The Haryana Veterinarian (June, 2025) **64(1),** 52-56 ISSN 0033-4359 (P) **Research Article**

HISTOCHEMICAL CHANGES OF SPLEEN IN PRE AND POSTNATAL AGE GROUPS OF SHEEP (*OVIS ARIES*)

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ABSTRACT

The present study was performed on spleen tissues from thirty-six animals, evenly divided between prenatal and postnatal stages, immediately post-slaughter and rinsed in normal saline. The study utilized cryosections to analyze various biochemical components, including glycogen, acid and neutral mucopolysaccharides, protein tyrosine, lipids, and enzymes like alkaline and acid phosphatase. In prenatal group, the superficial layer of the capsule showed a moderate Alcian blue reaction for acid mucopolysaccharides, while the deeper layers of the capsule and trabeculae exhibited moderate Periodic Acid Schiff's (PAS) reactions for neutral mucopolysaccharides. The central arteriole of the white pulp displayed a strong PAS reaction indicating the presence of glycogen. Notably, strong alkaline phosphatase activity was observed in the capsule and trabeculae, with no detectable activity in the splenic parenchyma. Conversely, strong reactions for acid phosphatase were noted in the capsule, trabeculae, red pulp, and marginal zone of the white pulp, but not in the germinal center. In postnatal group, extensive reactivity for tyrosine and glycogen was evident across the capsule, trabeculae, and associated arterial structures. Additionally, mild to strong reactions for acid and neutral mucopolysaccharides were noted. Similar to prenatal findings, strong alkaline phosphatase activity was present in the capsule and trabeculae, with consistent absence in the parenchyma and strong acid phosphatase activity in several regions except the germinal center. This study highlights significant biochemical variations across developmental stages, providing insights into the dynamic histochemical changes associated with spleen maturation and function.

Keywords: Histochemistry, Postnatal, Prenatal, Sheep, Spleen

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The spleen is the largest secondary lymphoid organ in the body, playing a pivotal role in the immune system by housing approximately 25% of the body's lymphocytes. It serves as a primary site for the proliferation and differentiation of immune cells and performs critical functions in blood filtration (Dharve *et al.*, 2020). As an essential component of immunological defence, the spleen is involved in several vital processes including blood purification, iron preservation, removal of encapsulated bacteria, destruction of senescent red blood cells, mediation of antigen-antibody interactions, protection against harmful cells and foreign substances and the production of opsonic antibodies (Bhagyalakshmi *et al.*, 2023).

The functional architecture of the spleen, particularly the interplay between its red and white pulp, is intricately linked to the arrangement of its blood vessels. This arrangement varies significantly across different animal species and can alter in response to immune challenges or disruptions in hematopoiesis (Hasan *et al.*, 2023). Understanding the structural and functional dynamics of the spleen is crucial, as these aspects are not only reflective of the genetic and environmental influences on an individual's development but also provide a foundation for clinical and surgical interventions (Alim *et al.*, 2012).

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Histochemical studies are particularly informative in revealing the distribution and activity of various enzymes within the spleen, some of which may be susceptible to degradation during conventional paraffin or plastic embedding processes. These studies have successfully demonstrated robust enzyme activities in cryosections, which preserve morphological details more effectively and allow for more accurate assessments of enzymatic patterns and cellular structures.

Given this context, the present study is dedicated to advancing our understanding of the spleen's development and functional attributes in sheep across prenatal and postnatal stages. By employing detailed histochemical analyses, this research aims to elucidate the complex biochemical and cellular landscapes that characterize the spleen during different phases of growth, thereby contributing to the broader field of immunological and physiological research. This represents a significant gap in our understanding of the organ structure and its function in this species.

MATERIALS AND METHODS

This research was conducted on thirty-six sheep, categorized into prenatal and postnatal groups, spanning from 0 to 150 days for prenatal individuals and from 1 month to over 2 years for postnatal individuals. The animals were

organized into six distinct groups, each comprising six sheep: Group-I (0 to 50 days), Group-II (51 to 100 days), Group-III (101 to 150 days), Group-IV (birth to 6 months, pre-pubertal), Group-V (6-8 months, pubertal) and Group-VI (2 years and above, adult).

