Scanning Electron Microscopic Observations of the Immunodefensive Systems with Special Reference to the Surface Morphology of the Non-Lymphoid Cells

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Summary. This paper reviews scanning electron microscopic observations of cellular elements forming various lymphoid organs.

The reticular cells in the secondary lymphoid organs are stellate, smooth-surfaced forms extending slender processes to comprise a three-dimensional network. The reticular fibers are usually covered by reticular cell processes, though they are naked in certain regions. Other types of reticular cells are observed in certain places: the “retothelial” type in the lymphatic sinus of the lymph nodes, and the “follicular dendritic” type in the germinal center of various lymphoid organs.

The thymic epithelial cells are divided into two main types: stellate cells which form a three-dimensional meshwork throughout the thymus parenchyma; and large vacuolated cells located in the medulla. A continuous single layer consisting of the processes of the stellate epithelial cells separates the parenchyma from the connective tissues of the capsule, septa and vessels.

The M cells in the epithelium of the gut-associated lymphoid tissues (GALTs) are cells with numerous irregular microprojections on the luminal surface. They often attach microorganisms to the luminal surface, reflecting their functions of antigen transport into the underlying lymphoid tissue. Lymphocytes of various shapes often cluster in the intercellular spaces under the M cells, a phenomenon believed to indicate direct stimulation of lymphocytes by certain transported substances.

Macrophages are amoeboid cells independent of and unable to transform into reticular and endothelial cells, at variance with prerequisites of the reticulo-endothelial system concept. Multiple features of macrophages probably reflect the presence of the subpopulations as well as the phases of their activity. The interdigitating cells (IDCs) are clearly distinguished by macrophages since they possess characteristic knob-like cytoplasmic processes gearing with each other. The IDCs often embrace lymphocytes, suggesting that the former cells may either nurse or deliver certain immunological information to the latter.

The endothelial cells in the postcapillary venules (high endothelial venules) in the secondary lymphoid organs (except the spleen) are dome-shaped and provide a special route for the circulating lymphocytes entering into the lymphatic parenchyma. The postcapillary venules in the thymus are composed of flattened epithelial cells and provide a specific site for lymphocytes “educated” in the thymus to pass into the general circulation.

The immunodefensive organs comprise various types of lymphoid and non-lymphoid cells, which are suitably arranged for immune response. Since these cells form complicated networks and labyrinths in the organs, it is often difficult to understand their precise shape and strategic arrangement through two-dimensional observations of thin sections by transmission electron microscopy (TEM). Scanning electron microscopy (SEM), when appropriately applied, affords a visualization of the three-dimensional fine structure of cells and tissues in such organs. Thus, this paper will review SEM studies by our research group on the thymus, tonsil, lymph node, Peyer's patch, appendix and spleen.

Special attention will be paid to the structure, function and nature of non-lymphoid cells as follows: various types of reticular cells; thymic epithelial cells; M cells in the gut-associated lymphoid tissue (GALT); macrophages; and interdigitating cells. Blood vessels and their endothelium are also demonstrated in relation to the migratory passage of lymphocytes.
MATERIALS AND METHODS

Of primary use were human and rat spleens, rat thymus and mesenteric lymph nodes, rabbit tonsils and appendix, and mouse Peyer's patches. The organs were arterially perfused with warmed Ringer solution or physiological saline and perfusion-fixed with 4% paraformaldehyde or 2% glutaraldehyde solutions (in 0.1 M phosphate or cacodylate buffer, pH 7.2-7.4). The fixed organs were cut into small blocks and kept in the same fixative for a few days. At this step, some of the specimens were sliced into sections (about 100-200 μm thick) using a microslicer. The tissue blocks and sections were then conductive-stained with the tannin osmium method by Murakami (1973), dehydrated with ethanol at increasing concentrations and transferred to isomyl acetate. The tissue blocks were freeze-cracked in absolute ethanol or isomyl acetate by the method described by Tokunaga et al. (1974). All tissues were critical point-dried using liquid CO₂. The dried tissues were then attached to aluminum stubs with adhesives, metal coated in an evaporator or ion coater, and examined in an SEM with an accelerating voltage of 10-15 kV.

