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Architecture of the Blood-Spleen Barrier in the Soft-Shelled Turtle, *Pelodiseus sinensis*

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ABSTRACT

We investigated the structure of the soft-shelled turtle, *Pelodiseus sinensi*, spleen and demonstrated that there were several microanatomical peculiarities by light and transmission electron microscopy. In the spleen, the white pulp of the spleen was composed of two compartments, the periarteriolar lymphatic sheath (PALS) and periellipsoidal lymphatic sheath (PELS). No lymph nodules and marginal zones were found. The spleen-blood barrier stood in the PELS and the ellipsoid. The high endothelial lining of penicilliform capillary contained small channels. These channels allowed circulating substances or lymphocytes to enter the ellipsoid. The distal portion of the penicilliform capillaries directly opened to pulp cords. The ellipsoid-associated cell (EAC) was located at the surface of the ellipsoid. Reticular fibers were mainly distributed in ellipsoid and the outer PELS. Both reticular cells and macrophages were distributed in the outer layers of PELS. S-100 protein positive dendritic cells were mainly distributed in out cells layer of the PELS and all over the PALS. Forty minutes after injection, carbon particles of Indian ink were mainly observed in the ellipsoid. Few carbon particles were observed in the outer PELS and fewer carbon particles in the red pulp. These findings suggested that a blood-spleen barrier indeed existed in the soft-turtle, *P. sinensi*, and it was a complex composed of an ellipsoid (including supporting cells, EAC, and reticular fibers) and the outer compartments of PELS (including dendritic cells, reticular fibers and cells, macrophages). Anat Rec, 292:1079–1087, 2009.

Key words: soft-shelled turtle; blood-spleen barrier; architecture

The spleen is the largest lymphoid organ and the most important organ for the immunological defense against particulate antigen in the blood (Pabst, 1993), as well as the only organ specialized for filtering blood. Splenic histology has been described in many classes of reptiles (Marchalonis et al., 1969; Borysenko and Cooper, 1972; Wetherall and Turner, 1972; Kanakambika and Muthukkaruppan, 1973; Borysenko, 1976; Hussein et al., 1978a,b, 1979a,b; Chen and Nie, 1993, 1995). However, there is very little information about the anatomy of the spleen in soft-shelled turtles. Detailed information about the splenic structure is important for the understanding of its immunological role and for the analysis of several infectious diseases.

The existence of the blood-spleen barrier (BSB) in the mouse spleen has been confirmed by light-microscopic, electron-microscopic (Zhu et al., 2005), and functional

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studies with various extracellular tracers (Jiang et al., 2001, 2002a). The BSB is only found in mice and rats spleen so far, and its information is lack in other species. In rodent spleens, the BSB surrounding the white pulp is composed of macrophagocytes, marginal-sinus-endothelial cells and their basement membrane, the reticular tissue, and collagen fibers.

In this study, we analyze, whether the BSB also exists in the soft-shelled turtles spleen and if there are microanatomical differences between the spleens of rats and turtles.

MATERIALS AND METHODS

Animals

Male and female adult soft-shelled turtles, *Pelodiseus sinensi*, weighing 650–720 gm/each, were purchased from a wild breeding base in Jiangsu province of China at the beginning of July. All protocols were approved by the Chinese Committee for Animal Use for Research and Education.

Light and Transmission Electron Microscopy

For light and transmission electron microscopy, soft-shelled turtles (5 males and 5 females) were comatosed via intraperitoneal administration of sodium Pentobarbital (1 mL/animal) and sacrificed by cervical dislocation or perfusion fixation, and the spleens were removed and cut in half. One half was fixed whole in 4% buffered paraformaldehyde and prepared routinely for light microscopy. Paraffin sections were cut at 5 μm and stained with hematoxylin and eosin (H and E). The other half was fixed by immersion in 2.5% glutaraldehyde buffered (PH 7.3), post-fixed in 1% osmium tetroxide in the same buffer for an hour, and dehydrated in acetone for embedding in Araldite. Semi-thin sections (thickness 1 μm) were stained with an alkaline solution of toluidine blue. Ultrathin sections were contrasted with lead citrate and uranyl acetate and examined with a JEM-1200EX electron microscope.

Alpha-Naphthylatesterase

For non-specific alphthylatesterase cytochemistry, the tissue blocks were fixed in 10% cold formalin PBS solution, frozen and paraffin sections were both used for the reaction, and then re-stained with methyl green. The basic method of non-specific alphathylatesterase cytochemistry as described by Mueller et al. (1975).

Silver Impregnation

To demonstrate the reticular fibers, silver impregnation was performed. The spleen was fixed with 4% buffered paraformaldehyde for 15 min. The tissue samples were further fixed in the same fixative overnight. After being washed in buffer, the tissue blocks were embedded in paraffin, and 12-μm-thick sections were cut and deparaffinized according to Du (1998).

Immunohistochemistry

Paraffin sections (6 μm) were placed on poly-L-lysine-coated glass slides and stained according to standard immunohistochemical techniques. Briefly, after deparaffinization and washing in phosphate-buffered saline (PBS), the sections were covered with 3% hydrogen peroxide in PBS for 15 min at room temperature to block endogenous peroxidase activity. The sections were blocked with 5% bovine serum albumin and incubated with rabbit anti-S-100 protein (1:100) (purchased from Boster Bio-Technology Co., LTD) in a moisture chamber at 4°C overnight. After washing, the sections were incubated with biotinylated goat anti-rabbit IgG (purchased from Boster Bio-Technology Co., LTD) for 1 hr at 37°C. The sections were then rehydrated in PBS (PH 7.2), incubated with avidin-biotinylated peroxidase complex for 45 min at 37°C. After being washed with PBS, peroxidase activity was revealed using DAB (purchased from Boster Bio-Technology Co., LTD) according to the manufacturer's instructions.

