Fine Structures of the Medulla-like Tissues and the Lymph Sinuses in the Lymph Nodes of Pig

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ABSTRACT. Fine structures of the “Medulla-like tissue (MT)” and the lymph sinuses facing the MT of pig lymph nodes were studied by electron microscopy. The MT consisted mainly of reticular cells and strands of collagen fibers, and there were no obvious structures of the so-called medullary sinuses. In the MT of the cortico-medullary junction and near the sub-capsular lymph sinuses, there found the clusters of dendritic cells, which were furnished with a number of thin, elongated cytoplasmic processes connected to one another or to macrophages to form fine networks. In the sub-capsular areas of the MT where the efferent lymph vessels were restrictively absent, no genuine lymphatic sinuses were identified. The endothelial cells which configure the trabecular side of the peri-trabecular lymph sinuses (PTLS) were constantly covered with a basal lamina and the surface of which has well-developed layer of collagen fibers. Inner walls of the sub-capsular lymph sinuses (SCLS) and parenchymal walls of the PTLS consisted of bi-layers of sinus lining cells with gaps and strands of collagen fibers. These results suggest that the MT in the pig lymph nodes has connective tissue-like structure, which is a characteristic ultrastructure, and enhances the filtration of materials and cellular elements in the pig lymph nodes.—**KEY WORDS:** lymph node, lymph sinus, pig, ultrastructure.

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The lymph nodes of pig are referred to as the “Reverse type of lymph nodes” in comparative histology [1, 9, 24]. More recently, it was clarified that the lymph nodes were not characteristic of simple reversal structures but consisted of several lymphoid segments, or “nodular units”, of various sizes. In addition, histological architecture of “medulla-like tissue (MT)” showed a marked difference from other mammalian lymph nodes, and a clear passing of colloidal carbon particles was not able to be observed in the MT in light microscopy as a result of histological reexamination of the structures, experiment of colloidal carbon treatment, and the intranodal distribution of T cells in the nodes [15] (Fig. 1). Up to now, however, there have been few investigations on the lymph nodes of pig in comparison with the other mammalian

lymph nodes, and especially, there are no detailed studies on the ultrastructural architectures of the lymph nodes of pig.

The purpose of the present study was to clarify the fine structures of the MT and of the lymph sinuses facing the MT.

MATERIALS AND METHODS

Lymph nodes: Mandibular, jejunal and popliteal lymph nodes from fifteen healthy pigs of Large White×Landrace, aged 6 months, were used.

Electron microscopy: The lymph nodes were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer solution (pH 7.4) at 4°C and then postfixed with 1% osmium tetroxide in 0.1 M phosphate buffer solution (pH 7.4) at 4°C. Dehydrated tissues with graded ethanol were embedded in Quetol-812

Fig. 1. A schematic drawing of the regional structural interrelations between the parenchyma and lymph pathways of a nodular unit in pig lymph nodes. AH: A-type hilus, ALV: afferent lymph vessel, CC: central cisterna, ITLC: intra-trabecular lymph channel, PTLS: peri-trabecular lymph sinus, SCLS: sub-capsular lymph sinus, Tr: trabecula.

Fig. 3. Medullary tissue in the mouse popliteal lymph node. Numerous free cells are found, such as lymphocytes (L) and plasma cells (PC). MS: medullary sinus, EC: sinus endothelial cell. $\times 1800$, (Bar=10 μm).

(NISSIN EM Co., Ltd., TOKYO). Ultrathin sections were stained with uranyl

Fig. 2. A few lymphocytes are present in the medulla-like tissue in the pig lymph node. The sinusoidal capillary is encircled by a pericyte (arrow). Reticular cells (arrowheads), CL: capillary lumen, E: eosinophil, M: macrophage. $\times 1800$, (Bar=10 μm).

acetate and lead citrate, and subsequently observed and photographed with a HITACHI HU-12A electron microscope. In order to make an ultrastructural comparison of the lymph nodes between pig and mouse, lymph nodes of mouse (mandibular, jejunal and popliteal lymph nodes) were sampled and the structures were observed following the same procedure as that used for pig.