The age of prenatal specimens was estimated using the crown-rump length (CRL) with the formula A = 2.1 (B+17), where 'A' represents the developmental age of the fetus in days, and 'B' is the CRL in centimeters (Noakes, 2009). For fetuses with a CRL up to 3 cm, standard values from Bryden *et al.* (1972) were utilized. The age of postnatal sheep was determined by analyzing the eruption pattern of their teeth according to Dyce *et al.* (1996). This methodological approach ensures precise age classification, facilitating a robust analysis of spleen development across various stages of sheep growth.

The spleens of these sheep were collected from slaughter houses in Andhra Pradesh and Tamil Nadu and were immediately preserved in thermoses filled with ice to maintain tissue integrity. These spleen samples, ranging from 1-2 mm in thickness, were sectioned at 10µ using a cryostat set to -20º C. The cryosections were subsequently processed using various histochemical techniques: Gomori's alkaline phosphatase cobalt method for alkaline phosphatase, Gomori's modified lead nitrate method for acid phosphatase (Carleton and Drury, 1980), and the Oil Red O method for lipids.

RESULTS AND DISCUSSION

Carbohydrate Distribution and Reactions in Prenatal Spleens

In prenatal age groups (group-II and group-III), histochemical analysis revealed a moderate Alcian blue reaction for acid mucopolysaccharides within the superficial layer of the spleen capsule when using combined Alcian blue–PAS staining (Fig. 1a). However, Yogesh (2007) noted a high to intense AB-PAS reaction in the outer fibrous layer of the splenic capsule and a moderate to high reaction in the red pulp during the prenatal period. Furthermore, the deep layer of the capsule and the trabeculae demonstrated a moderate PAS reaction for neutral mucopolysaccharides, indicating thepresenceofthese complex carbohydrates which are crucial for cellular functions such as adhesion and barrier formation (Supriya *et al.*, 2023). Notably, the central arteriole of the white pulp exhibited a strong PAS reaction for glycogen (Fig. 1b, Table 1), similar to observations in goat fetuses reported by both Yogesh (2007) and Gautam and Mishra (2015), suggesting a significant accumulation of energy reserves in these regions during prenatal development.

Protein Composition in Prenatal Spleens

A weak reaction for tyrosine was observed in the capsule, trabeculae, and parenchyma of the spleen (Fig. 1c). However, comprehensive literature review reveals that such protein components have not been widely reported in prenatal age groups (Table 1), indicating a possible underexplored area in fetal spleen biochemistry or differences in species-specific protein expression during development.

Lipid Content in Prenatal Spleens

Lipid deposits were notably absent in both the parenchyma and stroma across all prenatal age groups examined (Table 1). This lack of lipid detection aligns with the literature, where lipid components in prenatal spleens are rarely reported, suggesting minimal lipid storage or turnover during early spleen development.

Enzymatic Activity in Prenatal Spleens

Alkaline Phosphatase: Activity ranged from weak to moderate in the capsule, trabeculae, and white pulp (Fig. 1d). This contrasts with reports by Nishant (2017) and Geetha *et al.* (2016), who observed no alkaline phosphatase activity in the spleens of goat fetuses and small mammals respectively during prenatal stages. Alkaline phosphatase, known for its ability to hydrolyze phosphate esters in an alkaline medium (Dharve *et al.*, 2020), showed an increasing trend with advancing gestational age, indicating its role in maturation and possibly in mineralization processes.

Acid Phosphatase: The enzyme showed strong activity in the capsule, trabeculae, and white pulp, whereas the red pulp exhibited only a mild reaction (Fig. 2, Table 1). In stark contrast, Nishant (2017) found no acid phosphatase activity at any gestational age. This enzyme is thought to act as a chemotactic agent, attracting blood-born lymphocytes (Kapoor and Singh, 2016), and is frequently associated with monosaccharide transport in tissues (Kumar *et al.*, 2017). The observed enzyme activity might be intricately linked to the absence of an acid phosphate enzyme that hydrolyzes acid phosphatase esters, reflecting its specific biochemical roles in the fetal spleen environment.

