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The contribution of Bruce Glick to the definition of the role played by the bursa of Fabricius in the development of the B cell lineage

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Summary

In 1956, Bruce Glick and Timothy Chang reported that the bursa of Fabricius plays an important role in antibody production. Their demonstration that antibody responses are suppressed in the majority of bursectomized chickens became the cornerstone of modern immunology. Bursa research increased considerably during the 1960s and early 1970s.

Keywords: B cell, bursa of Fabricius, history of medicine, immunology, T cell

Fabricius ab Aquapendente

Fabricius ab Aquapendente was the student and successor of Andreas Vesalius (1514–64) and Gabriel Fallopius (1523–62). Girolamo Fabrici, or Fabrizio (Hieronymus Fabricius ab Aquapendente) (1533–1619), practised and taught anatomy at Padova for more than 50 years [1]. Harvey was one of his pupils. In addition to his demonstration of the valves of the vein, Fabricius is best known for his description of the bursa that bears his name.

A manuscript entitled *De Formatione Ovi et Pulli*, found among his lecture notes, was published in 1621. It contains the first description of the bursa [2]: 'The third thing which should be noted in the podex is the double sac [bursa] which in its lower portion projects toward the pubic bone and appears visible to the observer as soon as the uterus already mentioned presents itself to view' (p. 147).

The sac-like organ has been known ever since as the bursa of Fabricius (BF). It overlies the dorsal surface of the terminal portion of the gut in birds. In the 5–6-day-old chick embryo, it arises as a dorso-caudal outpouching near the cloaca that takes the form of a median lamina of endodermal epithelium permeated with spherical vacuoles of various sizes that eventually coalesce to create a lumen [3]. The bursa grows considerably during development and changes shape from round to oval. Hypertrophy of the mesoderm surrounding the bursal epithelium produces longitudinal plicae that project into its lumen [4].

Between the 13th and 15th days, epithelial cells lining the plicae thicken and extend into the tunica propria as epithelial buds. These buds then separate from the epithelium. Lymphopoiesis is active in those that form the medullus of the bursal follicle [5,6].

Follicles may be present during late embryonic development (after 16 days). Even so, they are best observed by light microscopy at hatching and during the early growth of the BF [7]. The BF has 8000–12 000 total follicles, each composed of a cortex, medulla, corticomedullary border and follicle-associated epithelium [8]. The presence of M cells within this epithelium explains the movement of antigen from the lumen into the medulla, where immature B cells develop [9].

The BF plays a major role in the development of antibody-mediated immunity

In December 1952 Bruce Glick, at Ohio State University, demonstrated that the BF grows most rapidly during the first 3 weeks after hatching. He thus became convinced that functional investigation of the BF would be successful only if it was removed (bursectomy, BSX) within this period.

In 1954 Timothy S. Chang, a graduate student, needed birds to develop antibodies against *Salmonella*; the only birds available were those of Glick. He therefore injected 6-month-old pullets with *Salmonella*-type O antigen to obtain serum with a high antibody titre. Several pullets died and none of

those that survived produced antibodies. It was then found that the entire batch had been BSX during the period of rapid bursa growth.

Glick deduced that the absence of BF was responsible for this failure, as non-BSX pullets produced normal antibody titres [10], and designed two experiments to substantiate this conclusion. Equal numbers of male and female white leghorns were BSX at 12 days of age and injected six times at intervals of 4 days with *S. typhimurium* O antigen. At 7 weeks, seven of 10 BSX birds and two of 10 controls failed to produce antibodies [10]. The second experiment employed larger numbers of birds and two breeds: 89·3% of the BSX birds failed to produce antibodies compared with only 13·7% of the controls [11,12].

BSX do not abrogate the antibody response to cellular antigens

Next, Chang *et al.* [13] showed that at 2 weeks BSX was more effective in suppressing antibody production than at 5 or 10 weeks of age. The failure of BSX to eliminate all antibody production suggested the existence of a brief period in embryo development during which the BF could be functional.

The first experiments to evaluate the existence of a functional period for the BF [14] took advantage of the regressive influence of androgens on the post-hatched BF [15,16]. Treatment of 9–12-day-old embryos with testosterone prevented immunoglobulin production and lymphoid development, and presumably destroyed the stem cells which are necessary for B cell production.

Subsequent injection of bovine serum albumin (BSA) into chicks hatched from eggs injected on day 5 of incubation revealed complete immunoglobulin elimination, while chicks from eggs injected with testosterone on days 12 or 13 possessed significantly reduced levels of antibody [17,18]. The BF was generally absent in 19-day embryos that had received testosterone prior to day 8 [19].

Various BSX methods cause more or less complete B cells defects and agammaglobulinaemia. They include testosterone treatment [14,16,19–22], cyclophosphamide administration [23,24], colchicine treatment [25], X-irradiation [26] and surgical operations [27,28].

Cooper *et al.* [26] showed that chickens irradiated at hatching and also subjected to total BSX develop peripheral small lymphocytes in a normal fashion, reject skin syngeneic grafts and display normal graft-*versus*-host reactions. They are, however, prevented from developing the two clearly definable immunoglobulins and are completely unable to form circulating antibodies, even in response to strong antigenic stimulation.

Immunoglobulin synthesis regulation

The BF as a site of antibody synthesis was investigated by Glick and his coworkers in the early 1960s. Two experiments gave conflicting results. In the first, pheasant bursa cells produced antibodies to bovine immunoglobulins [29], whereas in the second the BF was unable to produce plaque-forming cells to sheep red blood cells (SRBC) [30]. The reason that pure B cells did not produce antibodies against SRBC became obvious only later, when Henry Claman's group discovered the requirement of B–T cell cooperation for antibody production against this and other T-dependent antigens [31]. These authors investigated the participation of both T and B cells in the *in vitro* response of spleen cells from mice immunized with the hapten NIP coupled to a non-immunogenic isologous gamma globulin carrier (MGG) [31].

