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Supercritical drying and freeze cracking of lymph node of goats under scanning electron microscopy

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Abstract
Supercritical drying followed by freeze cracking of animal tissue rendered high quality cell surface for gold coating for precise observations under scanning electron microscope. The subpyloric lymph node of Jamieson and Dobson was subjected for this study. The lymph node contained reticular cells which were stellate plates oriented in a uniform direction. Large round macrophages were loosely fixed by the reticular cell processes. The parenchyma of the lymph node was made up of reticular cells, macrophages, lymphocytes, plasma cells and their intermediate types. Macrophages had few long tentacle-like projections that terminated in club shaped cytoplasmic processes. Plasma cells and lymphocytes also remained in the sinuses. The cortex of the node was built up by reticular cells, much smaller than those in the medulla and by densely packed round cells including a few macrophages. The trabeculae and the reticulum of the lymph node parenchyma formed a continuous structure. The continuity of sinus lining cells was confirmed in the present research.

Keywords: Supercritical drying, freeze cracking, lymph node, scanning electron microscope and goat

Introduction
The subpyloric lymph node of Jamieson and Dobson in goat was unpaired (single) which attracted the attention to explore it ultrastructurally. Supercritical drying followed by freeze cracking of animal tissue rendered the good quality cell surface for gold coating for precise observations under scanning electron microscope. Different climatic zones certainly have their influence in the immune status of animals to a variety of infections and infestation. This in turn decides the magnitude of normal physiological activity that any lymphatic organ should undergo. Therefore habitats of different geographical locations certainly show variation in ultrastructure of lymphoid organs during postnatal growth. Hence, the present study was designed to observe the scanning electron micrograph of the said lymph node in 2 year old goats. SEM revealed the surface anatomy, three dimensional morphology and the relations of the capsule, trabeculae, lymph nodules, reticular cells, macrophages, plasma cells, lymphocytes and other blood cells in the said lymph node. Present study would be of much use as normal reference in many clinical and pathological conditions.

Materials and methods
The lymph node under present study was collected from 6 numbers of 2 year old goats slaughtered in and around Thanjavur. The lymph nodes were washed in normal saline and a 5 millimeter long intact tissue piece was cut and fixed in 2.0% solution of glutaraldehyde, followed by post fixation in osmium tetroxide and was dehydrated in critical point dryer machine for 30 minutes. The specimen was first dehydrated in chilled acetone prior to supercritical drying using high pressure liquid carbon dioxide. The liquid carbon dioxide was then heated until its temperature goes beyond the critical point, at which time the pressure can be gradually released, allowing the acetone vapour to escape leaving a dried product [3]. In order to obtain good surface texture for SEM, freeze cracking of the critical point dried tissue was done by placing the tissue at -80 ^{o}C in a styrofoam container and gradually increasing the temperature by keeping the tissue over dry ice followed by application of gentle pressure [11]. The tissue surface to be observed was sputtered with gold-palladium by evaporation coating. The tissues were observed in a VEGE3 TESCAN in SASTRA University, Thanjavur with an accelerating voltage of 3 kV.

Results and Discussion

The lymph node tissue that was processed for electron microscopy when subjected to super critical drying and freeze cracking revealed capsule, trabeculea, lymph nodules, medullary cords, lymph sinuses and blood vessels with higher precision than the ordinary fracture line obtained with pressure.

Capsule and Trabeculae

The capsule revealed densely piled layers of collagen fibers. The inner surface of the capsule was attached to the spidery reticular cells by their feet traversing the marginal sinus. Trabeculea of variable thickness extended from the capsule into the sinuses. Cut end of each trabeculae resembled a stump of stiff wood under the SEM (Fig 1). Trabeculae had numerous branches. The trabecular ramification and the reticular cobweb of the sinus switched over to each other smoothly to form a continuous structure. The sinus lining sheet directly covered the trabeculae. Light microscope and TEM studies of lymph node sections have demonstrated that the inner surface of the capsule and the surfaces of the trabeculae were coated by a basement membrane like substance and a layer of flattened cells [10]. The reticular thread in the lymph node parenchyma were thus formed by a bundle of collagen fibrils with occassional elastic fibers covered by a sheath of reticular cell cytoplasm as also supported by some electron microscopists [1]. Based on light microscopic observations the trabeculae consisted of abundant collagen fibers and a few elastic fibers, covered by a basement membrane and flattened cells [4].

Lymphatic sinuses

The lymphatic sinuses formed tunnel like spaces running beneath the capsule of the node and was also surrounding the cortical nodules and medullary cords. The sinuses were lined by a thin cell sheet and contained a very loose network of spidery reticular cells (Fig 2). In some portions the sinus appeared like a hollow vessel because of the presence of few reticular cells in that portion.

Sinus Lining Cells

The lymph sinus was separated from the pulp by a sheet of flattened Sinusoidal endothelial cells called Sinus lining cells. The sinus lining cells were revealed in this study as flat type of reticular cells that retained their processes into the lumen of the sinus and to the reticular cells in the pulp [12]. The present study confirmed the continuity of the sinus lining cells [7]. Their cytoplasmic processes were connected to the processes of sinus reticular cells. The surface of the lining cells was equally rough as that of the reticular cells at higher magnification.