RESULTS AND DISCUSSION

Reticular cells in the secondary lymphoid organs

The lymphoid tissues are supported by a network of stellate cells. These cells are usually called "reticular cells" simply because they form a reticulum. The reticular cells in the secondary lymphoid organs are mesenchymal, whereas those in the thymus are epithelial in origin. In this study, we will restrict the term "reticular cells" to the mesenchymal cells in the secondary lymphoid organs. The thymic epithelial cells will be dealt with separately in the following chapter.

The reticular cells are stellate with slender processes extending in various directions (Fig. 1). These cell processes are often associated with reticular fibers, or bundles of collagen fibrils; the latter fibers are usually embraced by the former processes and are hindered from direct contact with free cells. Utilizing the reticular fibers as a scaffolding, the reticular cells connect their cytoplasmic processes to each other to form a reticular framework in the tissues. These morphological features of the reticulum are commonly found in the splenic red pulp (Miyoshi and Fujita, 1971; Fujita, 1974), inner cortex of the lymph node, and apical dome of the appendix and Peyer's patch.

The reticular cells in the medullary cord of the lymph node are somewhat different from those described above. These cells are irregular or stellate in shape and share features with fibroblasts rather than with reticular cells. They extend thin, irregularly branched processes which are in contact with or twined into reticular fibers. Thus, numerous reticular fibrils without any cellular coverage extend randomly to form a complex network in this region. Since intermediate forms of these reticular cells are found in the cortico-medullary area, the structural difference probably correlates with the speed of the extravascular fluid movement and the "busyness" of cellular traffic in the tissue; in the region where cells and fluid move rapidly, fibrocytic cells may transform into reticular cells.

The reticular cells in the lymphatic sinus of the lymph node are similar to the ordinary reticular cells in their surface structure (Fujita et al., 1972; He, 1985), but the former can be distinguished from the latter. The means for this is that the sinus reticular cells are continuous to a sheet of sinus lining cells, but are separated from the ordinary reticular cells in the lymphatic cord (Fig. 2). This implies that the former two are derived from lymphatic endothelium but not from fibroblasts. Mori and Lennert (1969) pointed out in their TEM studies that the sinus reticular cells are identical with the sinus lining cells, but are different from the reticular cells of the lymphatic cord. They proposed that the cells should be termed the "retothelial cells" instead of "reticular cells".

The reticular cells in the lymphoid follicles are still more peculiar in structure and function. Because of the presence of dendritic cytoplasmic processes, they have been called the "dendritic reticular cells" (Nossal et al., 1968) or "follicular dendritic cells (FDCs)" (Tew et al., 1982). Under the TEM, the FDCs reveal cell processes whose membrane forms a labyrinth of invaginations and infoldings. These cells are known to retain antigens on their membrane invaginations for lengthy periods of months or even years (Mandel et al., 1980).

By SEM, the FDCs extend many complicated dendritic processes in various directions (Fig. 3). These dendritic processes have a somewhat granular surface and often further extend filamentous projections from their margin.

The origin of the FDCs has been discussed by various investigators. Many authors have stated their possible transformation from reticular cells (Kamperdijk et al., 1978; Tykociński et al., 1983; Yoshida and Takaya, 1989), while Tew et al. (1984) pointed out the possibility that the FDCs might be
derived from non-reticular cells such as monocytic antigen-trapping cells. Our SEM studies could not provide any information concerning this point, and the controversy remains.

**Thymic epithelial cells in the thymus**

As mentioned above, thymic reticular cells are not mesenchymal in origin, but are derived from the epithelial outgrowth of the third branchial pouch and cleft (CORDIER and HAUMONT, 1980). To avoid terminological confusion, we will refer to these cells as the "thymic epithelial cells". The thymic epithelial cells show regional differences in shape (USHIKI, 1986). The cortical epithelial cells are stellate and have several long processes extending in various directions (Fig. 4). These processes are sheet- or string-like and connected with those of adjacent cells to form a coarse meshwork. Since few reticular fibers are associated with the epithelial meshwork, it is often

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**Fig. 1.** SEM image of typical reticular cells forming a wide-meshed network. Most of the free cells filling the meshes have been removed mechanically but some remain. From a dome of the rabbit appendix. \( \times 3,400 \)

**Fig. 2.** Retothelial cells lining and spanning a lymph sinus. From the medulla of a rat mesenteric lymph node. \( M \) macrophages. \( \times 2,000 \)

**Fig. 3.** Follicular dendritic cells in the germinal center of a rabbit appendix. \( \times 4,600 \)
called the cytoreticulum in contrast with the fibrous reticulum occurring in the secondary lymphoid organs.

