁶⁸; we have not tested the effects of the L22 and AV20 antibodies in causing apoptosis^{53, 57}. The L22 antibody has been used in studies involving the isolation of chB6⁺ cells by flow cytometry^{23, 24}. In our work chB6 appears to possess many of the characteristics of a death receptor on chicken B cells. When B cells are exposed to anti-chB6 antibody, rapid cell death results, and affected cells exhibit characteristic apoptotic cell morphology (FUNK et al., submitted for publication)⁵². In our initial studies we reported that phorbol esters did not protect primary B cells from chB6-initiated apoptosis. However, in these studies freshly isolated bursal B cells were placed in culture with PMA and anti-chB6 ascites added simultaneously. Subsequent studies by WEBER⁷⁰ have shown a protective effect on primary cells at higher concentrations of phorbol ester and when the cells are preincubated with the ester before the addition of anti-chB6 antibody. In recent studies using DT40 cells as a model of chB6-induced apoptosis we have confirmed that preincubation of cells with as little as 5 ng/ml PDBU can reduce apoptosis, as measured by TUNEL assay, as much as 50% (BECKER et al., unpublished observations). Accordingly, we feel that PKC-mediated signals can in fact suppress chB6-induced apoptosis. The mechanism of this suppression is not known, although phorbol esters have been reported to prevent the assembly of the death-inducing signal complex by causing the phosphorylation of the cytoplasmic domain of Fas⁴⁴.

Transfection of chB6 cDNA transferred this cell-death effect into other avian lymphocyte cell lines^{14, 52}. chB6 has been transfected into a murine cell line and shows similar function when bound by anti-chB6 antibody. This is suggestive of a conserved death mechanism.

ism. Using transfected murine FL5.12 cells we have shown that chB6 can mediate a signal that results in the cleavage of caspase 8 and caspase 3, both integral cysteine proteases commonly activated in apoptosis pathways⁵². Furthermore, this apoptosis could be regulated via signals from Bcl-x_L or growth factor. However, our studies on the growth factor-dependent murine cells are complicated by the necessity of removing interleukin 3 in order to allow chB6-initiated apoptosis. While we can detect increased cleavage of caspase 8 and 3 in these cells, it is always against a background of the caspase activation brought on by the removal of growth factor in the first place.

As a result of this complication we have elected to study chB6 signaling in the DT40 lymphoma cell line (FUNK et al., submitted for publication). DT40 is an avian leukosis virus-induced bursal lymphoma and possesses many of the characteristics of bursal lymphocytes. DT40 expresses the sLe^x carbohydrate and will home to the bursa, it undergoes gene conversion *in vitro*, and it expresses endogenous alleles of chB6.1 and chB6.2^{27, 40}. Therefore we can avoid artifacts due to transfection. Furthermore, DT40 is commonly used in studies of signal transduction via the BCR, so a number of signaling mutants are available²⁸. DT40 cells undergo rapid apoptosis after exposure to anti-chB6 antibodies as assessed by both their morphology and staining via the TUNEL procedure (FUNK et al., submitted for publication). We have confirmed that overexpression of Bcl-x_L inhibits chB6-mediated apoptosis in DT40. Furthermore, preincubation of DT40 cells with peptide inhibitors of caspase 8, caspase 9, or caspase 3 reduces apoptosis due to binding of anti-chB6 antibodies by nearly 50% (CRISAFI and FUNK, unpublished observation). Since chB6-induced apoptosis appears to be intact in DT40 and can be inhibited by phorbol ester, we can test whether Ig-derived signals can override death signals via chB6.

The signaling mechanism of chB6 remains a focus of our laboratory. Antibody binding to chB6 does not result in calcium mobilization⁵⁵ and we have been unable to detect phosphorylation events involving either tyrosine, threonine, or serine in DT40 cells (BECKER and FUNK, unpublished observations). Currently it seems likely that chB6 initiates signals via protein/protein interaction. Since we can detect activation of the initiator caspase 8 after chB6 is bound by anti-chB6 antibody, we favor a model where chB6 interacts with caspase 8 directly. There is no readily identifiable death domain in chB6 for the interaction of adaptor proteins and other instances of direct activation of caspase 8 signals have been reported^{15, 25, 67, 71}. We are currently testing this hypothesis. We have also determined that trun-

cation of the chB6 cytoplasmic domain at amino-acid 262 severely attenuates the ability of chB6 to induce cell death (ROBISON et al., unpublished observations). We are currently undertaking more detailed mutagenesis of the chB6 molecule.