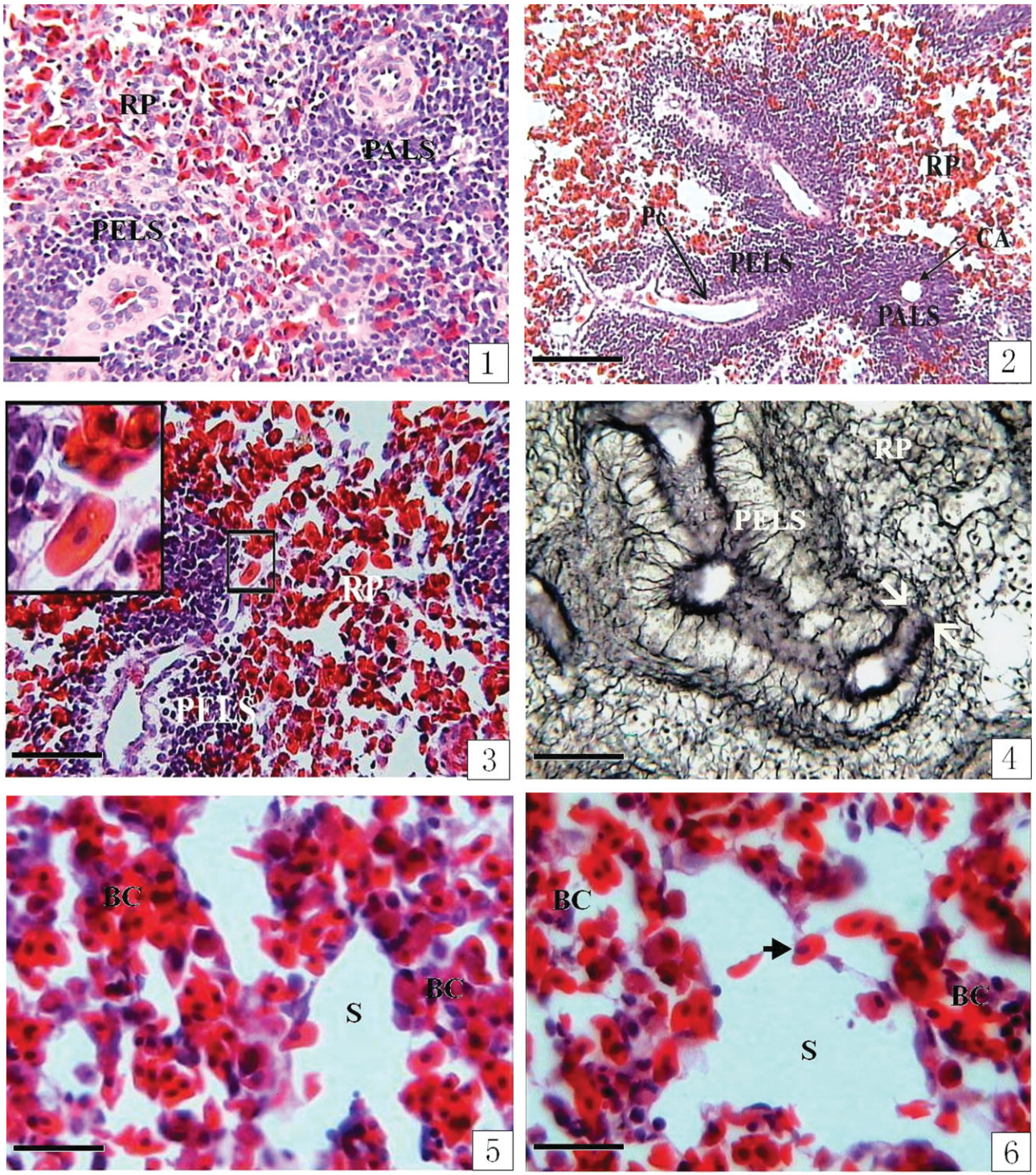
Injection of Indian Ink

To demonstrate the presence of BSB (Weiss, 1990; Zhu et al., 2005), suspension of Indian ink containing 50–100 nm particles was used. The soft-shelled turtles were injected by way of the aorta with 0.2 mL of a 40% solution of Indian ink in 0.9% NaCl per 100 g body weight. Forty minutes after injection, the turtles were killed. The plastron was removed and the spleen was dissected from the peritoneal cavity. Small fragments of the spleen were fixed whole in 4% buffered paraformaldehyde and prepared routinely for light microscopy. Paraffin sections were cut at 5 μm and stained with hematoxylin and eosin (H and E).

RESULTS

General Histology of the Spleen

The parenchyma of the spleen of this turtle showed a definite demarcation into a red and white pulp. The white pulp was composed of lymphoid tissue surrounding both central arterioles and ellipsoids, giving rise to the periarteriolar and periellipsoidal lymphocyte sheath (PALS and PELS), respectively (Fig. 1). PELS surrounding the ellipsoid was thicker in diameter and denser in lymphocytes assembling than that of PALS. The number of PELS was also more than that of PALS. The interstices of the white pulp were filled up by a system of venous sinuses and cords, constituting the red pulp. There was no histologically identifiable lymph nodules and marginal zone in this turtles. The splenic arteries entering the spleen at the hilus were surrounded by trabecular connective tissue (Fig. 23) just like other animals. When they left the trabeculae to enter the parenchyma, the arteries were immediately enveloped by lymphatic sheath (PALS). These vessels were known as central arteries (CA). No branches of the CA crossed the PALS (Fig. 2). The PALS usually ended where the CA makes a curve and became 2–6 straight penicilliform capillaries. As it was surrounded by the ellipsoid, the wall of the penicilliform capillary was thicker than that of the CA. The divisions could occur in the distal portion of the ellipsoid (Figs. 2, 23). Therefore, the actual shape of the ellipsoid was rarely ellipsoidal. It was usually bi-, tri-, or tetra-horned. The distal portions of the penicilliform capillaries continued into the red pulp cords (Figs. 3, 4). The number of red blood cells in the red pulp cords was more than that of red pulp sinus (Figs. 5, 6). Frequently,



Figs. 1–6. Fig 1. The parenchyma of spleen is divisible into red pulp (RP) and white pulp. White pulp is consisted of a periarterial lymphatic sheath (PALS) and periellipsoidal lymphatic sheath (PELS). H–E staining. Bar = 50 μm. Fig. 2. The central artery (CA) divided into two or three penicilliform capillaries (Pc). RP, red pulp; PALS, periarterial lymphatic sheath; PELS, periellipsoidal lymphatic sheath. H–E staining. Bar = 100 μm. Fig. 3. The distal position of penicilliform capillaries open into pulp cords, the inset is higher magnification. RP, red pulp; PELS, periellipsoidal lymphatic sheath. H–E staining. Bar = 50 μm. Fig. 4. At the border of PELS, the reticular fiber of distal position (➔) of penicilliform capillary continues into that of red pulp cords. RP, red pulp; PELS, periellipsoidal lymphatic sheath. Silver impregnation. Bar = 50 μm. Fig. 5. The number of red blood cells in the Billroth or pulp cord (BC) is more than in the sinus (S). H–E staining. Bar = 25 μm. Fig. 6. A red blood cell (➔) enters sinus (S) through the gap of their endothelial cells from Billroth or pulp cord (BC). H–E staining. Bar = 25 μm.

red blood cells entered sinus between their endothelial cells from the pulp cords (Fig. 6).