At the periphery of the cortex and around blood vessels, the epithelial cells extend attenuated processes forming a continuous limiting sheath, which separates the thymic parenchyma from the connective tissue territories of the capsule, septa and blood vessels (USHIKI, 1986).

Epithelial cells in the medulla can be classified into two main types: the stellate cells similar to the cortical epithelial cells, which extend thin thread-like processes to form the reticulum; and large cells showing complicated profiles of intercellular interdigitations. The latter type cells usually possess vacuoles of varying size in the cytoplasm. In their embryological studies of the mouse thymus, CORDIER and HAUMONT (1980) stated that the thymus has a mixed ecto-endodermal origin, the cortical cells being ectodermal, and the medullary cells endodermal in origin. The regional difference of the epithelial cell types may be related to such differing origins of the cells.

**M cells in the GALTs**

The lymphoid follicles in the gut-associated lymphoid tissues (GALTs) show a special close relation to the gut epithelium facing microbiological and immunological invasion from the gastrointestinal lumen. This follicle-associated epithelium is characterized by the presence of specialized cells which are believed to convey immunological information from the lumen to the underlying lymphoid tissue. They are usually referred to as “follicle-associated epithelial” (FAE) cells (BOCKMAN and COOPER, 1973) or “microfold” (M) cells (OWEN and JONES, 1974).

The M cells are varied in luminal surface structure, but most of the cells possess villous or lamellar microprojections of various sizes and populations on the luminal surface (Fig. 5). There are some cells extending tongue-like projections at the cell border, while others protrude smooth knob-like processes into the lumen. Globular or rod-like microorganisms are often attached to the surface of the M cells. It has been noted by various investigators that the M cells take up macromolecules and microorganisms from the intestinal lumen and transport them across their cytoplasm to the intercellular spaces (BOCKMAN and COOPER, 1973; SHAKHLAMOV et al., 1981; WOLF et al., 1981).

In fractured or cut surfaces of the epithelium, lymphoid cells are often found migrating in the epithelium (Fig. 6). These cells are basically spherical in shape and smooth in surface, but often equipped with some finger-like, or blebbed processes of their surface. These cells often cluster in intercellular spaces under the M cells. There are few macrophages in the intercellular spaces, though they are often found in the lymphoid tissue immediately beneath the epithelium.

In summary, the M cells apparently provide a specific route for antigen uptake through the epithelial barrier into the GALTs. Macrophages beneath the epithelium probably phagocytose and degrade complex microorganisms and particles to antigenic constituents. The functional significance of the M cell-associated lymphocytes remains unknown. The presence of the activated lymphocytes in this region suggests that they directly sample certain transported substances and are stimulated by them.

![Fig. 4. Thymic epithelial cells with typical, wing-like processes. Also mulberry-like lymphocytes are seen. From the deep cortex of a rat thymus. ×2,900](image-url)
Macrophages

Macrophages under the SEM are clearly distinguished from the reticular or epithelial cells, since the former cells show characteristic ameboid features and are densely covered by cytoplasmic microprojections. According to the "reticulo-endothelial system" (RES) concept originated by Aschoff (1924), reticular and endothelial cells in the spleen, lymphoid organs, and certain other tissues exert phagocytic and other protective activities against foreign body invasions, and the activated form of those cells represents the macrophages. However, a series of our SEM studies (MiYoshi and Fujita, 1971; Fujita, 1974, 1978; Fujita et al., 1982, 1985) has shown that the macrophages are independent of and untransformable to reticular and endothelial cells. These findings correspond well with those of Van Furth et al. (1972), who separated the macrophages from the reticular and endothelial cells by proposing a "mononuclear phagocytic system".

The surface structure of the macrophages, however, varies depending on where they are present. For example, the macrophages in the red pulp of human and rat spleens are usually rounded and covered by bubble-like, drumstic-shaped or spiny microprojections on their surfaces (Fig. 7). The macrophages in the marginal zone of the rat spleen are, on the other hand, rather flattened and extend a few large pseudopodia, forming a spindle or stellate cell shape (Fig. 8). They are covered by villous projections and ruffles and are often in close contact with several lymphocytes. Matsuno et al. (1986) demonstrated splenic macrophage heterogeneity in rats and mice by studying their phagocytic activities in response to carbon and/or neutral polysaccharide; in the rat, the red-pulp macrophages show a high phagocytic activity to carbon particles, while the marginal-zone macrophages take up neutral polysaccharide instead of carbon particles. Our SEM images probably reflect the presence of such macrophage subpopulations.