There are particular difficulties with the vision of chB6 as strictly a cell death-inducing molecule. First, chB6 is expressed on even the earliest B cell precursors²³. Since there are relatively few of these cells, one would expect them to be protected from cell death. Second, chB6 is expressed throughout B cell ontogeny, yet bursal cells are selectively susceptible to cell death after binding of anti-chB6 antibody, while splenic B cells are relatively unaffected^{14, 22, 70}. Bursal cells express particularly high levels of chB6, so perhaps the death signal is dependent on a threshold of chB6 signaling. Alternatively, the apoptotic signaling apparatus in particular B cell subsets may be inhibited, possibly by anti-apoptotic Bcl family members. A low level of chB6 expression has been detected on granular cells in the bursa and on a subset of macrophages in other tissues^{23, 70}. The function on these cells remains unexplored. Nevertheless, on bursal B cells chB6 seems to function in a death receptor-like fashion, particularly in the late embryonic and post-hatch period. It seems fitting that a death receptor would reside on the surface of lymphocytes to edit these cell populations in the most efficient and least detrimental fashion.

chB6 Ligand

Our previous studies support the hypothesis that chB6 can act as a death receptor for bursal lymphocytes with the anti-chB6 antibody acting as an agonist in place of a natural ligand. We therefore set out to determine if a natural ligand could be detected in the bursa. To investigate this we fused the cDNA encoding the extracellular domain of chB6.1 with an alkaline phosphatase (AP) reporter^{11, 12}. This fusion protein was then used to histochemically stain frozen tissue sections with alkaline phosphatase reactivity indicating the presence of chB6 ligand (Fig. 1) (PALMER and FUNK, manuscript in preparation). Little binding of either the chB6-AP fusion protein or the secreted AP was detected in thymus and liver. In the bursa, however, the chB6-AP fusion protein resulted in intense staining of the epithelial folds and a patchwork-like staining of follicles. Not all follicles stained and among those that did stain, the staining was often not uniform throughout the follicle. Rather, some follicles stained intensely throughout the medulla, whereas others stain only in a restricted area

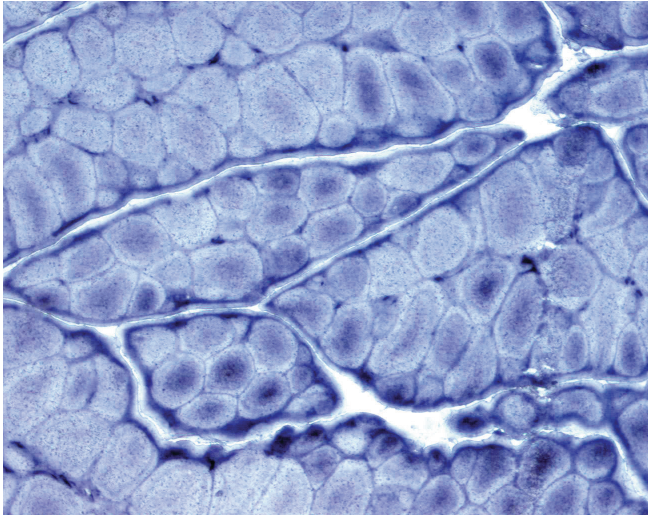


Fig. 1. chB6-APTag histochemistry in bursa. Low-power view of bursal section incubated with chB6-APTag supernatant as described, showing overview of putative ligand expression. Tissue from 1–2 week-old chickens was obtained and immediately frozen. A cryostat was used to obtain 20 μ m-thick sections of bursal tissue to mount on microscope slides. Tissue slides were then stained with chB6-APTag supernatant and binding visualized with NBT/BCIP substrate. Dark blue/purple staining can be seen along the epithelial folds of the bursal follicles, and inside the follicles on some of the B cells in sections stained with the chB6-APTag fusion protein. Tissue stained with APTag only (which does secrete AP) does not exhibit any specific cell staining (not shown)

of the medulla, sometimes giving a cap-like appearance. In the spleen, chB6-AP fusion protein stained distinctly in areas at the borders of germinal centers (PALMER and FUNK, manuscript in preparation). The locations in the spleen where this putative ligand is expressed correspond to areas where increased apoptosis of B cells has been reported⁶².

In the bursa, apoptotic cells have been observed in distinct clusters within follicles⁶⁰. Since relatively few cells initiate each follicle and undergo serial gene conversion events, independent antigen-reactive clones appear in distinct areas of each follicle^{21, 33, 37}. The patchwork appearance of apoptotic cells mirrors the patchwork appearance of antigen-reactive cells because as an autoreactive clone appears by gene conversion it must be eliminated. Likewise, the patchwork appearance of the presumptive ligand of chB6 could mirror the signals needed to begin the elimination of autoreactive or otherwise defective cells. Some bursal follicles have been noted to contain extensive numbers of apoptotic cells, suggesting a common signal for deletion¹⁹. This observation correlates well with our observation of the expression pattern of chB6 ligand. We are currently working to co-localize expression of the putative

chB6 ligand with TUNEL-positive apoptotic cells in the bursa. Nevertheless, the similarity in expression pattern is consistent with the idea that an external ligand binds to chB6 and can initiate apoptosis in B cells.