Pulp

The sponge like pulp tissue was comprised of lymph nodules and medullary cords. The framework of the pulp was formed by stellate reticular cells. The bodies of stellate reticular cells were smaller than those in the sinuses. The meshes were loosely filled with lymphocytes, plasma cells and macrophages. Secondary nodule was a dense, granular area that showed numerous, small round cells and appeared in the cortical portions of the pulp. Some smaller cells and large macrophages were found entangled in the meshes formed by the reticular cells.

Macrophage

The macrophages measured 10 -15 microns in diameter and appeared rough in surface as they were densely covered by clubbed cytoplasmic processes. Some of them had tentacle like projections with either clubbed or tapered endings. The macrophages were found to be tightly embraced by the reticular cell processes which appeared like broom stick ramifications. The macrophages in the lymph node, though they usually are round or ovoid, may extend pseudopodial processes during ameboid movement [8].

The medullary sinuses contain free macrophages [5]. The macrophages as seen under the SEM appear differently from the reticular cells in their shape and their relation to their neighbours (Fig 3) and there are no transitional forms between either. The rounded shape of the macrophages in our specimens may have made it easier to discriminate the two types of cells. Round macrophages appeared numerously and constantly in the sinus than in the pulp. The TEM study of the rat lymph node indicated that the sinus macrophages had irregular surface with many small finger-like micropseudopods [9]. This description was partly in accordance with our SEM observations.

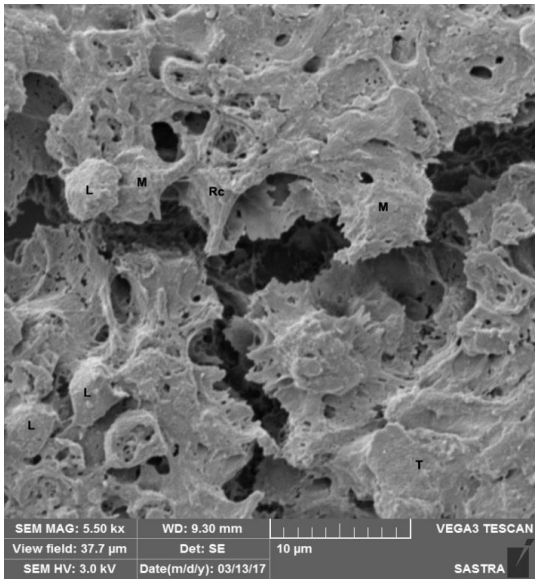

Fig 1: L – Lymphocyte, M – Macrophage, Rc – Reticular cell, T – Trabeculae

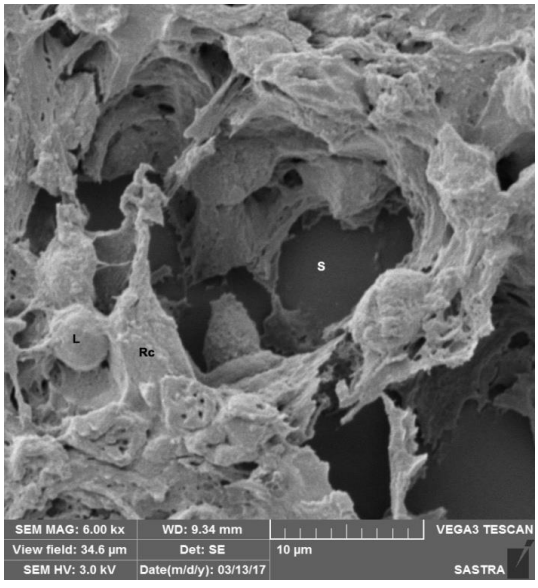

Fig 2: L – Lymphocyte, S – Sinusoid, Rc – Reticular cell

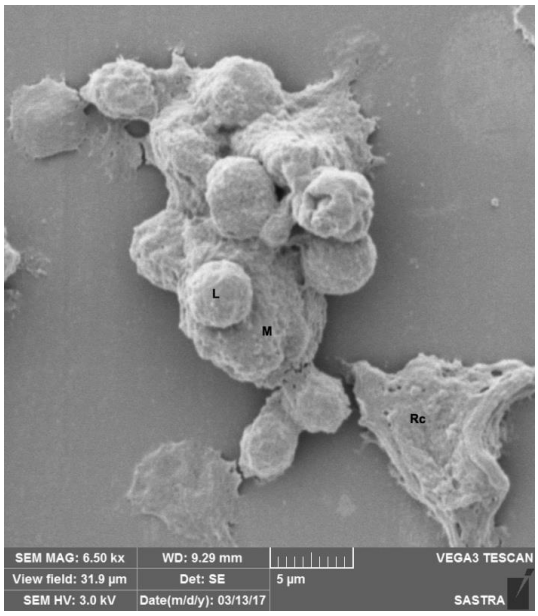

Fig 3: L – Lymphocyte, M – Macrophage, Rc – Reticular Cell

Reticular Cells

The reticular cells are polygonal or stellate in form with their thread-like processes. In most places the cell bodies were flat plates. The neighboring cell plates were arranged in uniform direction. The perikaryon of the reticular cell was thick. The cell surface appeared smooth when observed under lower magnification but was rough at higher magnification showing irregular elevations and tiny pits.

Present SEM observations indicated that the reticular cells, even though phagocytosed small particles such as the carbon particles of Indian ink [2] were specialized for the formation of the skeleton of the lymph node. The reticular cells of the lymph sinus were equivalent to the lymphatic valves [6].

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