The association between a macrophage and lymphocytes, which apparently represents the process of antigen presentation, is predominantly observed in the splenic marginal zone and the lymphatic sinus of the lymph node, though the phenomenon is also

Fig. 5. M cells in the epithelium covering a dome of the rabbit appendix. The M cells here are characterized by long, irregular microvilli. ×3,500

Fig. 6. Fractured aspect of a superficial portion of a rabbit appendix dome. M cells (M) basally extend cytoplasmic processes to form arcades, which embrace many lymphocytes (L). Lymphocytes are packed also in the subepithelial space. ×1,600
present in other regions. It is noteworthy that the macrophage-associated lymphocytes often project numerous and long lamellipodia and filopodia, which probably reflect an activated state of the cells.

**Interdigitating cells**

The interdigitating cells (IDCs) were first reported by VELDMAN (1970) in the paracortical area of the rabbit lymph nodes. By TEM, these cells are characterized by a clear cytoplasm that extends between the lymphocytes and forms interdigitating processes with neighboring cells both of the same type and also with lymphocytes (VELDMAN and KAISERLING, 1980). The IDCs have been demonstrated in the inner cortex and medulla of the thymus as well as in the periarterial lymphoid sheath (PALS) of the spleen (KAISERLING et al., 1974; VEERMAN, 1974; USHIKI et al., 1984).

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**Fig. 7.** A macrophage (M) covered by drumstick-like microprojections and basolaterally extending filopodia. From red pulp of a rat spleen. R reticular cells, S sinus rod cells. ×3,200

**Fig. 8.** A macrophage with a rather smooth surface, associated with several lymphocytes (L). R reticular cells from a marginal zone of a rat spleen. ×3,200

**Fig. 9.** An interdigitating cell (IDC) covered with characteristic cytoplasmic processes and associated with many lymphocytes (L). From the cortico-medullary boundary of a rat thymus. ×4,000
By SEM, the IDCs appear larger in size than the macrophages and have many spherical, stick-like or knob-like cytoplasmic processes entangling each other (USHIKI, 1986). The IDCs sometimes also extend veil-like processes which embrace or contact neighboring lymphocytes (Fig. 9).

The IDCs have been reported to be strongly positive for Ia antigen (BARCLAY, 1981; DUIJVESTIJN et al., 1983) and are considered to function as antigen-presenting and accessory cells in the immune response. The SEM images might visualize the delivery of immunological information from the IDCs to T-lymphocytes in the thymus as well as in the thymus-dependent areas of the lymph nodes and spleen.

**Blood vessels and their endothelial cells**

The postcapillary venules in the secondary lymphoid organs except the spleen are characterized by the peculiar architecture of its endothelium. Since the endothelial cells protrude into the lumen with their rounded bodies, the vessels are termed the “high-endothelial venules” (HEVs) (Fig. 10). The luminal surface of the HEVs is variable in appearance: some endothelial cells are smooth, while others are unevenly surfaced with granular microprojections (He, 1985). These morphological varieties may represent various functional states of the endothelial cells.

Many lymphocytes are attached to the luminal...
Whether the lymphocytes transverse the endothelium intracellularly or intercellularly has been contended by many investigators (ÜMETANI, 1977; CHO and DE BRUYN, 1979; YAMAGUCHI and SCHOFEL, 1983). Our SEM studies show the presence of lymphocytes penetrating the endothelial cells intracellularly as well as lymphocytes passing intercellularly. Both inter- and intracellular routes thus can be recognized in the HEVs.

The postcapillary venules in the thymus are quite different from those described above (Fig. 11). They are located in the cortico-medullary region and in the medulla of the thymus in the mouse, rat and guinea-pig. Their endothelium comprises flattened polygonal cells. In these venules, lymphocytes are frequently seen traversing the vessel wall via an intracellular route (USHIKI, 1986). These images represent the migration of T-lymphocytes into the blood circulation, though a possibility that some lymphocytes enter the thymus from the bloodstream can not be excluded in morphological studies.

REFERENCES


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