Scanning Electron Microscopic Study of the Thymus in Non Descript Goats in Thanjavur

S. Sivagnanam, S. Muthukrishnan, S. Paramasivan, J. Selvaraj and B. Samuel Masilamoni Ronald

Veterinary College and Research Institute, Orathanadu - 614625, Tamilnadu, India

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Abstract

The scanning electron micrograph of the goat thymus was studied under VEGA3 TESCAN in SASTRA University, Thanjavur. The thymus showed epithelial reticular cells (ERCs), lymphocytes (Thymocytes), macrophages, Hassal's corpuscles, interdigitating or Dendritic cells (IDCs) and Thymic nursing cells (TNCs).

The epithelial cells form a meshwork in the thymus parenchyma. Cortical epithelial reticular cells were stellate in shape, while the medullary epithelial reticular cells were of two types, stellate and large vacuolated elements. A continuous single layer of epithelial cells separates the parenchyma from connective tissue formations of the capsule, septa and vessels. Surrounding the blood vessels, this epithelial sheath was continuous in the cortex, while it was partly interrupted in the medulla, suggesting that the blood-thymus barrier might function more completely in the cortex.

Cortical lymphocytes were round and vary in size, whereas medullary lymphocytes were mainly small, although they vary considerably in surface morphology. Two types of large wandering cells, macrophages and IDCs, could be distinguished. Perivascular channels were present around venules and some arterioles in the cortico-medullary region and in the medulla. A few lymphatic vessels were present in extended perivascular spaces.

Keywords: *Thymus - Scanning electron microscopy - Hassals corpuscles - Interdigitating cells - Thymocytes - Lymphocytes - Goat*

Introduction

The thymus represented the central organ of the immune system, constituting a microenvironment for T-lymphocyte differentiation. It was now accepted that, in the embryo, precursor T-cells migrate into the thymus from the yolk sac and liver, and postnatally, from the bone marrow. They become mature T-cells after passing through the cortex and medulla of the thymus (Owen and Ritter 1969).

Scanning electron microscopy (SEM) has proven useful for revealing cell relationships showing microenvironment of T-cell differentiation and to the fine structure of interdigitating cells.

Materials and Methods

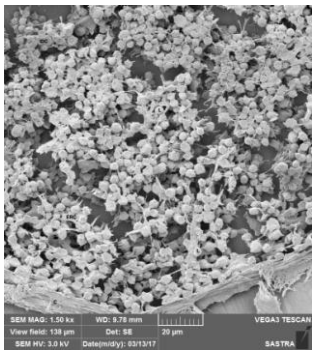
Thymus from 10 numbers of 2-3 year old goats slaughtered in and around thanjavur were collected. One millimeter cube tissue pieces were cut and fixed in neutral buffered formalin. The tissues were routinely processed and thick sections of 10 microns were made in Leica manual microtome. Obtained sections were

dewaxed and 3 millimeter square area of study was cut with glass cutter. Then sections were hydrated and treated with 2.0 % solution of glutaraldehyde. followed by post fixation in osmium tetroxide. and evaporation-coated with gold-palladium. The tissues were observed in a VEGE3 TESCAN in SASTRA University, Thanjavur with an accelerating voltage of 3 kV.

Result and Discussion

In low-magnification SEM views, the thymus was surrounded by a capsule (Fig 1) of connective tissue, which extended septa, dividing the thymic parenchyma into incomplete lobules (Wekerle *et al*., 1973). Each lobule consisted of the cortex and medulla.

Figure 1



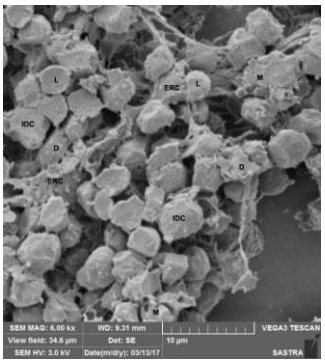
IDC – INTER DIGITATING CELL
L - LYMPHOCYTE
ERC – EPITHELIAL RETICULAR CELL
C – CAPSULE

Cells in the Cortex

In the cortex, epithelial cells, conventionally called "epithelial reticular cells", were stellate in shape (Fig 1) with a central thickening and several long processes extending in various directions (Ushiki *et al*., 1984). The cytoplasmic processes were string-like and connected with those of adjacent cells to form a reticulum. The coarse meshwork containing numerous spaces will hereafter be called the epithelial reticulum (Toro *et al* 1967). The epithelial cells were smooth in surface. Several vacuoles, 0.5-1.5 µm in diameter, were present in the cytoplasm. They usually contain fine-granular material (Tokunaga *et al* 1974). At the periphery of the cortex, the processes of the epithelial cells constitute a continuous single layer, which rests on a basal lamina and separates the thymic parenchyma from the connective tissue. The epithelial cells also anchor the capillaries (Schmitt *et al* 1980).

