



Scanning Electron Microscopic Study of the White Pulp of Spleen in Adult Goats

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ABSTRACT

The white pulp of the spleen in adult goats was thoroughly screened under the scanning electron microscope (VEGA3 TESCAN). The study revealed the presence of lymphoid and non-lymphoid cells. The T lymphocytes and B lymphocytes could morphologically be differentiated under the magnification of 8000 to 20000 times by scanning electron microscopy. The changes in number of cells in relation to the age was also recorded. The peri arterial lymphatic sheath and its structural connections with reticular cells and other non-lymphoid cells were clearly demonstrated. The types of lymphoid cells and their arrangement around the central arteries upto the marginal zones were observed in detail. A conclusion about the type of circulation in the spleen of goats was arrived after three dimensional observation of the tissue under scanning electron microscope.

Key words: Goat, Scanning electron microscopy, Spleen, White pulp.

INTRODUCTION

Scanning electron microscopy (SEM) has proven useful for revealing cell relationship at ultrastructural microenvironment of lymphoid and non-lymphoid cells with connective tissue framework as three dimensional images of spleen in goats. Scanning electron microscopy of the lymphoid organs under study revealed the difference in the surface ultrastructure of B and T lymphocytes. In the spleen free cell surface of the medium sized lymphocytes showed irregular blunt protrusions. The lymphoid cells included T and B lymphocytes, lymphoblasts and plasma cells whereas non lymphoid cells included reticular cells, macrophages, Interdigitating cells (IDC) and Dendritic cells (DC). Three dimensional ultrastructural observations suggested the occurrence of both closed and open circulation in the spleen of goat.

MATERIALS AND METHODS

The spleen from 10 numbers of adult goats slaughtered in the abattoirs in Namakkal were collected. The experiment was conducted in the department of Veterinary Anatomy, Veterinary College and Research Institute, Namakkal during 2017. The tissue pieces of 3 mm cube was initially fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer at pH 7.4 for 2 days. Then it was washed well with 0.1M phosphate buffer at pH 7.4 followed by post fixation in 1% osmium tetroxide.

The specimens were dehydrated in a series of ascending concentrations of acetone. Then the specimens were dried to the critical point. The specimens dehydrated with alcohol and immersed in amyl acetate were put in liquid carbon dioxide in a high pressure chamber. Then the chamber was warmed to 40°C and the high pressure carbon dioxide was slowly let out. Thus critical point dried and freeze fractured specimen was fixed on a metal stub with conductive paste holding the fractured surface upward. In order to obtain

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sufficient electrical conductivity and yield of secondary electrons, the fractured surface of the specimen was doubly vacuum evaporation coated with carbon and ion sputtered gold. The specimens were rotated and tilted during the gold sputtering. All the specimens prepared for SEM were observed and recorded using VEGA3 TESCAN with an accelerating voltage of 3 kV.

RESULTS AND DISCUSSION

The present research revealed the presence of lymphoid and non-lymphoid cells in the white pulp of the spleen in goats. The lymphoid cells included T and B lymphocytes, lymphoblasts and plasma cells whereas non lymphoid cells included reticular cells, macrophages, Interdigitating cells (IDC) and Dendritic cells (DC). There was increase in number of lymphoid cells upto three years of post-natal age. Thereafter number of non-lymphoid cells increased with decrease in number of lymphoid cells with age. Cheung and Nadakavukaren (1983) opined that the decreased cellularity and increased structural disturbance might be significant in the age-related decline of spleen lymphocyte functions. The

B lymphocytes showed a complex surface architecture with several microvilli of varying length, covering almost the entire exposed surface, whereas the T lymphocytes presented a smooth surface with moderate number of surface digitations that did not cover the entire exposed surface.

The reticular cells formed the basic framework of the white pulp. Reticular cells were spindle shaped and had long, slender cytoplasmic extensions which were accompanied by reticulin fibres as noticed by Saito *et al.* (1988) in rats. In the peripheral PALS the reticular cells were arranged like cylindrical shells (Fig 1). Ohta *et al.* (1977) observed in pigs that the lymph vessels originated from the shells formed by the reticular cells around the smaller arterioles that branched from the central arteriole. This suggested that lymph vessels formed a recirculation pathway for lymphoid cells.

A special type of mononuclear phagocyte the Interdigitating cells (IDC) (Fig 2) were found only in the central PALS region. Small lymphocytes (T lymphocytes) protruded into the cytoplasm of the IDCs. Ewijk *et al.* (1974) in mice interpreted that this type of cell contacts induced blast transformation of the lymphoid cells which resulted in the formation of medium sized T-cells. The central area of PALS formed the thymus dependent area of the spleen, the peripheral area of PALS contained both T and B lymphocytes where they interacted. Dendritic cells (DC) were found in the light zone *i.e.*, germinal centre and also in the peripheral PALS where they were often associated with capillaries. B-cells were found between the ramifications of the DC (Fig 1) as noticed by Jose *et al.* (1990) in pigs.

