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SPLEEN HISTOSTRUCTURE, FUNCTION AND FORMATION

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Abstract: The spleen is the largest secondary immune organ in the body and is responsible for initiating immune reactions to blood-borne antigens and for filtering the blood of foreign material and old or damaged red blood cells. These functions are carried out by the 2 main compartments of the spleen, the white pulp (including the marginal zone) and the red pulp, which are vastly different in their architecture, vascular organization, and cellular composition. The morphology of these compartments is described and, to a lesser extent, their functions are discussed. The variation between species and effects of aging and genetics on splenic morphology are also discussed.

Key words: spleen , function, morpholpogy, vascular organization, cellular composition.

Introduction

The spleen is a dark red to blue-black organ located in the left cranial abdomen. It is adjacent to the greater curvature of the stomach and within the omentum. It is an elongated organ, roughly triangular in cross section. The gross appearance and size of the spleen are variable, depending on the species and the degree of distension; nonetheless, spleen weights can be important in its evaluation. The ratio of splenic weight to body weight remains fairly constant regardless of age and, in rats, is typically around 0.2% (Losco, 1992).

The functions of the spleen are centered on the systemic circulation. As such, it lacks afferent lymphatic vessels. It is comprised of 2 functionally and morphologically distinct compartments, the red pulp and the white pulp (Figures 1 and 2). The red pulp is a blood filter that removes foreign material and damaged and effete erythrocytes. It is also a storage site for iron, erythrocytes, and platelets. In rodents, it is a site of hematopoiesis, particularly in fetal and neonatal animals. The spleen is also the largest secondary lymphoid organ containing about one-fourth of the body's lymphocytes and initiates immune responses to blood-borne antigens (Kuper et al., 2002; Nolte et al., 2002; Balogh et al., 2004). This function is charged to the white pulp which surrounds the central arterioles. The white pulp is composed of three sub-compartments: the periarteriolar lymphoid sheath (PALS), the follicles, and the marginal zone.

The spleen is surrounded by a capsule composed of dense fibrous tissue, elastic fibers, and smooth muscle. The outermost layer of the splenic capsule is composed of mesothelial cells, which may not be evident on histologic section. Irregularly spaced trabeculae of smooth muscle and fibroelastic tissue emanate from the capsule into the splenic parenchyma (Figure 10). These

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trabeculae also contain blood and lymph vessels and nerves. The lymph vessels are efferent vessels through which lymphocytes migrate to the splenic lymph nodes.

Being a blood filter, it follows that the spleen is a highly vascular organ. Blood flow through the spleen is rather complex, but is an important and sometimes controversial concept. Blood enters the spleen at the hilus via the splenic artery. The splenic artery divides into trabecular arteries located within the trabeculae entering the splenic parenchyma. Small arterioles branch from the trabecular arteries and enter the red pulp where they become central arterioles which are surrounded by lymphoid tissue. Smaller arterioles branch from the central arterioles and feed the white pulp capillary beds (Satodate et al., 1986; Valli et al., 2002). Some of these terminate in the marginal sinus at the junction of the white pulp and the marginal zone, others terminate within the marginal zone, and a few extend beyond the white pulp to terminate in the red pulp (Dijkstra and Veerman, 1990; Schmidt et al., 1985a). Blood entering the marginal sinus and marginal zone percolates through the marginal zone in the direction of the red pulp. Once through the marginal zone, the blood either flows directly into adjacent venous sinuses whose open ends are continuous with the marginal zone, the so-called “fast pathway,” or enters the reticular meshwork of the red pulp, the “slow pathway” (Schmidt et al., 1993). As much as 90% of the total splenic blood flow travels through the adjacent venous sinuses, bypassing the reticular meshwork of the red pulp (Schmidt et al., 1993). As the central arterioles continue, the white pulp wanes and they become the penicillar arteries surrounded by red pulp. These give rise to the arterial capillaries, which terminate in the reticular meshwork of the red pulp in rodents (open circulation; Mebius and Kraal, 2005). Blood from the red pulp collects in the venous sinuses which enter the trabeculae and merge into the trabecular veins (Figure 9). The trabecular veins then converge at the hilus to form the splenic vein which drains into the hepatic portal system.