Architecture of Blood-Spleen Barrier

BSB in the soft-shelled turtle was a complex structure located in the ellipsoid and the outer compartments of PELS.

Ellipsoid

The ellipsoid itself was embedded in the PELS, formed by supporting cell and ellipsoid-associated cell (EAC). EAC were found over the surface of ellipsoid, which were Alpha-naphthylatesterase (ANAE) positive cells (Fig. 7), and had an affinity for toluidine blue (Fig. 8). Frequently, one of the EAC processes crossed the ellipsoid (Fig. 9). In the ellipsoid, one layer of cells concentrically arranged around the penicilliform capillary (Figs. 8, 10). These cells were supporting cells, which were round or oval in shape and had a large vesicular nucleus (Fig. 8). They were ANAE negative cells (Fig. 7) with less toluidine blue affinity than EAC (Fig. 8). Mobile cells like small lymphocytes and red cells were occasionally present in the ellipsoid (Figs. 9, 10, 20). Lots of reticular fibers also appeared in the ellipsoid (Fig. 9). The ellipsoid which located centrally in the PELS is heavily stained with Sliver impregnation (Figs. 11, 12).

PELS

PELS and PALS consisted of the white pulp in the soft-shelled turtle spleen. There were some structure differences between the two parts of the white pulp. Elongated reticular cells were found at the border of PELS and red pulp (Fig. 13). Interestingly, no elongated cells could be detected in the PALS. Sliver impregnation revealed a dense reticular fiber network in red pulp. PELS outer was outlined by reticular fibers (Figs. 11, 12). There were, however, only a few heavily stained reticular fibers scattered over the entire PELS. In distinction to PELS, there was no clear border between red pulp and PALS. With the transmission electron microscopy, reticular cells and macrophages were found around the PELS. These reticular cells displayed numerous processes that surround reticular fibers (Fig. 14). The separation of the red pulp from the white pulp was well defined by the processes of reticular cells (Fig. 15).

The S-100 proteins were present in the cytoplasm and nucleus. At the outer border of the PELS, dendritic character cells could be identified by anti-S-100 protein, and S-100 positive satellite-shaped cells occurred all over the PALS (Fig. 16). And S-100$^+$ cells arranged in 3–4 concentric layers in the PALS that consisted of highly positive elongated (Fig. 17). Meanwhile, distribution, and stainability of S-100$^+$ cells were highly variable in the outer layers of the PELS (Fig. 18).

Localization of Carbon Particles of Indian Ink

Forty minutes after injection, the injected carbon particles could be demonstrated in the spleen of *P. sinensi*, on the H–E stained paraffin sections (Fig. 19). Carbon particles were mainly found in the processes of this part of inset into ellipsoid of EACs and supporting cells in the ellipsoid (Fig. 20). The sections showed a web-like distribution of carbon particles in the ellipsoid. Few carbon particles were found at the boundary between PELS (Fig. 21) and red pulp, and fewer particles in the red pulp (Fig. 22).

DISCUSSION

Architecture of BSB in the Rodent

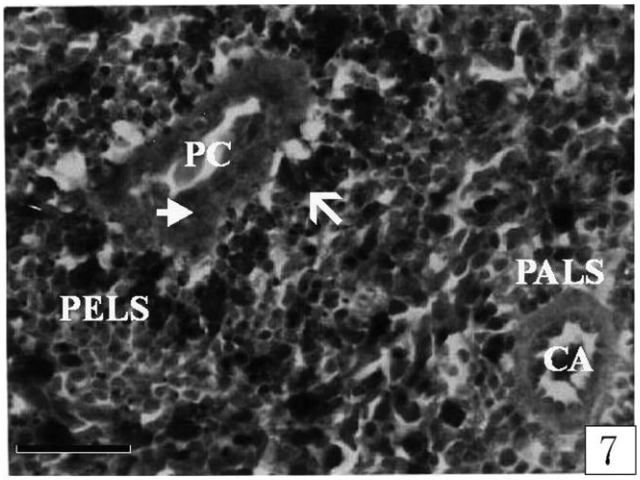
The BSB was first described in mice by Weiss (Weiss et al., 1986) in the non-lethal Plasmodium yoelii murine malaria. The course of non-lethal Plasmodium yoelii murine malaria might be divided into four phases. In the immediate post-infective phase, lasting several days, the filtration beds of the spleen were open. In the following precrisis phase, reticular cells, the stromal cells which form the splenic filtration beds, became activated, showing signs of intense protein secretion and increased branching and mitosis. The locules of the filtration beds appeared sealed off from the blood by branches of activated reticular cells. A BSB was thereby formed, protecting splenic hematopoiesis from the parasite (Weiss et al., 1986). Those stromal cells which branched and fused with one another, forming extensive, complex, irregular, syncytial membranous sheets, as a variety of barriers, which were called barrier cells (Weiss, 1989, 1990, 1991). However, Weiss didn't unequivocally point out the composition of BSB. In china, some investigators, by injecting ink from the tail vein in mice, observed that the ink particulated can be found in endothelial cells and macrophages and proposed that BSB is located in marginal zone and it is composed of macrophages, splenic sinus endothelial cells and their basement membrane, the reticular tissue and collagen fibers (Guo et al., 2000; Jiang et al., 2001; Jiang et al., 2002a,b; Zhu et al., 2005).