The lymphocytes in the central PALS were closely packed. The lymphocytes in the peripheral PALS were

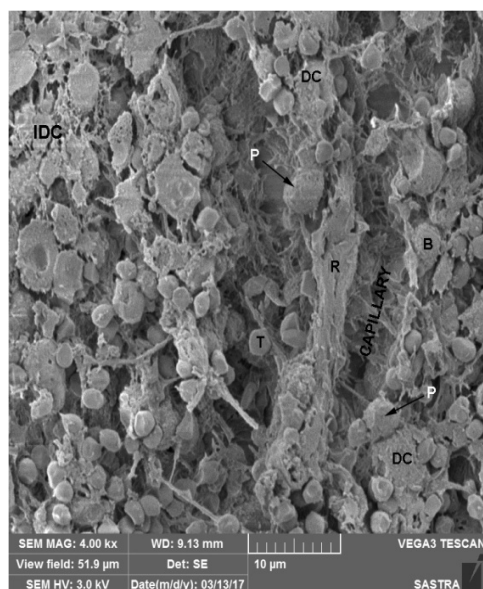


Fig 1: Scanning electron micrograph showing the white pulp of spleen in 1 to 2 years old goat at 4000 magnification. IDC- Interdigitating cell, DC- Dendritic cell, P- Plasma cell, T- T Lymphocyte, B- B Lymphocyte, R- Reticular cell.

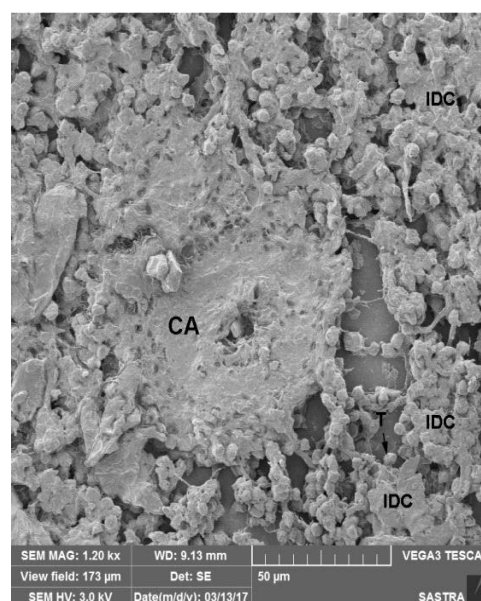


Fig 2: Scanning electron micrograph showing the central artery of spleen in 2 to 3 years old goat at 1200 magnification. IDC- Interdigitating cell, CA- Central artery, T- T Lymphocyte.

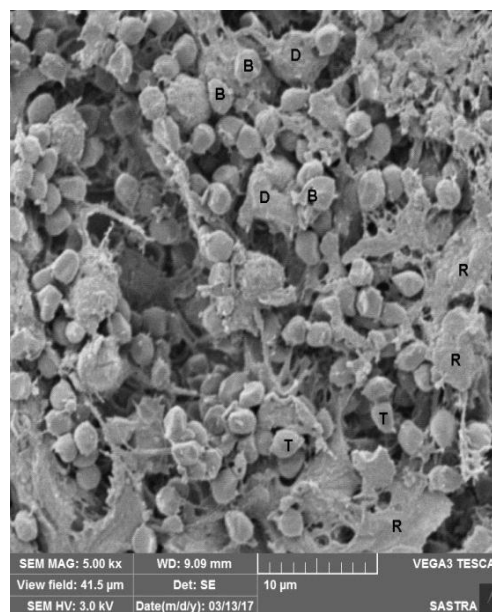


Fig 3: SEM showing the peripheral PALS of spleen in 3 to 4 years old goat at 5000 magnification. D- Dendritic cell, T- T Lymphocyte, B- B Lymphocyte, R- Reticular cell.

loosely packed (Fig 3). The number of small lymphocytes was found to decrease with increasing age. The marginal sinus surrounded the white pulp. Numerous macrophages occurred in the marginal zone. Veerman and Ewijk (1975) observed similar ultrastructure in the spleen of rat and mice and explained that lymphoblasts found in the peripheral PALS differentiated into plasma cells. The marginal zone contained predominantly medium-sized lymphocytes which were frequently found to be in contact with the reticular cells.

The free cell surface of the medium sized lymphocytes showed irregular blunt protrusions. This medium sized lymphocytes were found to cross the border between follicle and marginal zone.

Seki and Abe (1985) noted that the marginal sinus surrounded the follicles in rat, cat, dog, pig horse and cow. They also found the existence of a continuous pathway from the central arterioles via terminal arterioles to the red pulp sinuses and concluded that it was a closed circulation whereas the present ultrastructural study of spleen in goat revealed that the capillaries branching from the central arteriole traversed the peripheral PALS in an oblique fashion. In the follicles the capillaries form a demarcation between the follicle centre and the surrounding lymphocyte corona. They passed the corona and open freely in the marginal zone which suggested the occurrence of both closed and open circulation in the spleen of goat as observed by Schmidt *et al.* (1993) in mammals.

CONCLUSION

The white pulp of the spleen in goats is made of lymphoid and non-lymphoid cells. The lymphoid cells are T and B lymphocytes, lymphoblasts and plasma cells whereas non lymphoid cells included Reticular cells, macrophages, Interdigitating cells and Dendritic cells. The B lymphocytes have several microvilli on the outer surface. The T lymphocytes have smooth outer surface. A special type of mononuclear phagocyte the Interdigitating cells were found only in the central PALS region. In the peripheral PALS the reticular cells were arranged like cylindrical shells. Dendritic cells were found in the germinal centre. The circulation in goat spleen is both closed and open type.

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