Red Pulp

The red pulp is composed of a three dimensional meshwork of splenic cords and venous sinuses. The splenic cords are composed of reticular fibers, reticular cells, and associated macrophages (Saito et al., 1988). The reticular cells are considered to be myofibroblasts and may play a role in splenic contraction (Saito et al., 1988). With electron microscopy, it is apparent that the reticular fibers are actually ensheathed by the reticular cells and their processes (Saito et al., 1988). The reticular fibers are composed of collagenous and elastic fibers, microfibrils, reticular cell basal laminae, and unmyelinated adrenergic nerve fibers (Saito et al., 1988). For more information on the ultrastructure of the red pulp, see Saito et al. (1988) or Schmidt et al. (1993). Within the spaces between the cords are blood cells, including erythrocytes, granulocytes, and circulating mononuclear cells. Also associated with the splenic cords, are lymphocytes and hematopoietic cells as well as plasma cells and plasmablasts that migrate from the follicles and the outer PALS

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after antigen specific differentiation (Matsuno et al., 1989; Mebius and Kraal, 2005). The red pulp macrophages are actively phagocytic and remove old and damaged erythrocytes and blood-borne particulate matter. Extra medullary hematopoiesis is common in rodent red pulp, especially in fetal and neonatal animals. Any combination of erythroid, myeloid, and megakaryocytic cells may be evident (Figure 10).

Venous sinuses can be found throughout the red pulp, including, as mentioned previously, directly adjacent to the marginal zone (Figure 8). They are lined by loose network of endothelial cells which sit on a basement membrane that is sandwiched between the endothelial cells and reticular fibers of the red pulp (Saito et al., 1988). The penicillar arteries and arteriolar capillaries are also located in the red pulp, though they are more difficult to identify light microscopically.

Various pigments may be present in the spleen. Hemosiderin deposits in the cytoplasm of macrophages in the red pulp, and sometimes in the white pulp as well, are a typical finding (Figure 9). In fact, iron pigments (i.e., hemosiderin and ferritin) are the most common pigments in the macrophages of the red pulp (Losco, 1992). Iron from the hemoglobin of phagocytized erythrocytes is converted to hemosiderin for storage in the spleen. According to historical control data from the National Toxicology Program (NTP), hemosiderin pigmentation is more prevalent in females than in males (Ward et al., 1999). Ceroid and lipofuscin derived from oxidation of lipids is also typically found in the spleen, though they are less abundant than hemosiderin (Ward et al., 1999). Melanocytes containing melanin may be present in the spleen, particularly in black mice, usually in the trabeculae or focally in the red pulp (Ward et al., 1999).

White Pulp

The white pulp is subdivided into the PALS, the follicles, and the marginal zone (Figures 3, 4, and 5). It is composed of lymphocytes, macrophages, dendritic cells, plasma cells, arterioles, and capillaries in a reticular framework similar to that found in the red pulp (Saito et al., 1988). As the central arterioles enter the red pulp, they are surrounded by the PALS which are composed of lymphocytes and concentric layers of reticular fibers and flattened reticular cells (Dijkstra and Veerman, 1990; Satodate et al., 1986). The PALS are divided into the inner PALS and the outer PALS (Matsuno et al., 1989; Nicander et al., 1993; Van Rees et al., 1996). The inner PALS, a T-cell dependent region, may stain slightly more intensely than the outer PALS due to its cellular composition of predominantly small lymphocytes (Dijkstra and Veerman, 1990; Matsuno et al., 1989). The difference, however, is not uniformly present and is generally very subtle and difficult to detect by light microscopy (Stefanski et al., 1990). The cells of the inner PALS are largely CD4+ T-cells, though smaller numbers of CD8+ T-cells may also be present, as well as interdigitating dendritic cells, and migrating B-cells (Van Rees et al., 1996). The outer PALS is populated by small and medium lymphocytes (both B- and T-cells), macrophages, and, upon

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antigenic stimulation, plasma cells (Matsuno et al., 1989; Van Rees et al., 1996). It is an important site of lymphocyte traffic where the formation of plasma cells occurs (Dijkstra and Veerman, 1990; Matsuno et al., 1989).

The follicles are continuous with the PALS and are typically found at bifurcation sites of the central arterioles (Ward et al., 1999). They are composed primarily of B-cells with fewer follicular dendritic cells and CD4+ T-cells but typically do not contain CD8+ T-cells (Van Rees et al., 1996). The follicles have larger lymphocytes at the follicular center which is surrounded by a mantle zone or corona composed of small to medium lymphocytes (Ward et al., 1999). Follicles may contain germinal centers, which form upon antigenic stimulation, that stain less intensely due to the presence of fewer cells and contain tingible body macrophages and apoptotic B-cells.