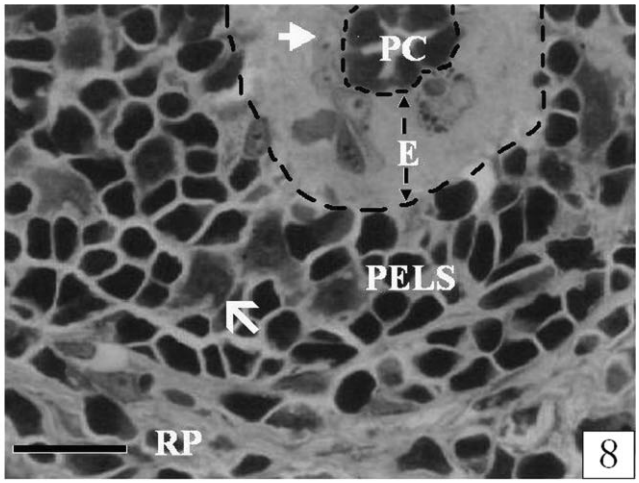
The Structural Elements and Framework of the BSB in the Soft-Shelled Turtle

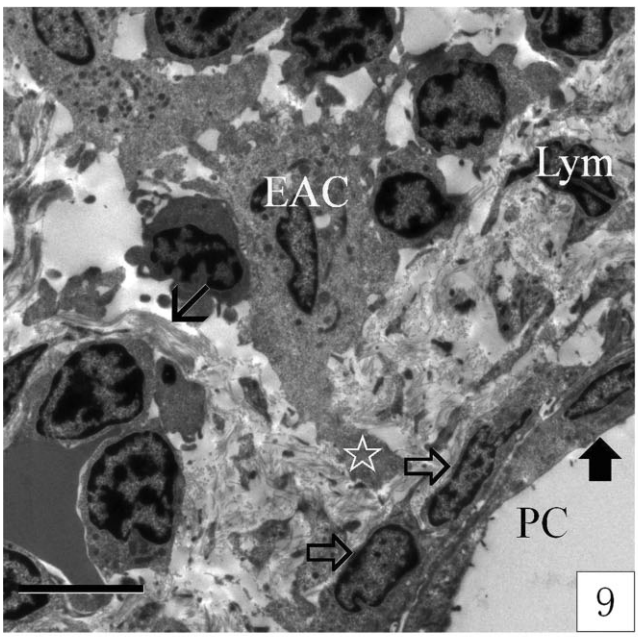
Our findings revealed that BSB consisted of two parts in the soft-shelled turtle, *P. sinens*.

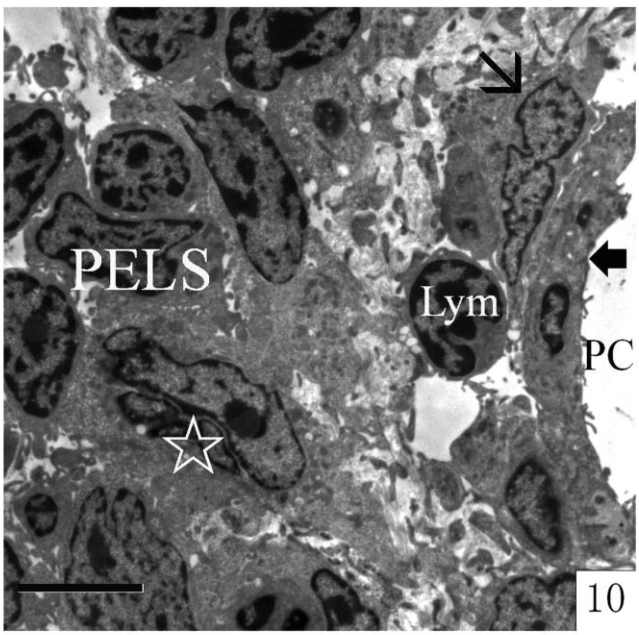
One Part of the BSB was Located in the Ellipsoid of the Turtle Spleen

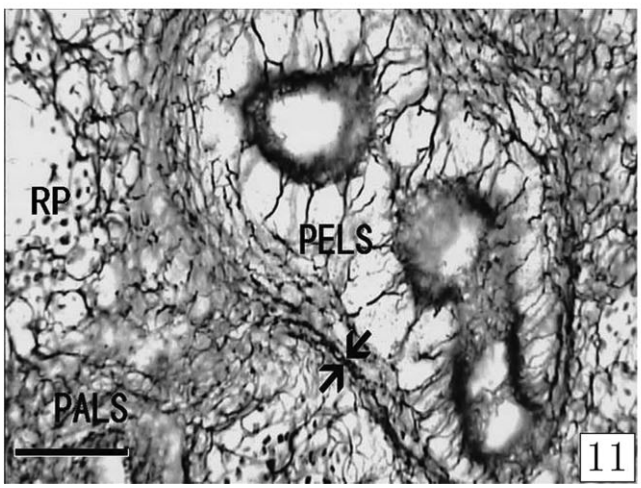
Our study reveals that the soft-shelled turtles lacked a marginal zone, separating the periarteriolar lymphatic sheath (PALS) from the red pulp. No branches of central artery (CA) crossed the PALS, which suggested that marginal sinus does not exist in the spleen of the turtle. The penicillar capillaries could freely open into the pulp cords from where the blood entered the sinuses through spaces between their endothelial cells. The penicilliform capillary was surrounded by the ellipsoid or Schweigger-Seidel sheath. In the chicken, the endothelial lining of this part of the penicilliform capillary contains small channels, formed between neighboring endothelial cells. These channels allow circulating substances to accumulate in the ellipsoid (Olah and Glick, 1982). Our findings indicated that lymphocytes were occasionally present between endothelial cells of penciliform capillary or in the ellipsoid as what Chen observed (Chen and Nie, 1995), which suggested that penicilliform capillary also had small channels in the *P. sinensis* spleen. Figure 23 indicated the general histology and microcirculation of