RESULTS

The medulla-like tissues (MT) were poor in cellular components, especially in lymphocytes, in contrast to the medulla of the mouse lymph nodes of packed wandering cells (Figs. 2, 3). The MT was mainly composed of fixed cellular elements, reticular cells and strands of collagen fibers. The reticular cells formed sparse networks throughout the MT, among which there were collagen fibers (Fig. 2). This histological feature distinguished the MT from the cortex-like tissue (CT) in pig lymph nodes. No distinct lymph sinuses (or spaces) or medullary cord-like structures, which are

Fig. 4. Dendritic cell from the medulla-like tissue exhibits numerous thin, elongated interdigitating cytoplasmic processes. $\times 9000$, (Bar=1 μm).

Fig. 5. Dendritic cells are in close association with junctional structures (arrowheads). $\times 5000$, (Bar=1 μm). The inset shows higher magnification of junctional structures. $\times 9000$, (Bar=1 μm).

Fig. 6. An area of sub-capsular lymph sinus (between arrowheads) facing the medulla-like tissue (MT). Cap: capsule. $\times 1900$, (Bar= $10\ \mu\text{m}$).

Fig. 7. The trabecular wall of the peri-trabecular lymph sinus (PTLS) lined by sinus endothelial cells (EC) associated with reticular fibers (arrowhead). $\times 5000$, (Bar= $1\ \mu\text{m}$). The inset shows higher magnification of inter-sinus endothelial cell junction. Junction (arrowhead) and constant basal lamina (arrow) are seen. Co: collagen fiber. $\times 15000$, (Bar= $1\ \mu\text{m}$).

Fig. 8. The parenchymal wall of the medullary sinus (MS) in the mouse lymph node. The reticular cell (RC) extends a sheet of cytoplasm, to just beneath the sinus lining endothelial cell (EC). A thin collagenous layer (arrowheads) is sandwiched between this sheet and sinus lining. $\times 10000$, (Bar = $1\ \mu\text{m}$).

ordinarily found in mouse lymph nodes, were recognized in the MT areas by electron microscopy (Figs. 2, 3). In addition, numerous dendritic cells in clusters were located exclusively in the MT side of the cortico-medullary junction and also in the MT near the sub-capsular lymph sinuses. These cells were characterized by a number of thin and elongated cytoplasmic processes which combined with one another to form a distinct network (Fig. 4). Occasionally, their cytoplasmic processes were joined together or with macrophages tightly by attenuated structures (Fig. 5). The nuclei of these dendritic cells were generally round or oval in shape, where the nucleoplasm contained a prominent nucleolus, a large amount of euchromatin, and only a narrow rim of heterochromatin was seen along the inner leaflet of the nuclear membrane. The cytoplasm consisted of a less electron-dense hyaloplasmatic matrix in which dispersed free ribosomes, vesicles, and a few lysosome-like bodies were contained (Figs. 4, 5).

In the restricted sub-capsular areas facing the CT (near the A-type hilus [15]), and the MT where efferent lymph vessels were found, sub-capsular lymph sinuses (SCLS) were clearly identifiable (Fig. 6). However, in the areas of sub-capsules where the efferent lymph vessels were absent, no lymph sinuses with endothelium were demonstrated.