Histochemistry

Carbohydrate Dynamics in Postnatal Spleens

In postnatal sheep, significant glycogen accumulation was observed in the capsule, trabeculae, trabecular arteries and central arterioles (Fig. 3a), as evidenced by strong histochemical reactions. This observation aligns with findings in other species such as guinea fowl and guinea pigs, where similar patterns were noted by Parmar *et al.* (2020) and Geetha and Vijayaraghavan (1999). Additionally, Venkatesan *et al.* (2004) identified PAS-positive activity across the capsule, macrophages, vascular structures, and the reticular network in the spleen of Japanese quail, underscoring the conserved nature of glycogen storage across diverse species. The capsule and trabeculae in sheep also exhibited mild to strong reactivity for acid and neutral

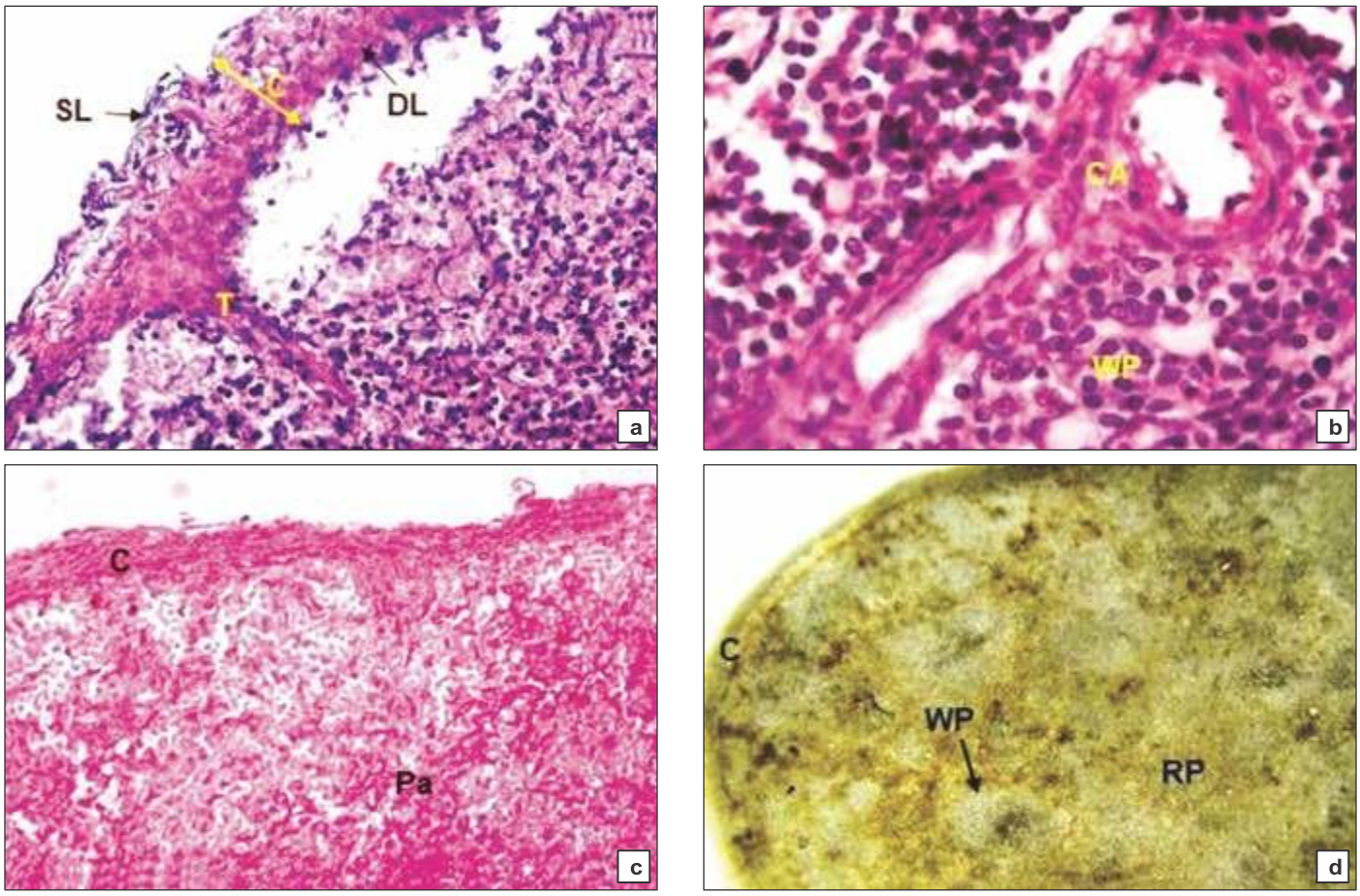

Fig. 1. **(a)** AB-PAS positive reaction at 103 days of gestation (Combined Alcian blue-PAS X400), **(b)** PAS positive reaction at 103 days of gestation (PAS X 400), **(c)** localization of tyrosine at 126 days of gestation (Millon's reaction X 400), **(d)** Alkaline phosphatase reaction at 84 days of gestation (Alkaline Phosphatase X 40). [C- Capsule; SL- Superficial Layer; DL- Deep Layer; T- Trabeculae; CA- Central Arteriole; WP- White Pulp; Pa- Parenchyma; RP- Red Pulp]

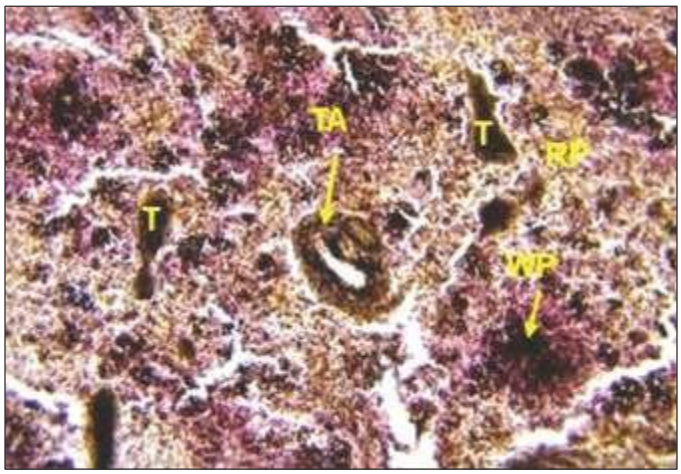