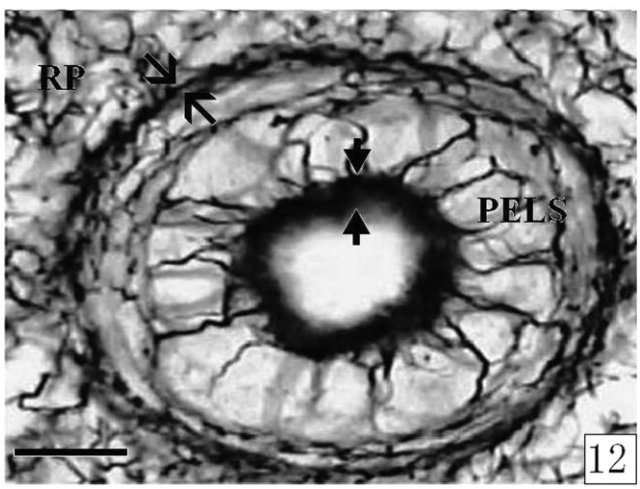




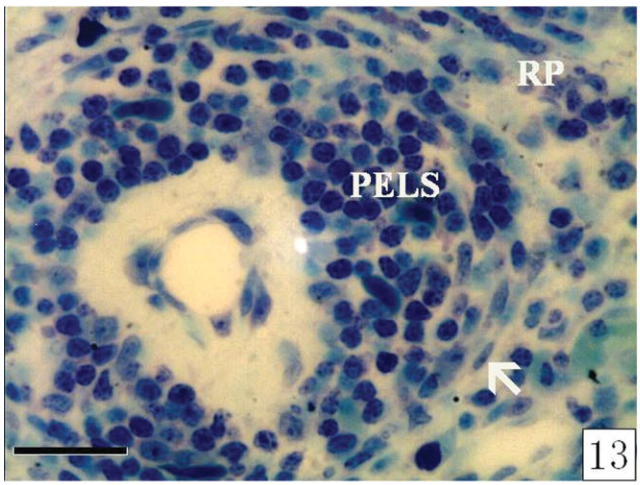


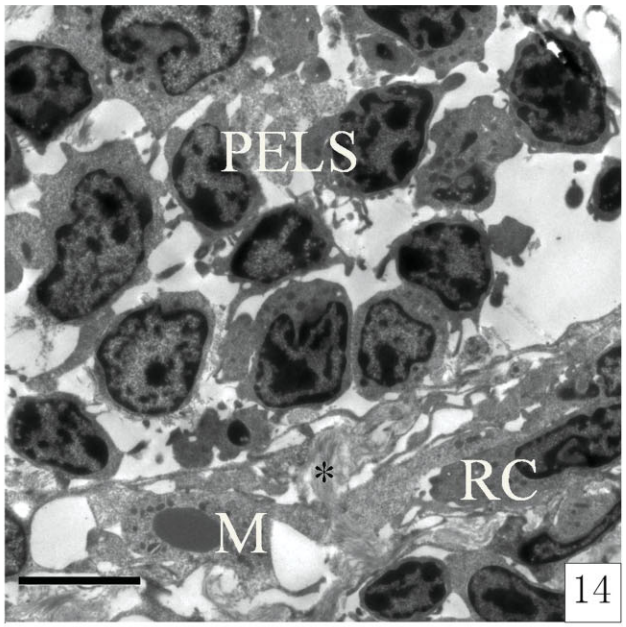


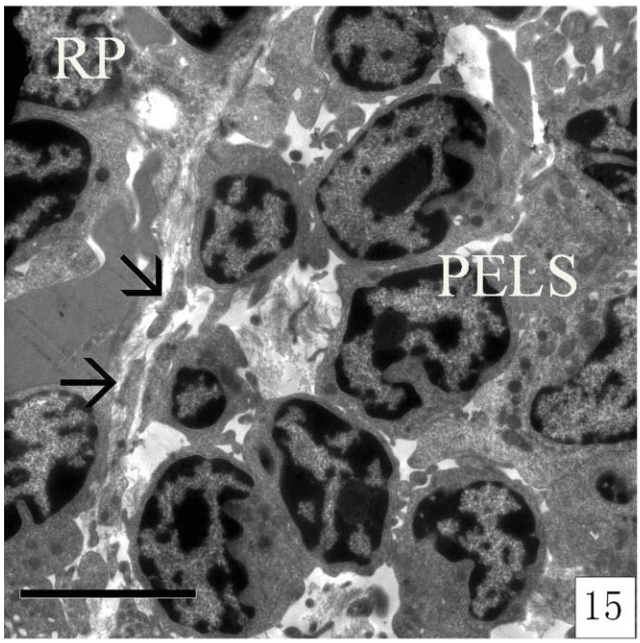


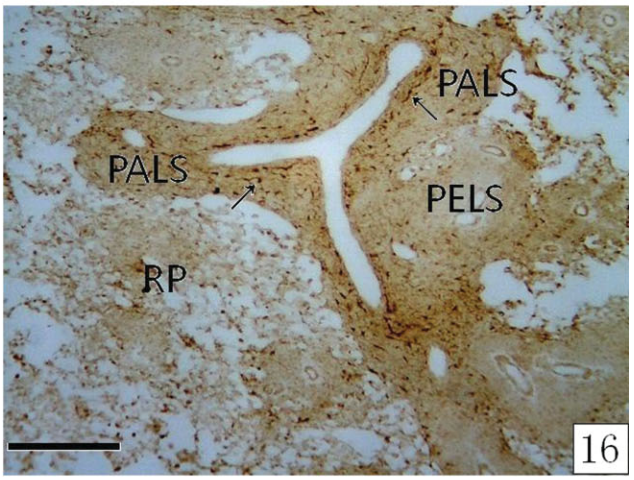


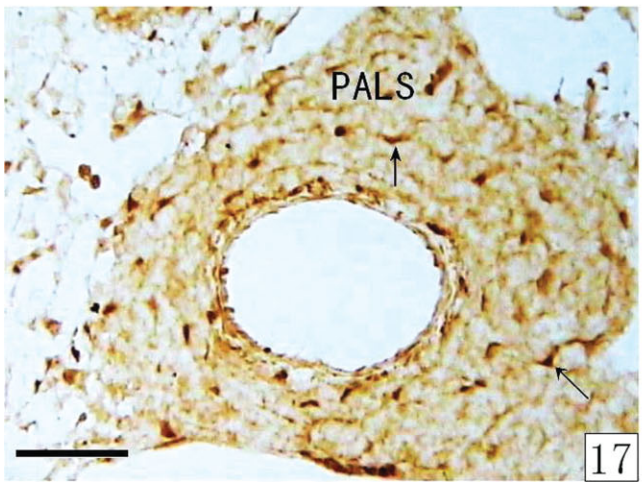
Figs. 7–12. Fig. 7. The penicilliform capillary (PC) is surrounded by a capillary sleeve embroidered by ANAE + EACs (➔), while supporting cells (➡) show a negative reaction. PALS, periarterial lymphatic sheath; PELS, periellipsoidal lymphatic sheath; CA, central artery. Alpha-naphthylatesterase cytochemistry. Bar = 25 μm. Fig. 8. Bodies of EACs (➔) in PELS, surrounding ellipsoids (E), have an affinity for toluidine blue. Supporting cells (➡) are located juxtapositioned to the penicilliform capillary (PC) and have less toluidine blue affinity than the EAC. PELS, periellipsoidal lymphatic sheath; RP, red pulp. Toluidine blue staining. Bar = 7 μm. Fig. 9. Electron micrograph shows one of the EACs. Its processes (☆) cross the ellipsoid and end on the supporting cells (⇒) that surround the penicilliform capillary (PC). Lymphocyte (Lym) and lots of reticular fibers (➔) appear in the ellipsoid vascular endothelial cell (➡). Bar = 5 μm. Fig. 10. Electron micrograph shows the supporting cells (➔) are located close to the outer sheath of penicilliform capillary (PC). Lymphocyte (Lym) is present in the ellipsoid. PELS, periellipsoidal lymphatic sheath; EAC (☆); vascular endothelial cell (➡). Bar = 5 μm. Fig. 11. Sliver impregnation reveals a dense reticular fiber network in the red pulp (RP) and periarterial lymphatic sheath (PALS). There is no clear border between RP and PALS. Conversely, there is a distinct border (➔) between RP and periellipsoidal lymphatic sheath (PELS). Bar = 100 μm. Fig. 12. Higher magnification showing the single, strongly stained reticular fibers with prominent ring structure in the outer periellipsoidal lymphatic sheath (PELS) (➔) and the wall of ellipsoid (➡). RP, red pulp. Silver impregnation. Bar = 25 μm.