The peri-trabecular lymph sinuses (PTLS) were narrow and their locations were obscure in comparison with those in the mouse medullary sinuses. The endothelial cells in the trabecular side of the PTLS were generally furnished with a round or pleomorphic nucleus. The cytoplasm was elongated and thin, so the nuclear portion of the cell bulged into the sinus lumen or into the surrounding parenchyma. A few cytoplasmic components such as mitochondria, granular endoplasmic reticulum, free ribosomes or lysosomal-like bodies were contained in the cytoplasm (Fig. 7). Endothelial cells were joined by junctional structures. A basal lamina seemed to be almost constant (Fig. 7). Along the inner wall of the SCLS and the parenchymal wall of the PTLS of the pig lymph nodes, a bi-laminal structure, composed both of sinus lining cells and layers of collagen fibers was noted (Figs. 6, 9). Whereas in the mouse lymph nodes, the inner wall of the marginal sinuses and the parenchymal wall of the medullary sinuses were furnished with a tri-laminal structure: flattened sinus endothelial cells, connective tissue composed of collagen fibers, and reticular cells (Fig. 8). Occasionally, endothelial cells of the medullary sinuses in the mouse lymph nodes were similar to the elongated macrophages with well-developed Golgi complexes, numerous vesicles and vacuoles of various densities, and lysosomes. In the lymph nodes of pig, however, frequent clefts in the parenchymal (MT) wall of the PTLS containing lymphocytes in transit were detected (Fig. 9).

Fig. 9. A lymphocyte (L) is seen in the gap of the parenchymal wall of the peri-trabecular lymph sinus (PTLS). Note the macrophage (M) extending toward the sinus. Co: collagen fiber, EC: sinus endothelial cell. $\times 3000$, (Bar= $10\ \mu\text{m}$).

Fig. 10. Macrophages (M) adhere to the surface of the endothelial cells which form the parenchymal wall of the peri-trabecular lymph sinus (PTLS), and collagen fibers (Co) in endothelial gaps (arrowheads). $\times 6000$, (Bar= $1\ \mu\text{m}$).

Macrophages, however, were frequently found in the clefts or on the surface of the sinus lining layers which formed the inner wall of the SCLS and parenchymal wall of the PTLs (Fig. 10).

DISCUSSION

Since the ultrastructural architectures of lymph nodes were first analysed by electron microscopy [5], minute structures of reticular networks and sinuses of lymph nodes have been described in various animals [6, 7, 13, 14, 16, 23, 28–31]. However, only one ultrastructural investigation of the structure in the lymph nodes of pig has been reported [26]. More recently, although studies in the field of physiology suggested that the migration of lymphocytes from lymph to blood also occurs within pig lymph nodes [2, 3, 22, 25], the morphological basis accounting for these mechanisms still remains unknown.

It is known that the lymph nodes of pigs have an unusual structure in comparison with those of other mammalian species. The MT in the pig lymph nodes were generally located in peripheral regions in the nodes in contrast with the CT, except in the region of A-type hilus, and were composed mainly of meshworks of reticular fibers and rich collagen fibers, which were finer and denser than those of the CT in light microscopy [15]. The results of our previous and present examinations clarified that among reticular cells which formed sparse networks throughout the MT, there were abundant collagen fibers instead of lymph sinuses; therefore, the MT in the pig lymph nodes revealed a connective tissue-like structure. This finding suggests that the MT impedes the passage of cellular elements and particle substances, and may support the concept that the lymphocytes traffic to the outside of the nodes would be caused mainly via the intranodal veins, and partly via the efferent lymph vessels after across the MT [2, 3, 22,

25].

In the present study, the dendritic cells located in the MT were characterized by a number of thin and elongated cytoplasmic processes that combined one another or with macrophages to form a complicated labyrinth-like fine network. Up to now, the non-lymphoid mononuclear cells with “dendritic” features, Langerhans cells (LC) [12, 17–19], interdigitating cells (IDC) [11, 19], and follicle dendritic cells (FDC) [11, 17, 20] have been identified in the lymph nodes. The cytoplasmic processes of the LC, IDC and FDC were much thicker and “stellate” in form in comparison with those of the present dendritic cells in pig nodes. In addition, the nuclei of LC and IDC were irregular in shape and their cytoplasm contained specific “Birbeck granules” [11, 18, 19], which distinguished them from usual macrophages [4, 11]. Furthermore, IDC is considered to be a derivative of LC and to be distributed exclusively in the thymus dependent areas in the lymph nodes. On the other hand, the FDC with round or oval nuclei are found in the germinal centers in contrast to the present dendritic cells located in the MT.