A Model of Bursal Signaling

Signals emanating from Ig would appear to be critical to the establishment of B cell precursors in the bursa. Immigrant B cells express Ig and a distinct interaction with bursal epithelial cells leads to the initiation of a bursal follicle with explosive proliferation of B cells. As part of this interaction, only cells with productive rearrangements, at least at the light-chain locus, are selected to proliferate⁴³. B cells within the bursa are competent to transduce signals via surface Ig and the machinery needed to propagate these signals is present and functional⁵⁹. The dramatic proliferation of B cells after seeding the bursa is suggestive of Ig-driven proliferation. In a model originally proposed by THOMPSON⁶⁵, bursal immigrants bind to a putative self antigen present on the surface of bursal epithelial cells. Engagement of surface Ig by this self antigen stimulated proliferation and gene conversion in these cells. Loss of signal, coincident with sufficient gene conversion to mutate the surface Ig to abolish binding the self antigen, would cause these cells to exit the cell cycle and make them competent to exit the bursa for the peripheral immune system. However, this model did not provide a mechanism of distinguishing cells that had sufficient gene conversion from those that had lost Ig expression via defective conversion events. It now appears that these cells would comprise a minority of the population in the bursa⁵⁹. We know that most lymphocytes will never leave the bursa³², yet the majority of gene conversion events preserve the reading frame⁵⁹, meaning that most of the cells will die with the capability of producing a functional Ig. In addition, the model does not provide a mechanism to eliminate self-reactive lymphocytes created by gene conversion. The finding that cells lacking V(D)J-encoded determinants can colonize the bursa would seem to require the revision of this model⁵⁸.

A schematic of our working model is given in Fig. 2. The model presented is simply a way to consider B lymphocyte development and is best thought of as a way to formulate hypotheses and design experiments. In this model, cells enter the bursa and initiate follicle formation by virtue of their expression of Ig on the cell surface. This engages a receptor on the epithelium that binds to non-V(D)J determinants, yet still stimulates

