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Identification and structural composition of the blood–spleen barrier in chickens

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ABSTRACT

To identify the existence and composition of the blood–spleen barrier (BSB) in chickens, the microanatomical features of the spleen were investigated by light and transmission electron microscopy, intravenous injection of ink, acid phosphatase reaction, and silver impregnation. The results showed that the white pulp in chicken spleen consists of lymphoid nodules, periarteriolar lymphatic sheaths (PALS) and periellipsoidal lymphatic sheaths (PELS). There was no evidence for the presence of a marginal zone.

The splenic ellipsoid was a unique structure, which functioned as a barrier for filtering and phagocytosis. Uptake of carbon particles was limited to the ellipsoid and PELS, 60 min after injection of carbon particles. Reticular fibres were densely distributed in the ellipsoid and extended into the PELS. Ellipsoid-associated cells (EACs), reticular cells and macrophages were acid phosphatase positive. The sheathed capillaries, surrounded by the ellipsoid, were similar to high endothelial venules (HEVs). These findings suggest that the BSB of chickens is present in the ellipsoid and PELS, protecting the spleen from invasion from circulating pathogens. The BSB was a reticular framework, between the arterial and venous vessels, which included cuboidal-shaped endothelial cells, supporting cells, EACs, macrophages, reticular cells and fibres. Lymphocyte migration into the spleen parenchyma is most likely via the HEV-like vessels. These research findings contribute to better understanding of avian immunology and provide an insight into evolutionary differences in the immune system.

Introduction

As the primary site for immunocyte proliferation and differentiation, the spleen is the largest secondary lymphoid organ in chickens, containing around 25% of the total number of lymphocytes. During embryonic development, the spleen functions as a haemopoietic organ (Fukuta and Mochizuki, 1982). Subsequently, with formation of red and white pulp, it plays a crucial role in immune responses, particularly to blood-borne antigens (Brendolan et al., 2007). There have been an increasing number of immunological studies in chickens, focused on responses in the spleen, as a consequence of poorly developed lymphatic vessels and lymph nodes in avian species (Nagy et al., 2005; Yang et al., 2010; Zhang et al., 2010; Kita, 2014).

Unlike in mammals, the spleen in avian species has no marginal zone containing macrophages and marginal reticular meshwork (Jeurissen, 1991). The ellipsoid, also known as the Schweigger–Seidel sheath, is a specialised capillary segment in the spleen of chickens (Kasai et al., 1995). Supportive cells and phagocytic cells are present within the ellipsoid, allowing the spleen to have the ability of clearing pathogens from the circulation. However, the formation of the ellipsoid structure varies between species. Ellipsoids are common in avian species, turtles, cats, dogs and humans, but not in rodents and lagomorphs (Hatae, 1978; Kroese and Van Rooijen, 1983; Kasai et al., 1995; Onkar and Govardhan, 2013). Specific antigen-trapping areas of the ellipsoid in the spleens of chickens are thought to be functionally similar to the marginal zone in mammalian spleens (Jeurissen et al., 1992; Kasai et al., 1995). Thus, the splenic ellipsoid, populated with lymphocytes and macrophages, is likely to play a crucial role in the development of specific immune responses in chickens.

Although the immune functions of the chicken spleen are well described, the structural characteristics of the blood–spleen barrier (BSB) are poorly defined. The BSB in the spleens of mice is characterised by a filtration bed, creating a microenvironment between lymphoid and non-lymphoid cells (Weiss et al., 1986; Weiss, 1988). However, there are notable differences in the structure of the BSB between species; whereas in rodents, the BSB is located in the splenic marginal zone (Weiss, 1991; Cubas et al., 2000), in turtles, it is located in the ellipsoid region of the spleen (Bao et al., 2009).

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Such information regarding the morphological features and immunological mechanisms of the BSB in avian species is lacking.

The aim of the present study was to identify the location, composition and ultrastructural characteristics of the BSB in chickens. Better understanding of the histological structure and immune function of the BSB could improve our understanding of the aetiology and pathogenesis of infectious diseases in chickens.

Materials and methods

Study population

Adult male and female Sanhuang broiler chickens, 60–65 days of age and weighing 1.5–2.0 kg were used for this study. Light and transmission electron microscopy, ink injection, acid phosphatase histochemistry and silver impregnation were undertaken in separate groups of birds ($n = 10$ per group). All chickens were euthanased by cervical dislocation, following intravenous (IV) administration of 3% sodium pentobarbital (25 mg/kg; Sigma-Aldrich). The procedures were approved by the Nanjing Agricultural Veterinary College Experimental Animal Ethics Committee (Approval number SYXK (SU) 2010-0005; Date of approval, 18 June 2010).

Light and transmission electron microscopy

The spleen samples were obtained immediately post-mortem and sectioned into two parts. One part was fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 5 μm thickness. These sections were stained with haematoxylin and eosin (H&E) and assessed by light microscopy using an Olympus BX53 microscope. The other part was cut into 1-mm^3 blocks, immersed in 2.5% glutaraldehyde fixative in 0.01 M phosphate-buffered saline (PBS; pH 7.4) at 4 °C overnight, then submerged in 1% osmium tetroxide in the same buffer for 60 min. Samples were dehydrated in ascending concentrations of ethyl alcohol, infiltrated with a propylene oxide-Araldite mixture, and embedded in Araldite. Ultrathin sections (50 nm) were stained with uranyl acetate and lead citrate for 20 min each. The sections were examined and photographed with a transmission electron microscope H-7650 (Hitachi).