In the meshes of the epithelial reticulum, numerous free cells were found, most corresponding to thymic lymphocytes, which were round and vary in size from 4 to 8 µm in diameter (Raviola *et al* 1972). Small lymphocytes predominate, while large lymphocytes were sparsely scattered throughout the cortex (Fig 1), the latter being more numerous in the outer cortex (Owen *et al* 1983).

Figure 2



L - LYMPHOCYTE
M - MACROPHAGE
D – DENTRITIC CELL
ERC – EPITHELIAL RETICULAR CELL
IDC – INTER DIGITATING CELL

Both types of lymphocyte were smooth in surface; they possess a few villous microprocesses and occasionally exhibit knob-like cytoplasmic protrusions (Murakami *et al* 1974).

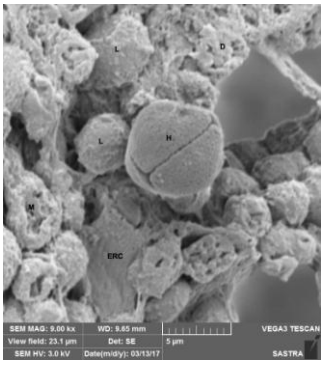
Another type of free cell was scattered in the cortex, especially around small vessels. These cells measure 10-15µm in diameter and correspond to cortical macrophages (Mosier *et al* 1972). They were covered by numerous villous or bubble-like microprojections and ruffles, which sometimes contact both epithelial cells and lymphocytes (Moore *et al* 1967). Sometimes, the macrophages enclose one or two lymphocytes with their pseudopods (Minoda *et al* 1983). Some fractured macrophages also reveal large vacuolar or cavernous inclusions, which probably correspond to phagosomes derived from the internalized lymphocytes (Kotani *et al* 1967).

Cells in the Medulla

Epithelial cells in the medulla, as in the cortex, form a reticulum, although the meshwork was more complicated than in the cortex. These cells present several morphologic forms, which can be classified into the following two main types. The first was stellate in shape, extending thin, thread-like processes which form the reticulum (Kostowiecki *et al* 1967). These cells display a smooth surface. The second type was a large cell usually possessing some vacuoles of varying size in the cytoplasm. These cells sometimes show a complicated profile of cellular interdigitation with the adjacent cells of the same type (Kessel *et al* 1979).

This cell type often accumulates in small groups. Transitions between the two types were also present. Hassall's corpuscles, which were formed by a concentric arrangement of epithelial cells (Fig 3), were also encountered in the present study.

Figure 3



L - LYMPHOCYTE
D – DENDRITIC CELL
H – HASSELS CORPUSCLE
M - MACROPHAGE
ERC – EPITHELIAL RETICULAR CELLS

The epithelial cells form a distinct sheath covering the perivascular channels surrounding vessels in the medulla and the cortico-medullary region (Kaiserling *et al* 1974). Probable lymphocytes were abundantly found within the reticulum formed by the stellate epithelial cells (Itoh *et al* 1981). These cells were primarily small type, about 5 µm in diameter, and vary in surface structure, some showing slight undulations while others project pseudopods, The lymphocytes were also covered with microvillus-like projections of varying numbers (Hoshino *et al* 1963).

Large wandering cells were found in this area and in the cortico-medullary region. They appear to be divided into two subtypes, although there were intermediate forms. One of these was densely covered

by spinous, filamentous microprojections (Hirokawa *et al* 1982).

The endothelial cells were elongated in shape, and the surface was provided with a few microvillous projections, especially at the cell margin. The arterioles were tightly surrounded by a single layer of slender cells, presumably smooth muscle cells, and these further surrounded by a single layer of epithelial cells. Occasionally, a distinct space, i.e., a perivascular space or channel, can be seen between the vessel and epithelial sheath. The perivascular space may be quite narrow or very spacious (Gaudecker *et al* 1980). The channel contains many round cells, mainly lymphocytes. The epithelial sheath was sometimes interrupted.

Capillaries, 3-5 µm in diameter, were present in the cortex and medulla. Some cortical capillaries directly drain into the capsular veins. The endothelial cells were spindle shaped and provided with marginal folds. These cells have no fenestrations. Immediately beneath the endothelium lies a continuous basal lamina, which was surrounded by a layer of epithelial cells and their processes.

Postcapillary venules, 10-50 µm in caliber, were located in the cortico-medullary region and in the medulla. Their endothelial cells were flattened, except for round swellings at sites of nuclei. The cells were polygonal in outline and the cell border was fringed with a marginal fold. The postcapillary venules were also surrounded by a perivascular channel bordered by a layer of epithelial cells. The epithelial sheath was often interrupted, and lymphocytes and IDCs were seen passing through these interruptions.

Cell types

The present study revealed the three dimensional ultra structure of the four main cell types and few other types of cells in the goat thymus. The main cell types were found to be epithelial cells, thymic lymphocytes (Thymocytes), macrophages, and interdigitating cells.

Based on their TEM studies, several investigators described cortical epithelial cells as stellate in shape with long processes (Duijvestijn and Hoefsmit 1981). Using SEM, Hwang *et al* . (1974) first demonstrated an epithelial meshwork in the rat thymus, although a precise observation was hardly possible because of the damage arising from the use of an ultrasonic cleanser to dislodge the lymphocytes.