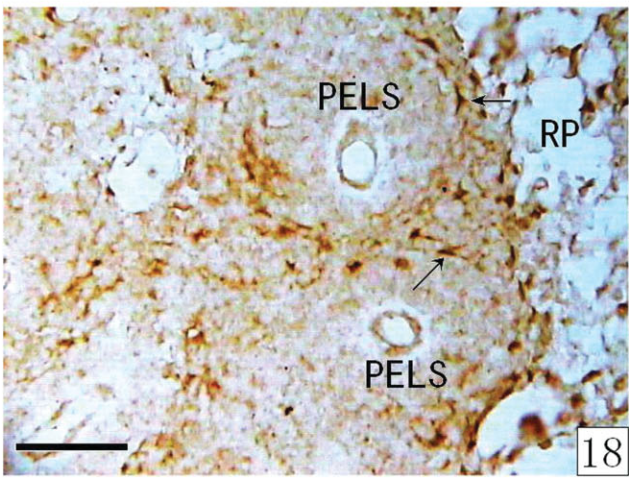




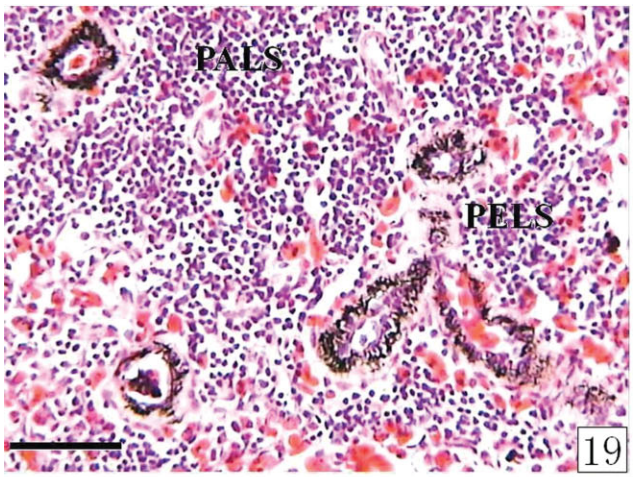


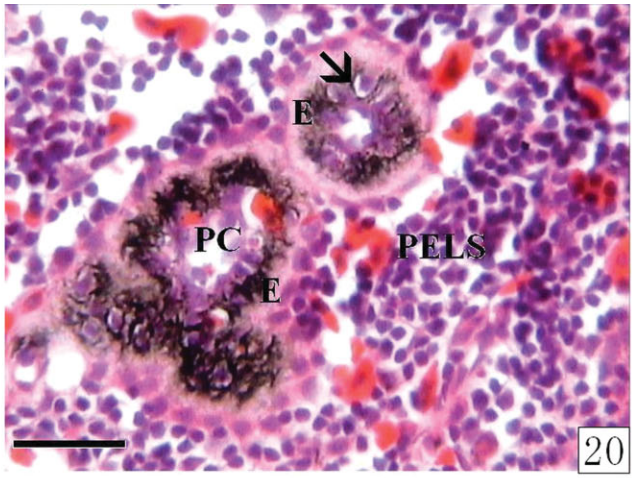


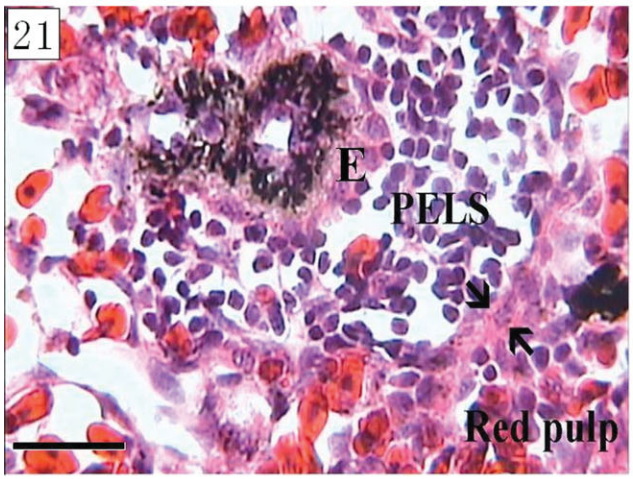


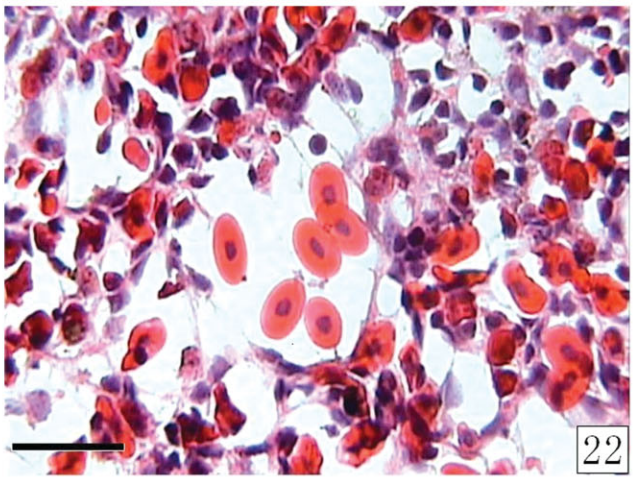


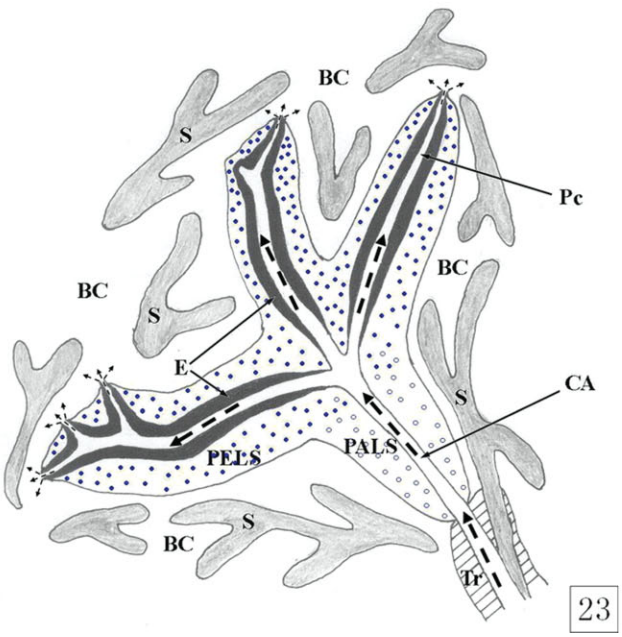
Figs. 13–18. Fig. 13. The single layered elongated reticular cells (➔) forming boundary between the periellipsoidal lymphatic sheath (PELS) and red pulp (RP). Toluidine blue staining. Bar = 25 μm. Fig. 14. Transmission electron micrograph showing the reticular cells (RC) and macrophages (M) localized around the periellipsoidal lymphatic sheath (PELS). Between these reticular cells and the cells of red pulp, there is a space filled with some reticular fibers (✱). Bar = 5 μm. Fig. 15. The red pulp (RP) is separated from periellipsoidal lymphatic sheath (PELS) by well defined processes (➔) of reticular cells. Bar = 5 μm. Fig. 16. Strongly S-100-positive dendritic cells (➞) are enormously distributed in the periarterial lymphatic sheath (PALS). RP, red pulp; PELS, periellipsoidal lymphatic sheath. Immunohistochemistry. Bar = 100 μm. Fig. 17. S-100 protein is expressed in both; the cytoplasm and nucleus of cells. These S-100^{+}cells (➞) are located in the periarterial lymphatic sheath (PALS). Immunohistochemistry. Bar = 50 μm. Fig. 18. Variable appearance of S-100^{+} cells (➞) found around the periellipsoidal lymphatic sheath (PELS). RP, red pulp. Immunohistochemistry. Bar = 50 μm.

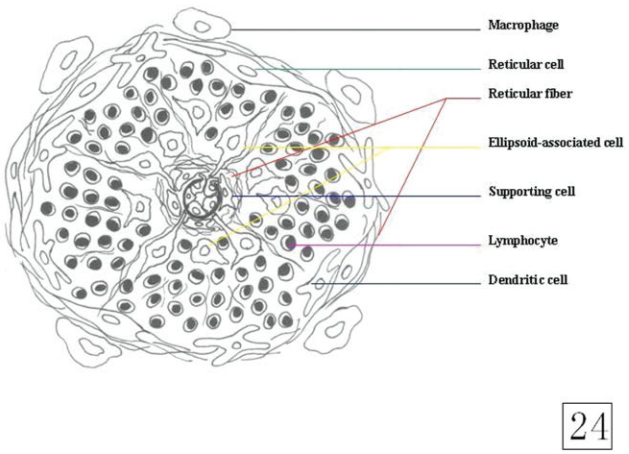












