Direct Contact between Reticular Fibers and Migratory Cells in the Paracortex of Mouse Lymph Nodes: A Morphological and Quantitative Study

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Summary. Transmission electron microscope observation of mouse lymph nodes demonstrated that reticular fibers in the paracortex were invested not only by reticular cells, but occasionally also by migratory cells such as interdigitating cells, macrophages, and lymphocytes. Quantitative analysis of electron micrographs covering wide areas revealed that about 90% of the surface area of the reticular fiber was enclosed in the sheath of reticular cells in both nude and hetero mice, whereas the rest of the surface area was associated with migratory cells. In nude mice, whose lymph nodes contain more numerous interdigitating cells than hetero animals, about 9% of the surface area was occupied by interdigitating cells including Langerhans cells; in hetero mice only about 3% was associated with the interdigitating cells. Actively phagocytizing macrophages occupied about 3% of the surface area in both nude and hetero mice. Contact between lymphocytes and reticular fibers was observed in hetero mice, whereas this relation could not be demonstrated in nude mice whose lymph nodes contain very few lymphocytes. These results suggest that the association between reticular cells and reticular fibers in the paracortex of lymph node is flexible, allowing for the interposing of migratory cells.

The basic framework of the lymph node is constructed of reticular cells and reticular fibers. The reticular cells are connected to each other by desmosome-like junctions and extend along the extracellular skeleton of the reticular fibers. The mesh of the framework is occupied by lymphocytes and non-lymphocytic migratory cells such as macrophages and interdigitating cells. Early studies by electron microscopy demonstrated that the reticular fibers were regularly surrounded by a sheath of reticular cells, and thus separated from the migratory cells within the mesh of the framework (Sorensen, 1960; Han, 1961; Clark, 1962; Moe, 1963; Bairati et al., 1964). Following these early works, there has been little further study on the fine structural relationship between the reticular fibers and migratory cells in different locations in the lymph node.

In 1966, it was shown that the paracortex of the lymph node, an inner part of the cortex, is a specialized region where thymus-derived T-lymphocytes are accumulated (Parrot et al., 1966). Langerhans cells with an antigen-presenting function (Shelly and JuHLIN, 1976; Stingl et al., 1977) were recognized in the paracortex of lymph nodes (Kondo, 1969; Hoshino et al., 1971; Kobayashi and Hoshino, 1976). These cells have been shown to come from the skin via lymphatics (Kelly et al., 1978). Interdigitating cells, which were first described as a type of reticular cell in the paracortex (Veldman, 1970), have been categorized as a type of migratory cell which has no contact with the reticular fiber (Kamperdijk et al., 1978; HoeFSMIt et al., 1980; Hendriks et al., 1981).

In the course of our fine structural observations on the lymphocyte-depleted paracortex of athymic nude mice, we often observed reticular fibers directly covered by cells other than reticular cells, i.e., interdigitating cells, Langerhans cells, and macrophages. In order to obtain better insight into the basic structure of the reticular framework in the paracortex of the lymph node, we attempted a morphological and quantitative study on the association of reticular fibers with different cell types, using nude and hetero mice.
MATERIALS AND METHODS

Nude mice (nu/nu, aged 6-12 weeks) and hetero mice as control (nu/+, aged 8 weeks) of both sexes were supplied by the Institute of Laboratory Animal Research, Nagoya University School of Medicine. Under anesthesia with ether, cervical lymph nodes were removed and cut into halves, fixed with 4% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2-3 days and then fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2 h, both at 4°C. They were postfixed with 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4) for 90 min at room temperature, dehydrated with graded concentrations of ethanol and embedded in Quetol 812 (Nissin EM). Ultrathin sections were cut on a Porter-Blum MT-1 ultramicrotome using glass knives. The sections were stained with uranyl acetate and lead citrate and examined in a Hitachi electron microscope (H-800).

For quantitative studies, a total area of at least 32,000 \( \mu m^2 \) of the paracortex for one mouse was photographed at 3,000 \( \times \) magnification. The total length of the cell membranes which contacted the reticular fiber was calculated for different cell types using a photopattern analyzer (Digigrammer, Mutoh) on the electron micrographs of 9,000 \( \times \) magnification. Five nude and three hetero mice were used for this determination.

RESULTS

1. Identification of reticular and other cell types

Non-lymphocytic cells other than the vascular components in the paracortex could be divided into three types; reticular cells, macrophages, and interdigitating cells, based on their morphology, as follows. Dendritic reticular cells were not recognized. The same three cell types were identified in both nude and hetero mice.