Glick failed to identify antibody to BSA in the BF from 3-week-old intravenously immunized chickens [32]. The B cell differentiates in the BF and is able to produce immunoglobulins on the 14th day of embryo development. The first immunoglobulin is the large 1 000 000 molecular weight molecule called immunoglobulin M (IgM), followed by IgG on the 20th day and then by IgA [33,34]. Two equally plausible explanations of this sequence were advanced. One held that IgM B cells give to the IgG and IgA B cells, the other proposed sequential intrabursal development of isotype-committed sublineages. Kincade and Cooper [35] found that the anti-μ-mediated inhibition of IgM B cells also inhibited the development of IgG and IgA B cells. Moreover, the combination of embryonic anti-μ administration and post-hatching BSX resulted in permanent agammaglobulinaemia. These experiments indicated that while all chicken B cells express IgM initially, they can switch to the production of other isotypes. Neonatal anti-μ antibody treatment also inhibited mouse B cell development and antibody production of all Ig isotypes [36].

Delineation of the thymic and bursal lymphoid systems in the chicken

Functional dissociation of the chicken immune system based on differences in thymic and bursal influences was suggested originally by Szenberg and Warner [37].

Following Glick's demonstration of the crucial function of BF in the development of antibodies and the immune responses related to their production, in 1958 Francis A. P. Miller in Australia discovered the role of thymus-derived cells in cellular immunity [38]. Miller's experiments indicate that: (a) thymectomy is associated generally with a diminution in the lymphocyte population and (b) the earlier in life thymectomy is performed, the greater the deficiency of lymphocytes in other lymphoid organs [38].

Robert Good and his collaborators (notably Max D. Cooper) developed the idea of the B and T cell concept, demonstrating the essential role of the thymus in the development of cellular immunity functions other than antibody production in chickens [26,39]. Chickens were thus the first source of the two-component concept of immunity.

Sublethal X-irradiation of newly hatched chickens was needed to clarify the roles of the thymus and the BF in development of the two separate and functionally different lymphoid systems [39]. The BSX and irradiated birds were completely devoid of germinal centres, plasma cells and the ability to produce antibodies, yet they had perfectly normal development of thymocytes and lymphocytes elsewhere in the body that mediated cellular immune reactions; while the thymectomized and irradiated birds were deficient in lymphocytes that mediated cellular immunity as assessed by skin graft rejection, delayed-type hypersensivity and graft-*versus*-host reaction, but still produced germinal centres, plasma cells and circulating Igs. Van Alten *et al.* [27] used BSX within the eggs to show that the two-component concept was clearly evident even in the absence of X-irradiation. The BF and the thymus are central lymphoid organs in the chicken, essential for the ontogenetic development of their adaptive immunity. Surgical removal of one or both organs in the newly hatched chickens, followed by sublethal X-irradiation, led to the recognition of two morphologically distinct cell systems in the peripheral lymphoid tissues of the spleen, gut and other organs, and clear differentiation of their functions.

The thymus controls the development of all cell-mediated immunities, including delayed reactions, allograft immunities and other immunological functions. In addition to being a basic immunologist, Good also held the position of professor of Pediatrics at the School of Medicine in Minneapolis, Minnesota. He thus had access to the various cases of immunodeficiencies that had led him to recognize similarities between Bruton's agammaglobulinaemia and Glick's BSX, on one hand, and Di George syndrome and Miller's thymectomized mice on the other hand.

In fact, removal of the BF from the egg inhibits germinal centres and plasma cells and prevents antibody production [40]. BSX chickens are strikingly similar to patients with Bruton's X-linked agammaglobulinaemia [41], and *in ovo* thymectomized chicks are strikingly similar to those with Di George syndrome, while patients with severe combined immunodeficiency disease (SCID) are similar to chickens bursectomized and thymectomized in the newly hatched period [41]. The major immunodeficiencies, Bruton's disease, Di George syndrome and SCID, are thus mimicked by BSX or thymectomy *in ovo*.

BF equivalent in mammals and other vertebrates

The BF is present in all avian orders, but is absent in mammals. Several structures, however, have been identified as 'bursa equivalents', such as gut-associated lymphoid tissues in rabbits and ungulates and bone marrow in rodents and primates, including humans.

Archer *et al.* [42] found that the rabbit sacculus rotundus located at the ileo-coecal valve, like the BF, develops within follicular outpouchings of the lower gut. Immediate extirpation of this organ in neonates resulted in an impressive and lifelong immunodeficiency of antibody production [40,42]. Knight and Crane [43] have since demonstrated that the BF and the appendix-sacculus rotundus mediate very similar influences on the humoral system. However, the sacculus rotundus has not emerged as the BF equivalent organ.

Owen *et al.* [44] found that Ig-bearing cells first appear in the liver during mouse embryogenesis and employed fetal liver organ cultures to show that B cells are generated in the haematopoietic tissue. Moreover, Owen *et al.* [45] found that, after their colonization with haematopoietic stem cells, fetal long bones can also generate B cell *ex vivo*.

This finding suggested that mammalian B cell generation is a multi-focal process that shifts from one haematopoietic environment to another during development, to continue throughout life in the bone marrow. It is now clear that in mammals, B cells remain and differentiate in the bone marrow, a most convenient etymological coincidence, as the nomenclature for B cells as bone marrow-derived cells does not need to be changed.

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