Ink injection

Chickens were injected with Indian ink (Aolindan) containing 50–100 nm carbon particles. Three to five millilitres of ink, diluted 1:10 with 0.9% NaCl were injected IV after anaesthesia induced with 3% sodium pentobarbital. The control group was injected with a similar volume of 0.9% NaCl. Spleens were removed 60 min after injection and formalin-fixed, paraffin-embedded sections (5 μm) were assessed by light microscopy with and without staining by H&E as described previously.

Acid phosphatase histochemistry

Spleen blocks were fixed in 4% buffered paraformaldehyde at 4 °C for 3 h; then cryostat sections (12 μm) were prepared by use of a Jung-2700 freezing microtome. Sections were air-dried and exposed to cold acetone for 10 min. Acid phosphatase was demonstrated using the Gomori acid phosphatase method (Waters and Butcher, 1980). Briefly, cryostat sections were incubated in a solution containing 3% sodium-β-glycerophosphate (Acros Organics), 50 mM acetate buffer (pH 5.0) and 4 mM lead nitrate at 37 °C for 1 h, then sections were treated with 0.2% ammonium sulfide for 1 min.

Silver impregnation

The spleens were fixed in 10% neutral buffered formalin for over 24 h. Paraffin sections (12 μm) were assessed by the silver impregnation method specified by Gordon and Sweets (1936). Briefly, deparaffinised sections were rinsed in distilled water between each incubation step of 1% acidified potassium permanganate for 3 min, 1% oxalic acid for 2 min, 2.5% iron alum for 15 min, ammoniacal silver solution for 2 min, 10% aqueous formalin for 2 min, 0.2% gold chloride for 2 min, and 5% sodium thiosulfate for 2 min.

Results

Histological characteristics of the chicken spleen

The histological structure of the chicken spleen was different from that of mammals. Two distinct compartments of the chicken spleen were observed, namely red pulp and its interwoven white pulp, with no distinct marginal zone between them (Fig. 1a). The chicken white pulp was composed of the periarteriolar and periellipsoidal lymphocyte sheath (PALS and PELS), which surrounded central arteries and ellipsoids, respectively, and lymph nodules were sometimes observed attached to the PALS (Fig. 1b). Unlike in mammals, no trabeculae were evident in chicken spleens.

The trabecular artery continued as a central artery which was enveloped by the PALS. Lymph nodules were found at the border of the central artery and trabecular artery, usually at the beginning of the central artery (Fig. 1b). The central artery extended into penicilliform capillaries, the mid-portion of which (sheathed capillary) was encircled by the ellipsoid (Schweigger-Seidel sheath) (Fig. 1d). There was a larger amount of PELS surrounding the ellipsoid, compared with PALS around the artery, indicating that the chicken spleen was rich in sheathed capillaries, and the number of the branched sheathed capillaries was greater than the central arteries. Compared with the artery, the sheathed capillary was devoid of a smooth muscle layer (Fig. 1c, d).

The location of the BSB in the chicken spleen

Carbon particles appeared in the splenic ellipsoid 1 h after injection of Indian ink. Most of the carbon particles showed a web-like distribution in the ellipsoid. They were mainly deposited specifically in the position of ellipsoid-associated cells (EACs) and supporting cells of splenic ellipsoid. Few carbon particles were found at the border of PELS and red pulp, and fewer particles were found in the red pulp (Fig. 2a). Under higher magnification, carbon particles seemed to leave the blood stream from the vascular channels of the sheathed capillary (Fig. 2b). It was apparent that the BSB exists in the chicken spleen, which prevented carbon particles from entering the red pulp.

Following H&E staining, the PALS, lymph nodules and red pulp were seen to be free of carbon (Figs. 3a, b). Carbon particles were predominantly found in the extracellular space in the ellipsoid (Fig. 3c). EACs were observed to have an irregular shape and a relatively large amount of carbon on their surface (Fig. 3d). In addition, carbon particles were detected in the intercellular spaces between the supporting cells. A limited number of carbon particles infiltrated the PELS region, outside of the ellipsoid (Fig. 3d).

The cellular composition of the BSB in chickens

Since acid phosphatase (ACP) is mainly located in the lysosomes, assessment of splenic tissues by the ACP reaction revealed phagocytic cells of the BSB. ACP-positive cells were present in the ellipsoid and PELS (Figs. 4a, b). PALS and red pulp were ACP-negative (Fig. 4a). The PELS contain lymphocytes, reticular cells, EACs and macrophages. The ACP-positive cells in the PELS were different from those in PALS, with ACP activity in the PELS associated with cell clusters (Fig. 4b). EACs, macrophages, and reticular cells on the surface of ellipsoid were positive in the ACP reaction at the site where carbon particles were found (Fig. 3c).

Silver impregnation revealed the reticular fibres of the supporting framework of the BSB. In contrast to the single layer of the central artery, two layers of fibres were present on the ellipsoid, which were consistent with the basement membrane of the sheathed capillary and the discontinuous capsule of the sheath (Figs. 5a, b). In cross-section, two layers of fibres of the ellipsoid were observed, arranged in concentric circles. Within the ellipsoid, some filamentous fibres were vertically distributed between the inner and outer circle (Fig. 5d). Reticular fibres in the PELS formed a dense network. A discontinuous reticular fibre border between the PELS and red pulp was seen (Fig. 5b). The distal portion of the penicilliform capillary continued as red pulp sinus (Fig. 5c).

The ultrastructure of the spleen barrier cells

Ultrastructurally, the sheathed capillary was seen in the centre of the ellipsoid, supported by a discontinuous basement