The present study revealed that the cortical epithelial cells extend laminar or string-like processes in various directions to form a cytoreticulum. Wekerle *et al*. (1980) isolated thymic nurse cells (TNCs) by fractionated trypsin dissociation of the mouse thymus. In their TEM observations of TNCs, they reported that these elements resembled cortical epithelial cells.

Epithelial cells also form a reticulum in the medulla. With the TEM, several investigators have distinguished a reticular and hypertrophic type of epithelial cell (Duijvestijn and Hoefsmit 1981). The present SEM study confirmed the two main types of epithelial cells described by previous authors. One of these was the stellate cell. The latter form

a fine reticulum in the medulla, although Duijvestijn and Hoefsmit (1981) stated that, in TEM, the epithelial reticulum in the medulla was widely meshed. The other type demonstrated by SEM was the large cell, corresponding to what has previously been identified as a hypertrophic or vacuolated epithelial cell.

In their SEM study, Bhalla and Karnovsky (1978) delineated cortical lymphocytes as polyhedral cells, apparently due to pressure from adjacent cells. In the present study, the cortical lymphocytes retained their round shape.

Large lymphocytes seen in the outer cortex were believed to be lymphoblasts. Van Haelst (1967) reported in his TEM study that some lymphocytes of the thymus form blebs, which may correspond to the knob-like protrusions of the cortical lymphocytes in this study. Newell *et al*. (1978) demonstrated such blebs on the surface of B-lymphoblasts in vitro. We assume that the blebs on the surface may reflect certain stages in differentiation.

Medullary lymphocytes were mainly of a small type, varying considerably in surface morphology, as described in the SEM study by Bhalla and Karnovsky (1978). The slight undulations, ruffles or pseudopods of the lymphocytes may possibly reflect the acquirement of motility for the cells.

Based on lightmicroscopic and TEM studies, several investigators have described different types of mesenchymal cells. Important among these classifications were the two types proposed by Duijvestijn and Hoefsmit (1981): macrophages and interdigitating cells (IDCs). The present study confirmed these two cell types, as well as the occurrence of intermediate forms.

Both macrophages (Fig 3) and IDCs (Fig 2) were not involved in the formation of a meshwork comparable to that of the thymic epithelial cells. Duijvestijn *et al* . (1983) suggested that IDCs might be involved in the last stage of lymphocyte maturation. Furthermore, Ewijk (1984) proposed that the distribution of IDCs in the medulla might correlate with the antigen-dependent differentiation of helper T-lymphocytes. The present study demonstrated that the IDCs sometimes embrace or contact lymphocytes, and it seems reasonable to presume that such SEM images might visualize the intercellular delivery of immunological information from the IDCs to lymphocytes.

From their experiment in vitro, Beller and Unanue (1977) suggested that the maturation of thymic lymphocytes might be induced by macrophages.

Blood-thymus barrier

Clark (1963) and Weiss (1963) described a blood-thymus barrier as a continuous investment of epithelial cells around blood vessels. However, Ito and Hoshino (1966) demonstrated that the epithelial cells were not completely continuous around the blood vessels in the mouse. The present study confirmed the occurrence of a continuous single layer of epithelial cells, which separates the thymic parenchyma from the connective tissue territories of the capsule, septa and blood

vessels in the cortex. No interruptions of the epithelial cell layer could be seen in the cortex, whereas in the medulla and in the cortico-medullary region, the epithelial cell sheath was often interrupted. Therefore, we support the morphological existence of a blood-thymus barrier, particularly in the cortex.

Perivascular channel

Light-microscopic studies have demonstrated a perivascular space in silver-impregnated sections of the thymus (Sainte-Marie and Leblond 1964). In their TEM study of the human thymus, Bearman *et al* . (1975) visualized a perivascular space in the cortico-medullary region. The present study more clearly demonstrated these spaces, which were limited by a distinct lining of epithelial cells.

In their SEM observations of the rat thymus, Irino *et al* . (1981) reported that the perivascular spaces were present only around the postcapillary venules. In the present study, however, we ascertained the existence of these spaces around arterioles as well as venules in the cortico-medullary region and in the medulla. Furthermore, as demonstrated by Pereira and Clermont (1972) in their histochemical study of the epithelial cells, continuity between the perivascular spaces and extrathymic connective tissues was present, and lymphocytes were accumulated in tissue spaces. Therefore, we prefer to use the term "perivascular channels" for the perivascular spaces. In their light-microscopic studies of the guinea-pig and rat thymus, Kotani *et al* . (1966, 1967) demonstrated lymphatic vessels within or near accumulations of lymphocytes in the interlobular connective tissue, and suggested that the lymphatics might play an important role in the transport of lymphocytes into the general circulation. Our observations clearly show the existence of lymphatic vessels in the large perivascular space. Lymphatics, then, appear to be one of the pathways for thymic lymphocytes entering the general circulation. Furthermore, it was possible that the IDCs observed in the lymphatics also enter these vessels from the thymic parenchyma.

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