1) Reticular cells (Figs. 1a and 3a, 2a and 3b)

These were stellate with slender cell processes. The nuclei were large, oval and occasionally showed a prominent nucleolus. The cytoplasm was dark with many profiles of rough-surfaced endoplasmic reticulum, a large Golgi complex, many primary and secondary lysosomes, and some mitochondria. The reticular cells were closely associated with reticular fibers, enwrapping the fibers with their cell membrane. These cells corresponded to the cells designated as the "reticulum cell" (STUART, 1975; HOEFSMITH, 1975; KAMPERDIJK et al., 1978; HOEFSMITH et al., 1980; HENDRIKIS et al., 1981) or "fibroblastic reticulum cell" (MÜLLER-HERMELINK and LENNERT, 1978).

2) Macrophages (Figs. 1a and 3a)

These were large, round or elongated cells with a high cytoplasm-to-nucleus ratio. The nucleus was oval or elongated, usually with a prominent nucleolus in the center, and often eccentrically located. The cytoplasm contained much debris of degenerating cells, presumably phagocytized lymphocytes, and variable numbers of primary and secondary lysosomes. Smooth and rough-surfaced endoplasmic reticulum, free ribosomes, and a prominent Golgi complex were also observed. These cells corresponded to the histiocytic reticulum cells categorized by MÜLLER-HERMELINK and LENNERT (1978).

3) Interdigitating cells (Figs. 1a and 3a)

These were large-sized cells with a high cytoplasm-to-nucleus ratio. The nucleus was often deeply invaginated at two or three sites, and occasionally showed a small nucleolus. These cells extended their cytoplasmic processes to interdigitate with those of other interdigitating cells. The processes had few cell organelles. The cytoplasm around the nucleus, however, contained small Golgi complexes, many small vesicles, slender mitochondria and a variable amount of rough-surfaced endoplasmic reticulum. The interdigitating cells corresponded to the cells identified by VELDMAN (1970) and KAISERLING and LENNERT (1974). Langerhans cells have been included in the interdigitating cells because they showed the same cellular appearance except that they contained Birbeck granules, and it was impossible to distinguish either cells from the other when Birbeck granules were absent from the sectional profile of a Langerhans cell (HOEFSMITH et al., 1982; KOBAYASHI and HOSHINO, 1983).

Fig. 1. a. Low magnification electron micrograph of the paracortex of a nude mouse. R reticular cells, I an interdigitating cell in contact with a reticular fiber, M a macrophage in contact with a reticular fiber. \( \times5,700 \).

b. Higher magnification of the area labeled b in Figure 1a. A reticular fiber (arrowheads) associated with a macrophage \( (M) \) and a reticular cell \( (R) \). \( \times21,600 \).

c. Higher magnification of the area labeled c in Figure 1a. A reticular fiber \( (arrow) \) associated with an interdigitating cell \( (I) \) and reticular cells. \( \times21,600 \)
Fig. 1. Legend on the opposite page.
Fig. 2.  

a. Low magnification electron micrograph of the paracortex of a hetero mouse.  
R a reticular cell,  
L a lymphocyte in contact with a reticular fiber.  \( \times 5,700 \).  

b. Higher magnification of the area labeled b in Figure 2a.  
A reticular fiber (arrow) associated with a lymphocyte (L) and a reticular cell (R).  \( \times 21,600 \)
Fig. 3, a and b. Traces of electron micrographs corresponding to Figures 1a and 2a, respectively. Interdigitating cell, lymphocyte, reticular cell, reticular fiber, macrophage. Large arrow associations of interdigitating cell-reticular fiber, arrowhead macrophage-reticular fiber associations, small arrow lymphocyte-reticular fiber associations.
2. The relationship of reticular fibers with reticular cells and other cell types in the paracortex of lymph nodes

In low magnification electron micrographs and corresponding drawings of the paracortex, profiles of reticular cells associated with reticular fibers were seen in nude mice (Figs. 1a, 3a) and hetero mice (Figs. 2a, 3b). The reticular fibers also associated with interdigitating cells, macrophages and lymphocytes. In nude mice there were a few lymphocytes and many interdigitating cells in the paracortex. We therefore could easily recognize reticular fibers associated with interdigitating cells. A small part of these interdigitating cells abutted on the reticular fibers between the reticular cells (Figs. 1a, 1c, 3a). Direct contact between reticular fibers and Langerhans cells containing Birbeck granules was also recognized (Figures not shown). In hetero mice, many more lymphocytes were present in the paracortex, and contact between reticular fibers and cell membrane of lymphocytes was occasionally seen (Figs. 2a, 2b, 3b). Actively phagocytizing macrophages usually occurred in the paracortex of both nude and hetero mice. A small part of the cell membrane of these cells occasionally made direct contact with the reticular fibers (Figs. 1a, 1b, 3a).

