

Postnatal development of dendritic reticulum cells and their immune complex trapping ability

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Summary. The postnatal development of dendritic reticulum cells in the rat popliteal lymph nodes was electron microscopically investigated in relation to the appearance of immune complex trapping capacity. The popliteal lymph nodes of neonatal rat consisted of loosely arranged fibroblastic reticulum cells. In the following stage, the peripheral cortex and paracortex became distinguishable. The former was made up of an accumulation of small lymphocytes, scattered within a framework of reticulum cells. On the 28th day, the first primary follicle appeared in the peripheral cortex. Simultaneously the immune complex could be trapped on the cytoplasmic membrane of reticulum cells, which were located in the central portion of the primary follicles. The early image of germinal centers appeared corresponding to immune complex trapping areas. In the well-developed secondary follicles, the immune complex trapping cells were mainly localized in the cap area. Their cytoplasmic membranes formed the dendritic processes, on which the distinct ability of trapping of the immune complex was recognized. It was demonstrated that the fibroblastic reticulum cells, forming the stroma of lymph nodes, were transformed into the typical dendritic reticulum cells with labyrinth structures in the cap area.

Desmosomal junctions were often found, not only between the dendritic reticulum cells themselves, but also between the dendritic reticulum cells and lymphocytes. We suggest that the desmosomal junctions play a role as the channel for a transmission of immunological information.

Key words: Rat lymph node · Dendritic reticulum cells · Immune complex trapping · Postnatal development

Introduction

Recently much effort has been devoted to the study of morphology and functions of non-lymphoid cells in the lymphoid tissues. Among the non-lymphoid cells, interdigitating cells (IDC) and dendritic reticulum cells (DRC) form the special microenvironment. They play an important role in homing, development and interactions of T and B lymphocytes. With regard to DRC, Chen et al. (1978) showed the retention of horseradish peroxidase (HRP)-anti-HRP immune complex in ultrastructural observation. It has been shown that the DRC plays an important role in follicular trapping of antigenic materials, although the origin of DRC and the trapping mechanisms have not been verified.

Our purpose in this study is to investigate the origin of DRC and the temporal development of immune complex trapping ability of DRC. Therefore, we observed the postnatal development of the DRC in the popliteal lymph nodes of rats, in relation to the appearance of immune complex trapping ability. Furthermore, we made an attempt to show a significance of desmosomal junctions, which were often found on the processes of the DRC in the secondary follicles.

Materials and methods

Pregnant Wistar rats (Charles River Japan Inc.) were kept under the routine laboratory conditions. Popliteal lymph nodes were extirpated from at least two rats, at the following stages: 0, 1, 2, 3, 4, 6, 8, 10, 14, 16, 18, 21, 24, 28, 35, 42 and 49 days postnatally. For the purpose of

Origin and function of DRC

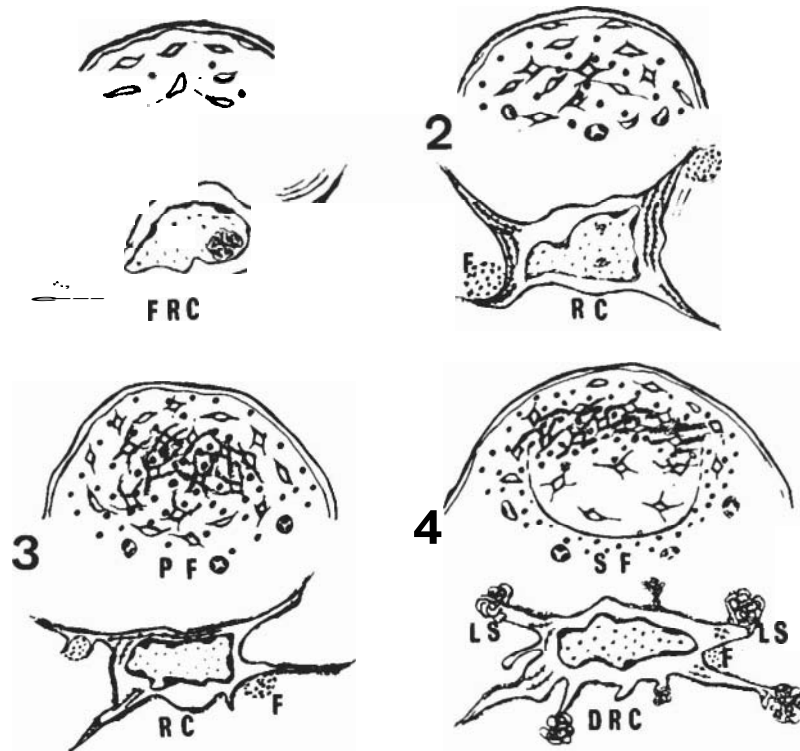


Fig. 1. This schematic diagram summarizes the postnatal development of lymph follicles and the transformation of the reticulum cells. 1. One day after birth, the cortical area is poorly developed. The basic framework consists of the fibroblastic reticulum cells (FRC). 2. Two weeks after birth, the peripheral cortex shows a broad expansion with the accumulation of small lymphocytes. Generally, reticulum cells (RC) are polygonal and display well-developed rER. The cytoplasmic processes often embrace bundles of reticulin fibers (F). 3. Four weeks after birth, the first primary follicles appear in the peripheral cortex, having nodular accumulation of small lymphocytes. The polygonal reticulum cells (RC), in the central portion of the primary follicle (PF), shows the distinct PAP trapping. 4. Seven weeks after birth, the well-developed secondary follicle (SF) shows a clear polarization. PAP trapping cells in the cap area are the typical dendritic reticulum cells (DRC) with complicated labyrinth structures. PAP trapping is especially remarkable on the labyrinth structures (LS).