Fig. 2. Acid phosphatase reaction at 110 days of gestation (Acid Phosphatase X 100). [T- Trabeculae; RP- Red Pulp; WP- White Pulp; TA- Trabecular artery]

mucopolysaccharides, respectively, as shown with combined Alcian blue-PAS staining (Fig. 3b, Table 2). This is consistent with findings by Dahariya *et al.* (2020), who observed varying degrees of mucopolysaccharide activity in the splenic capsule of chickens. Contrasting these, Yogesh (2007) reported high to intense AB-PAS reactions across all postnatal age groups in goats, highlighting species -specific variations in mucopolysaccharide deposition.

Protein Composition in Postnatal Spleens

A robust reactivity for tyrosine was detected in the capsule, trabeculae, and parenchyma of postnatal sheep (Fig. 3c, Table 2), indicating active protein metabolism. This contrasts with prenatal observations where protein components were notably absent, suggesting developmental changes in protein expression and utilization post-birth.

Lipid Content in Postnatal Spleens

Moderate reactions for lipids were recorded in both the white and red pulp areas of postnatal spleens (Fig. 3d, Table 2), pointing to lipid involvement in postnatal metabolic processes. Notably, such lipid components were not detected in prenatal spleens, possibly reflecting an increased metabolic demand and immune activity following birth.

Enzymatic Activity in Postnatal Spleens

Alkaline Phosphatase: Strongactivityofalkaline phosphatase was observed predominantly in the capsule and trabeculae,