3. Quantitative studies on the fiber-cell relationship

Measurement of the length of the membrane profile of different cell types which directly associated with reticular fibers on the photographs is schematically shown in Figure 4. Table 1 shows the proportion of the total length of membrane profiles of the four types of cells associated with reticular fibers. The length of the membrane profile is proportional to the surface area (WEIBEL et al., 1966) of the reticular fiber enclosed in the cellular sheath. Around 10% of the surface of the reticular fiber was associated with cells other than reticular cells in both nude and hetero mice. In nude mice, the surface area of the reticular fiber which was associated with interdigitating cells was about 9%, being three times that in hetero mice. Furthermore, about 3% of the surface area of the reticular fiber was associated with actively phagocytizing macrophages, in both nude and hetero mice. The association area between lymphocytes and reticular fibers was about 2% in hetero mice, but not observed in nude mice.

Table 1. Proportions of the reticular fibers associated with the 4 types of cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Nude mouse (%)</th>
<th>Hetero mouse (%)</th>
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<tbody>
<tr>
<td>Reticular cell</td>
<td>87.4</td>
<td>93.0</td>
</tr>
<tr>
<td>Other cells</td>
<td>12.6</td>
<td>7.0</td>
</tr>
<tr>
<td>Interdigitating cell</td>
<td>9.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Macrophage</td>
<td>3.3</td>
<td>2.5</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>0.0</td>
<td>1.7</td>
</tr>
</tbody>
</table>

DISCUSSION

The basic structure of the reticular framework of the lymph node was demonstrated by early electron microscopic studies carried out around 1960. These studies showed, in various experimental animals, that argyrophilic reticular fibers, as extracellular components of fibrous connective tissue, are completely enclosed in a sheath of reticular cells. In some of those studies, it was pointed out that there were occasional small gaps between the reticular cells where the reticular fiber abutted on a lymphocyte or a plasma cell (BAIRATI et al., 1964; MOVAT and FERNANDO, 1964). This problem, however, was not further investigated, because it was difficult at that time to distinguish such gaps from the artifacts.
caused by the shrinkage of cells during specimen preparation. In the present study, the cell membranes of neighboring cells in the paracortex closely conformed to each other, and no unfavorable shrinkage of cells or expansion of intercellular space was recognized around the sites of the fiber-cell association in question. The associations, therefore, could be considered as actual cases of contact occurring in situ.

Interdigitating cells were first described in the lymph node paracortex of the rabbit by Veldman (1970) as non-phagocytic reticular cells. Kaiserling and Lennert (1974) also described a corresponding cell type in the paracortex of the human lymph node. Later, extensive studies by Kamperdijk et al. (1978), Hoefsmit et al. (1980), and Hendriks et al. (1981) reached the conclusion that the interdigitating cells are migratory cells coming from the outside of the lymph nodes and accumulating in the paracortex. These cells are located within the mesh of the reticular framework, and make close contact with lymphocytes. We have observed the paracortex of lymph nodes in nude mice in which few lymphocytes but many interdigitating cells were present. We thus gave evidence that the interdigitating cells often directly contacted with the reticular fibers. The same contacts could also be found in hetero mice, but the percentage of contact area was much lower than in nude mice. Langerhans cells, which were included in the interdigitating cells, were also associated with the reticular fibers. These cells are considered migratory cells of bone marrow origin (Tamaki and Katz, 1980), traveling through the epidermis to the paracortex of the regional lymph nodes (Kelly et al., 1978; Kobayashi and Hoshino, 1976, 1983).

Macrophages in the lymph node are generally considered migratory cells, which are independent from the reticular cells in origin, structure, and function (Van Furth et al., 1972; Fujita and Kashimura, 1981). In the paracortex of the lymph node, we observed macrophages making direct contact with reticular fibers. The percentage of the contact area was about 3% in both nude and hetero mice. These results indicate that, despite remarkable changes in the population of lymphocytes and interdigitating cells in nude mice, macrophages maintain their relationship to the reticular fiber. The lymphocytes in the paracortex of hetero mice were also found to make direct contact with the reticular fibers, although the frequency was only 1.7% of the surface area of the reticular fiber. This contact could not be found in nude mice as lymphocytes were very few in the paracortex.

Our quantitative observations demonstrate that around 90% of the surface area of the reticular fiber in the paracortex is covered with reticular cells of fibroblastic appearance in both nude and hetero mice. The components of the reticular fibers are most likely produced by these reticular cells. Unlike fibroblasts in the connective tissue, these cells enwrap their products with a membrane. The fact that about 10% of the surface area of the reticular fiber was in contact with migratory cells would indicate that the association between reticular cells and reticular fibers is not rigid but rather flexible. The migratory cells, probably moving actively in the paracortex, may be able to thrust themselves between the reticular cells and reticular fibers.

REFERENCES