From their morphological features and the regional cell locality, the dendritic cells in the MT of the pig lymph nodes were obviously different from the LC, IDC and FDC. In other characteristic features for cell classification, Fc and C3b receptors, surface cell markers, show similar features to LC, FDC, monocytes, and phagocytes, except for IDC [27]. The results of the present study indicated that dendritic cells may be poor in migratory activity due to their gap junctions, belonging to the mononuclear phagocytic family in morphology. However, the immunocytochemical nature of the pig dendritic cells was not clearly elucidated, as it was in the cell types mentioned above. Therefore, further immunohistochemical studies are needed to clarify whether the

dendritic cells differ from the other cell types of mononuclear phagocytes or not, and what is their origin in pig lymph nodes.

The cells of the inner walls of the lymph sinuses have been regarded traditionally as reticular cells arranged in an endothelial position and having a phagocytic nature [8]. Extensive ultrastructural investigations of the inner-wall cells of other mammalian lymph nodes [16, 23] have supported the classical concept that the reticuloendothelial cells line the sinuses, and the so-called stellate cells, which bridge the sinuses, share the functions of both endothelial cells and macrophages [7, 23]. More recently, it is reported that the walls of the medullary sinuses consist of flattened endothelial cells and macrophages [8, 21].

In the present work, the outer wall of the SCLS and the trabecular side of the PTLs were composed of an intact continuous endothelium with flattened endothelial cells and a layer of basal lamina. While there were numerous gaps observed exclusively in the inner wall of the SCLS as well as in the parenchymal wall of the PTLs. These gaps in the sinus lining are reported in many perforations in human [10] or small pores in rat [14]. Therefore these walls did not form a barrier because the walls possessed interruptions, allowing for the passage of cells, and presumably other components such as antigens. The present findings on the cellular architectures of the sinus walls of pig lymph nodes are in agreement with the results of Clark, Jr. [7] and Moe [23] in mice. In our previous study of colloidal carbon treatment in the popliteal lymph nodes of pig [15], no accumulations of carbon particles either in the SCLS or in the efferent vessels, despite their presence both in the PTLs and in the MT, were found even at 60 hr after treatment. From these facts and the present results, the alternative accumulations of foreign materials in pig lymph nodes were considered to be caused

by macrophages in the sinus lining layers and gaps, or by dendritic cells in the MT. It is possible to consider that the cellular elements and the particle substances which filled PTLs in the lymph can not pass easily through the interstitium of the MT, while the lymph passes exclusively through these tissues in order. This view would be interesting to follow in the future studies on intranodal localities and immunobiological functions of the lymphocytes pathways in pig lymph nodes, comparing the processes of production of immunocompetent cells and their systemic peripherizations.

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要 約

ブタリンパ節の髄質様組織およびリンパ洞の微細構造について：星 信彦・橋本善春・北川 浩・昆 泰寛・工藤規雄（北海道大学獣医学部家畜解剖学講座）——ブタリンパ節の「髄質様組織」とそれに面するリンパ洞の微細構造を電顕的に検索した。髄質様組織は主に細網細胞と膠原線維束からなり、いわゆる髄洞構造はみとめられなかった。皮質・髄質様組織境界部の髄質様組織側および被膜下洞付近の髄質様組織には、多数の細長い細胞質突起を相互に、あるいは大食細胞の細胞質突起と複雑迷路状にからめあい、密な網工を形成している樹枝状細胞が多数観察された。輸出リンパ管の存在しない被膜下領域では、明瞭なリンパ洞の形態を備えた被膜下洞はみとめられなかった。梁柱周囲洞の梁柱側を構成する内皮細胞はほぼ連続した基底膜で覆われ、その外側にはよく発達した膠原線維束が観察された。梁柱周囲洞の実質側および被膜下洞の内壁は不連続な洞内皮細胞と膠原線維束の2層構造を呈した。これらの結果から、ブタリンパ節のリンパ洞には他動物のリンパ節にはみられない微細構造が存在するとともにその髄質様組織は強い結合組織性の性格を有し、リンパ節に流入する物質や細胞成分の濾過作用を増強させているものと推察された。