Figs. 19–24. Fig. 19. Light micrograph shows the distribution of carbon. PALS, periarterial lymphatic sheath; PELS, periellipsoidal lymphatic sheath. H–E staining. Bar = 50 μm. Fig. 20. The section shows a web-like distribution of carbon (➔) in the ellipsoid (E) after 40 min of injection. PELS, periellipsoidal lymphatic sheath; PC, pencilliform capillary. H–E staining. Bar = 25 μm. Fig. 21. Carbon particles are mainly found in the ellipsoid (E), and few at the boundary (➔) between periellipsoidal lymphatic sheath (PELS) and red pulp (Red Pulp). H–E staining. Bar = 25 μm. Fig. 22. There are fewer carbon particles in the red pulp. H–E staining. Bar = 25 μm. Fig. 23. Diagrammatic representation of the spleen. PALS, periarterial lymphoid sheath; PELS, periellipsoidal lymphoid sheath; CA, central artery; Pc, penicillar capillary; BC, Billroth pulp cord; Tr, trabecule; S, sinus. Dash line indicates direction of blood circulation. Fig. 24. Diagrammatic representation of the BSB.

the spleen in the soft-shelled turtle. In rodents, antigens and recirculating lymphocytes entering the spleen via blood vessels are first retained in the marginal zone because of the presence of a marginal sinus, which allows extravasation of blood borne antigens and recirculating cells (Humphrey, 1981; Humphrey and Grennan, 1981; Kraal et al., 1989). As the presence of the carbon particles on the ellipsoid after Indian ink injection, it was clear that ellipsoid first received antigens in the spleen of the soft-shelled turtle.