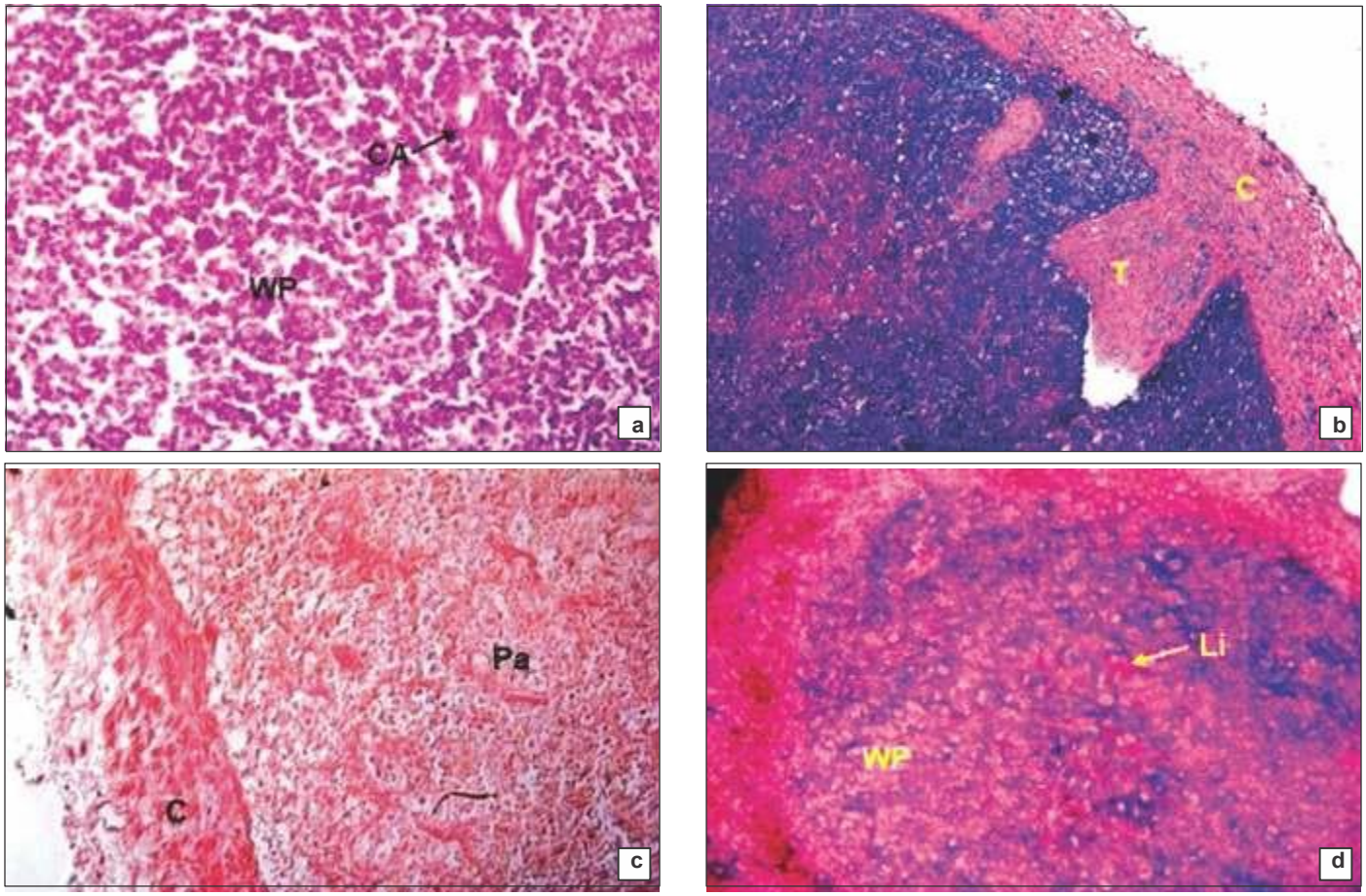

Fig. 3. **(a)** PAS positive reaction at 4 months of age (PAS X 400), **(b)** AB-PAS positive reaction at 4 months of age (AB-PAS X 100), **(c)** Localization of tyrosine in spleen of sheep at 2 years of age (Millon's reaction X 400), **(d)** Lipid deposits at 4 months of age (Oil Red O X 400). [CA- Central Arteriole; WP- White Pulp; C- Capsule; T- Trabeculae; Pa- Parenchyma; Li- Lipids; GC- Germinal Centre]