Marginal Zone

The marginal zone is a unique region of the spleen situated at the interface of the red pulp with the PALS and follicles (Figure 6). Considered by many to be a separate compartment rather than part of the white pulp, it is designed to screen the systemic circulation for antigens and pathogens and plays an important role in antigen processing (Kuper et al., 2002; Mebius and Kraal, 2005). A band of macrophages, the marginal zone metallophilic macrophages, and the marginal sinus (Dijkstra and Veerman, 1990; Mebius and Kraal, 2005; Satodate et al., 1986), separate the marginal zone from the PALS and follicles. The marginal zone metallophilic macrophages are a unique subset of macrophages at the inner margin of the marginal zone adjacent to the PALS and follicles (Dijkstra and Veerman, 1990; Matsuno et al., 1989; Mebius and Kraal, 2005). They can be visualized by silver staining and with the monoclonal antibody MOMA-1 (Mebius and Kraal, 2005). Adjacent and peripheral to the marginal zone metallophilic macrophages is the marginal sinus. It is continuous with vessels that feed the capillary beds of the PALS and follicles and is lined by MADCAM1+, sinus-lining endothelial cells (Mebius and Kraal, 2005). Peripheral to the marginal sinus, is the thick outer ring of the marginal zone, composed of reticular fibroblasts, marginal zone macrophages, dendritic cells, and medium sized marginal zone B-cells (Dijkstra and Veerman, 1990; Mebius and Kraal, 2005). The marginal zone blends into the red pulp. The marginal zone macrophages are another population of splenic macrophages that stain with the monoclonal antibody ERTR-9 (Van Rees et al., 1996). While all the potential functions of the marginal zone metallophilic macrophages are not known, the marginal zone macrophages are important in clearance of microorganisms and viruses. They express a number of pattern recognition receptors such as toll-like receptors (TLRs) and the macrophage receptor with collagenous structure (MARCO), which are important in the uptake of various bacteria (Mebius and Kraal, 2005). The marginal zone B-cells are a unique subset of noncirculating B-cells that

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have an IgM+/IgD− phenotype as opposed to follicular B-cells which are IgM+/IgD+ (Van Rees et al., 1996).

Factors Affecting Splenic Morphology

Species

There are a number of species differences in the gross and histologic appearance of the spleen. In dogs, for example, the spleen is somewhat dumbbell shaped, while in mice and rats, it's more uniform along the longitudinal axis. The spleen in dogs is able to expand to store large numbers of erythrocytes, but it is also capable of rapid contraction. Therefore, its gross appearance is quite variable, ranging from large and dark red to blue-black to smaller and lighter red. The capsule and trabeculae of dogs contains more smooth muscle than that of mice and rats, so the spleens of rodents do not contract as rapidly and tend to vary less in their gross appearance (Valli et al., 2002). The splenic artery also differs among species. In dogs, it branches into as many as 25 smaller branches prior to entry into the spleen (HoganEsch and Hahn, 2001), while in the rat, there are as many as eight branches (Satodate et al., 1986).

Vascular arrangements are perhaps the greatest source of species variation in splenic architecture. Species variation in the structure and morphology of the venous sinuses forms the basis for the classification of spleens into two groups, sinusal spleens and nonsinusal spleens (Schmidt et al., 1985a). Sinusal spleens are found in rats and dogs and nonsinusal spleens are found in mice (Schmidt et al., 1985a). The venous sinuses of sinusal spleens are larger, more abundant, make numerous anastamoses, and have a characteristic wall structure relative to the venous sinuses of nonsinusal spleens (for an in depth description of these differences, see Snook (1950) and for more detail on the wall structure of each vessel type, see Blue and Weiss (1981) (Schmidt et al., 1985a). The venous sinuses of nonsinusal spleens are so different, in fact, that some investigators use the term pulp venules rather than venus sinuses (Schmidt et al., 1985a). The larger venous sinuses of the rat spleen are far more conspicuous than those of the mouse spleen.

There are also species differences in the arterial vasculature. Schmidt et al. have reported that, in dogs, the arterial capillaries both terminate in the reticular meshwork (open circulation) and empty directly into the venous sinuses with no interruption of the endothelial lining (closed circulation) (Schmidt et al., 1982, 1983, 1993). In dogs, but not rats, the arterial capillaries are surrounded by dense, circumferential clusters of macrophages known as ellipsoids or periarterial macrophage sheaths (PAMS) (Blue and Weiss, 1981; Satodate et al., 1986). In dogs, there are very few capillaries within the PALS, as opposed to rats and mice where the PALS have abundant capillaries (Schmidt et al., 1983, 1985a, 1985b, 1993).

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Extra medullary hematopoiesis is more prevalent in spleens of mice than rats. In dogs, hematopoietic tissue is present in the spleen in pathologic conditions such as neoplasia and anemia, but may be present in the absence of underlying disease (HoganEsch and Hahn, 2001). When the hematopoietic tissue is predominantly myeloid, the term myeloid hyperplasia may be applied. The incidence of splenic myeloid hyperplasia in the absence of underlying disease was 4% in beagle dogs in one study (HoganEsch and Hahn, 2001).