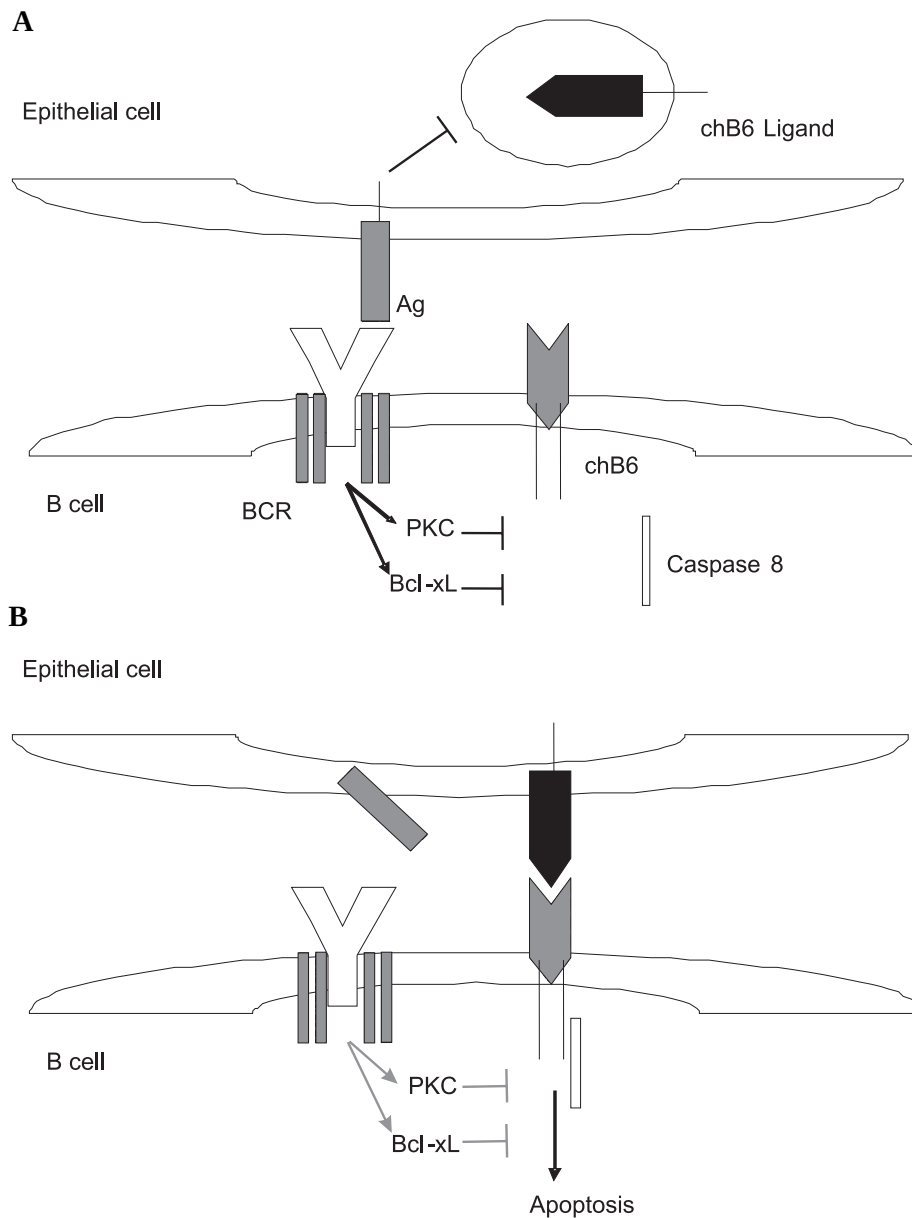


Fig. 2. Hypothetical model of chB6 signaling. **A** – B cells developing in the post-hatch bursa are dependent on BCR-derived signals, potentially derived from response to exogenous antigen (Ag) sequestered on bursal epithelial cells. BCR signaling acts to prevent chB6 signals from triggering apoptosis. In addition, antigen sequestered as immune complexes on the epithelial cells serves to retain chB6 ligand in intracellular vesicles; **B** – upon the loss of BCR signals, chB6 is no longer inhibited and can initiate apoptotic signals. It does not lead to apoptosis, however, until the translocation of chB6 ligand to the surface of the epithelial cells. We hypothesize that this occurs when the epithelial cell loses signals derived from the sequestered antigen

BCR-dependent signals. Alternatively, basal signaling via BCR may be sufficient to maintain these cells even in the absence of an external ligand⁵⁹. As gene conversion proceeds, only those cells that maintain Ig expression continue to be stimulated. At hatching, exogenous antigens derived from the gut flora are present in the follicular medulla in the form of immune complexes^{8, 9}. Around the time of hatch a second BCR-derived signal, likely involving exogenous antigens, selectively drives the proliferation of cells responding to foreign antigens (Fig. 2A). Interestingly, it is not clear from published reports whether the exogenous antigens favor expansion or deletion of specifically reactive cells^{2, 10}. In either case, the BCR signals serve to suppress apoptosis via chB6. Our working hypothesis is that chB6 ligand

is held internally in medullary epithelial cells. A loss of Ig binding to ligands, possibly immune complexes bound to the surface of epithelial cells by FcR, allows for the surface translocation of chB6 ligand. By engaging chB6 on the B lymphocytes, the chB6 ligand will trigger apoptosis of those cells that have lost the ability to generate BCR signals. Importantly, continued generation of BCR signals will prevent or delay the apoptosis of nearby lymphocytes; only the conjunction of loss of BCR signaling and initiation of chB6 signals leads to apoptosis. In this case, chB6 would be seen as an apoptosis accelerator since cells losing BCR signals would undergo apoptosis eventually. By accelerating the removal of these cells, space and resources are devoted to new cells that still maintain Ig expression and bind

foreign antigens. While the model presented is speculative, it does allow for the involvement of exogenous antigens in the Ig-driven proliferation of B cells, it explains the patchwork pattern of expression of chB6 ligand, and it provides a possible explanation for the role of chB6 in eliminating defective cells.

While it is unclear if there is a molecular homologue of chB6 in mammals, the idea of a multisignal mechanism to monitor lymphocyte development may provide a new insight into the development of B cells in mice and humans. The rapidity of chB6-induced death allows for rapid removal of cells. The apoptotic transit time of B cells in mouse bone marrow is estimated to be very short, on the order of 30 min³⁵. The ability of chB6 to kill mammalian cells and to be regulated in those cells suggests that it operates via a conserved pathway, yet chB6 itself appears to be a uniquely avian molecule^{52, 66}. Perhaps there are highly divergent molecules in mice that perform a similar function but are so distinctive as to no longer resemble one another.

The chicken remains an interesting and informative model for the study of lymphocyte development. As presented here, the chicken is hardly an ignored model of the immune system and investigators continue to learn more about the dynamics of B cell development in the bursa. Clearly, there are more questions than answers about lymphocyte development in the bursa. These include: how are distinctly self-reactive cells detected, is there an Ig ligand needed for effective colonization of the bursa, and what is the role of exogenous antigens? The development of novel molecular and genetic methods promises to open new avenues of experimentation and, without doubt, the posing of new questions.

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