The ellipsoid itself is embedded in the PELS. In mammals, Blue and Weiss (1981a,b) using a transmission electron microscope, characterized the sheath as a laminated structure consisting of supporting cells and macrophages. In avian species, their results were confirmed, but the arrangement of the two cell types is different and the processes of phagocytic cells appear to be inserted into the sheath, which in turn is formed by supporting cells. These special phagocytic cells are called EACs which are only found in the sheath of spleen (Oláh and Glick, 1982). In the present study, ellipsoid of the soft-shelled turtle was morphologically similar with that of avian, formed by supporting cells and EACs. Between these supporting cells, there were lots of reticular fibers. At the surface of ellipsoid were EACs. It is now clear that the ellipsoid or Schweigger-Seidel sheath is a filtering apparatus (Blue and Weiss, 1981a, 1981b), as originally proposed by Schweigger-Seidel (Schweigger-Seidel, 1863) and confirmed by light-microscopic, electron-microscopic (Oláh et al., 1975; Oláh and Glick, 1982), and functional studies with various extracellular tracers (Oláh et al., 1985). Our results showed that carbon particles of Indian ink, 40 min after injection, entered the ellipsoid through the penicillar capillaries (perhaps through the stomata or fenestrations on the endothelium), but the supporting cells, EAC and extracellular matrix (such as reticular fibers) of the ellipsoid prevented them from spreading freely into the PELS, serving as a biological and mechanical filtering apparatus. Therefore, the ellipsoid was the first barrier for the immunological defense against 50–100 nm particulate antigens in the blood in the spleen of the soft-shelled turtle.

The Second Part of the BSB Stood in Outer Layers of the PELS

During evolution, the morphology of the spleen has been changed considerably and there is an increasing compartmentalization of the white pulp from the lower to the higher vertebrates (Secombes and Manning, 1980; Imre and Bruce, 1982; Kroese and Van Rooijen, 1982; Hao and Nie, 1999; Cheng et al., 2003). In reptilian, however, the organization of the splenic white pulp seems to be different due to the different orders or families. In squamate reptiles and in the tuatara, the white pulp of the spleen is only located around a central arteriole (Murata, 1959; Marchalonis et al., 1969; Wetherall and Turner, 1972; Kanakambika and Muthukkaruppan, 1973; Hussein et al., 1978a,b, 1979a; El Ridi et al., 1981). A centriole arteriole is, however, not found in the snake *Elaphe quadrivirgata* (Murata, 1959). Ellipsoids seem to be absent in the spleen of lizards and snakes (Tischendorf, 1969). Sheathed capillaries are, however, found in the spleen of the lizard *Uromastyx aegyptia* (Hussein et al., 1978b), and these are not encircled by lymphocytes as in the chelonian. More developed white pulp including PELS and PALS occurs in the red-eared slider, *Chrysemys scripta elegans* (Kroese and Van Rooijen, 1982). Our finding indicated that the white pulp of the spleen of the soft-shelled turtle, was composed of two distinct lymphoid compartments, the periarteriolar lymphocyte sheath (PALS) and periellipsoidal lymphocyte sheath (PELS). Follicles and germinal centers were not found.

Two types of dendritic cells occur in the periarteriolar lymphocyte sheath (PALS) and lymph nodes of mammals or in the germinal centres of the chicken spleen: interdigital (IDC) and follicular dendritic cells (FDC), respectively (Igyártó et al., 2007). Dendritic cells represent a heterogeneous cell population, which has an essential role in the initiation and regulation of immune responses (Nagy et al., 2004). Using a polyclonal antibody against whole bovine S-100 protein, it was shown that S-100 protein-containing cells were observed in the locations which have been reported to contain avian dendritic cells such as the medulla of the bursal follicles, and the germinal centers and T-dependent areas in the spleen, and indicate that S-100 protein can be considered as a cell marker for the identification of the chicken dendritic cell (Gallego et al., 1992). Although trapping of immune complexes is restricted to non-lymphoid cells of dendritic character, situated only in one of the white pulp compartments: the periellipsoidal lymphocyte sheath in the turtle Chrysemys scripta elegans spleen (Kroese and Van Rooijen, 1983), our results of immunohistochemistry displayed that S-100 protein positive dendritic cells were found in the outer layers of the PELS and all over the PALS by using rabbit anti-S-100 protein.

Possibly, there were two reasons. One was that antigen not entering the ellipsoids was carried into the red pulp where it could be filtered by the BSB of the *P. sinensi*, which led the dendritic cells in the PALS to have no chance to contact with antigen. Two was that dendritic cells in the PALS were unable to bind antigens. Therefore, antigen-trapping cells were only found in the outer layers of the PELS.

Our result revealed that, except for S-100 protein positive dendritic cells, macrophages, reticular fibers, and cells were also found in the outer layers of the PELS. Few tracers or antigens escaped capture of ellipsoid and enter PELS were filtered again by PELS outer (including dendritic cells, reticular fibers and cells, macrophages). Thus, no carbon particles of 50–100 nm were found in the red pulp.

Differences of the BSB Between the Soft-Shelled Turtle and the Rodent

In rodent spleen, BSB develops in the marginal zone and it is composed of macrophages, plenic sinus endothelial cells and their basement membrance, the reticular tissue and fibers (Weiss et al., 1986; Zhu et al., 2005). On the basis of our studies, it is clear that BSB indeed exist in the soft-turtle, *P. sinensi*, and it was composed of two parts. One was ellipsoid; the other was outer compartments of PELS (including dendritic cells, reticular fibers and cells, macrophages). Figure 24 indicated the architecture of BSB in the turtle spleen. It revealed that

the situation, composition, and structural detail of the BSB in the soft-shelled turtle spleen are different from that of the rodent one.

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