Though there is a lot of individual variation, mice tend to have a greater proportion of white pulp than rats, but the follicles and marginal zone of mice are less distinct than those of rats (Figures 1, 2, and 7) (Ward et al., 1999). In rats, the marginal zone comprises up to 28% of the splenic volume and is the largest B-cell region in the spleen (Dijkstra and Veerman, 1990; Schmidt et al., 1993). Approximately one-third of the B-cells in the rat spleen have the marginal zone B-cell phenotype, whereas in the mouse, only 15% of the splenic B-cells have this phenotype (Van Rees et al., 1996). Though the region where the marginal sinus is located is more consistently discernible in rats, electron microscopic studies show that the marginal sinus is up to 6 times larger in mice (Schmidt et al., 1993).

Age

In the fetus, the spleen begins as a collection of primitive reticular cells in the dorsal mesogastrium. The first cells to appear are hematopoietic, which are evident by gestation day 17 in the rat (Losco, 1992). In the mouse, splenic tissue can first be identified, light microscopically, at gestation day 12.5 and the first hematopoietic cells can be seen at gestation day 15.5 (Seymour et al., 2006). In the dog, lymphocytes first appear in the spleen at gestation day 52, while the rodent spleen contains little or no white pulp at birth (HoganEsch and Hahn, 2001; Van Rees et al., 1996). The first lymphocytes to appear are T-cells that accumulate in the PALS regions (Losco, 1992; Van Rees et al., 1996). In rats, this begins by 2 days of age, by day 5, dendritic cell precursors appear, after which B-cell follicles begin to develop, and immunologic function begins at 14 days of age when cell to cell contact of antigen presenting dendritic cells becomes apparent (Losco, 1992). The spleen reaches peak development at puberty, in rats, followed by gradual involution (Losco, 1992). In dogs, the spleen increases in weight during the first 6 months of life (HoganEsch and Hahn, 2001).

Numerous references discuss the effects of aging on lymphocyte function and changes in the distribution of lymphocyte subsets. Lymphocyte numbers, however, may also decrease with age. One study showed a greater than 80% decrease in lymphocyte numbers in the white pulp of Fisher rats between 4 and 30 months of age (Cheung and Nadakavukaren, 1983). This change corresponded, light and electron microscopically, to a decrease in lymphocyte density in the white pulp (Cheung and Nadakavukaren, 1983). There was also an increase in the number of

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reticular cells and macrophages in the same regions (Cheung and Nadakavukaren, 1983). Some degree of white pulp atrophy is also a common aging change in Sprague–Dawley rats (Losco, 1992). The spleens of older dogs and rodents typically have fewer germinal centers (HoganEsch and Hahn, 2001; Losco, 1992).

Extra medullary hematopoiesis tends to be decreased in adult animals, but can increase in any animal when there is increased demand for these cells as in cases of anemia, inflammation, decreased production by the bone marrow, or in cases of neoplasia (Losco, 1992). The amount of hemosiderin present in the spleen tends to increase with age in both rodents and dogs (HoganEsch and Hahn, 2001; Losco, 1992; Van Rees et al., 1996) and, in mice, is more prevalent in females than males (Ward et al., 1999).

Genetics

Genetic mutations in rats and mice, either spontaneous or engineered, resulting in immunodeficiency markedly affect the morphology of the spleen. Among the immunodeficient strains, nude rats and SCID (severe combined immunodeficiency disease) mice are perhaps the best known and most commonly used in scientific studies. Nude rats are congenitally athymic and so are deficient in T-lymphocytes. The spleens of nude rats (and mice) are smaller than those of their wild-type counterparts. They have sparsely populated PALS regions and, since T-cell activity is required for the formation of germinal centers, lack secondary follicles (Figures 11 and 12) (Bell et al., 1987; Hanes, 2005). SCID mice are homozygous for the $Prkdc^{scid}$ mutation, a mutation in the gene encoding the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) (Perryman, 2004; Seymour et al., 2006). This results in a defect in V(D)J recombination of T-cell receptors and B-cell immunoglobulin receptors and a lack mature B- and T-cells (Perryman, 2004). The spleens of SCID mice are smaller than those of wild-type mice and all three regions of the white pulp contain few lymphocytes but do contain macrophages (Custer et al., 1985). The follicles are variable in size and contain occasional plasma cells, however, follicular dendritic cells are absent since B and T cells are required for the development of these cells (Custer et al., 1985; Seymour et al., 2006). The marginal zone is markedly decreased in size and is poorly demarcated from the PALS and follicles (Figure 13). In both the SCID and nude mutants, the reticular framework of the sparsely populated white pulp is intact .

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