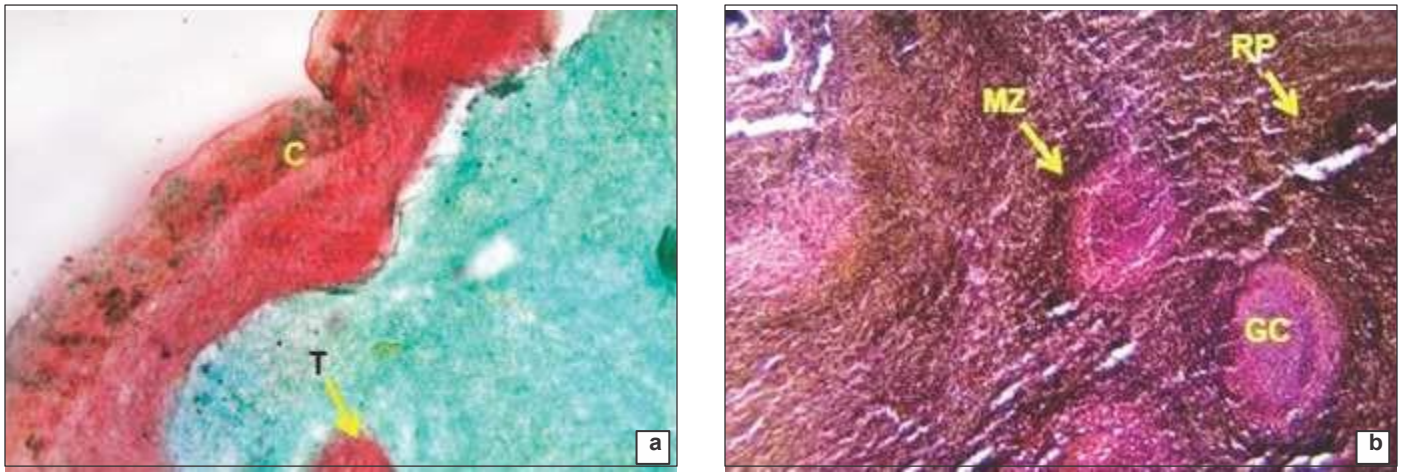

Fig. 4. **(a)** Alkaline phosphatase reaction at 2 years of age (Alkaline phosphatase X 400), **(b)** Acid phosphatase reaction at 7 months of age (Acid phosphatase X 100). [C- Capsule; T- Trabeculae; RP- Red Pulp; GC- Germinal Centre; MZ- Marginal Zone]

with no detectable activity in the splenic parenchyma (Fig. 4a). This enzyme profile differs from that observed in pigs by Kaur *et al.* (2019), where activity ranged from weak to strong across various spleen sections. Alkaline phosphatase has been linked to monosaccharide metabolism and nutrient transport, as discussed by Bachhawat *et al.* (1972) and Kumar *et al.* (2017), suggesting its integral role in cellular processes within the spleen.

Acid Phosphatase: The activity of acid phosphatase was strongly pronounced in the capsule, trabeculae, red pulp, and marginal zone of the white pulp, while areas around the arterioles showed no reaction (Fig. 4b, Table 2). This pattern is consistent with observations in other species, such as guinea pigs and chickens, reported by Geetha *et al.* (2016) . The presence of acid phosphatase is critical for degrading phosphoester bonds, which is vital for various

Table 1. Histochemical observations of spleen in prenatal age groups of sheep

SPLEEN	Prenatal age groups					
	PAS	Combined Alcian blue-PAS	Tyrosine	Lipid	Alkaline Phosphatase	Acid Phosphatase
Capsule	-	++	+	-	+ to ++	+++
Trabeculae	-	++	+	-	+ to ++	+++
Red pulp	-	-	+	-	-	+
White pulp	-	-	+	-	+ to ++	+++
Trabecular artery	-	-	+	-	-	-
Central arteriole	+++	-	+	-	-	-

+++ Strong; ++ Moderate; + Weak; - No reaction

Table 2. Histochemical observations of spleen in postnatal age groups of sheep

SPLEEN	Prenatal age groups					
	PAS	Combined Alcian blue-PAS	Tyrosine	Lipid	Alkaline Phosphatase	Acid Phosphatase
Capsule	+++	+ to +++	+++	-	+++	+++
Trabeculae	+++	+ to +++	+++	-	+++	+++
Red pulp	-	-	+++	++	-	+++
White pulp	-	-	+++	++	-	-
Trabecular artery	+++	-	+++	-	-	-
Central artery	+++	-	+++	-	-	-

+++ Strong; ++ Moderate; + Weak; - No reaction

cellular functions including the degradation of cellular debris and the immune response.

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