detection of the temporal follicular trapping ability, the single injection of immune complex (rabbit peroxidase-anti-peroxidase soluble complex, PAP) was administered to each serial rat in footpads, one day before the extirpation of popliteal lymph nodes. After extirpation, lymph nodes were fixed in the mixture of 4% paraformaldehyde and 2% glutaraldehyde, buffered by 0.1 M phosphate buffer (pH 7.4) at 4°C for 4 hours. The fixed tissues were sliced into approximately 30 µm sections with the Microslicer (Dosaka EM Co., LTD). After rinsing three times with 0.05 M Tris-HCl buffer, the lymph node sections were incubated in 0.05% 3,3'-diaminobenzidine tetra-hydrochloride (DAB) containing 0.003% H₂O₂ for 20 minutes at room temperature. Following postfixation with 1% OsO₄, each section was dehydrated through graded alcohol and propylene oxide, and embedded horizontally in Epon columns. Ultrathin sections were stained with uranyl acetate and lead citrate for electron microscopical observations.

Results

The postnatal development of the popliteal lymph nodes of rats was divided into the following serial stages: 1) early development, 2) development of cortical area, 3) development of primary follicles, 4) development of

secondary follicles. In the schematic diagrams (Fig. 1), the histogenesis of lymph follicles and the transformation of reticulum cells are summarized. In the present study, stage three was closely related to the appearance of follicular trapping ability of PAP.

1. Early development

The popliteal lymph nodes of newborn Wistar rats were immature; however, the nodal parenchyma was already divided into two compartments, the cortex and the medulla. The basic framework of the cortex consisted of fibroblastic reticulum cells. A few lymphoid cells were scattered among the fibroblastic reticulum cells. The medulla was composed mainly of undifferentiated lymphatic sinuses and blood vessels. In the sinusoidal space, mononuclear phagocytes were recognizable. In electron microscopical observation, the fibroblastic reticulum cells displayed a spindle-shaped nucleus with dispersed heterochromatin and a large prominent nucleolus. Their cytoplasm showed the bipolarly elongated profiles, in which small aggregated bundles of reticulin fibers were often embraced. The rough endoplasmic reticulum (rER) was poorly developed and slightly dilated. It contained some electron-lucent materials (Fig. 2).

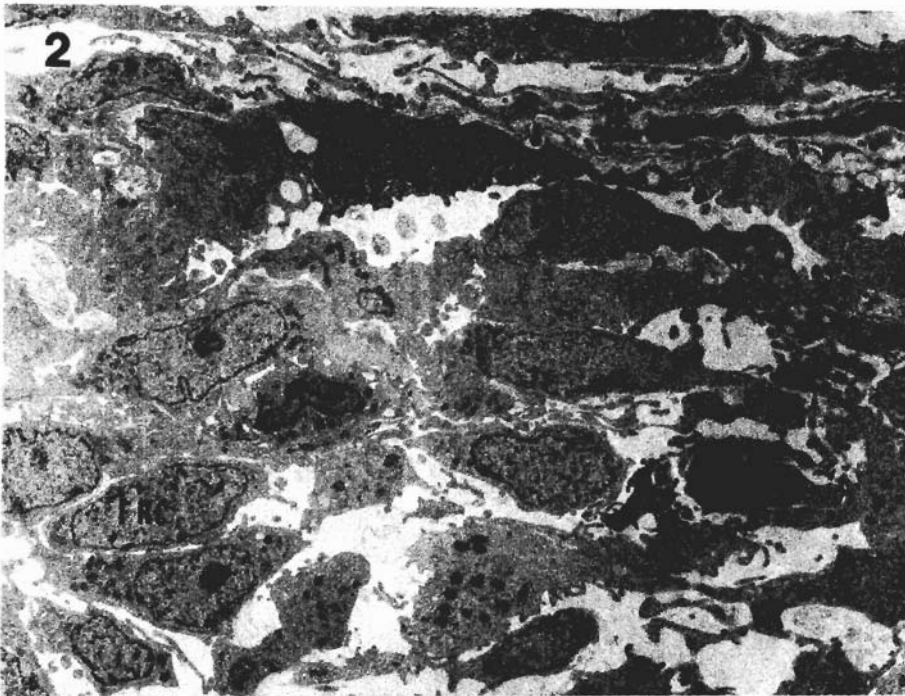


Fig. 2. One day after birth, the fibroblastic reticulum cells (FRC) form the reticular network in the cortical area where a few lymphoid cells (L) are scattered. x 2,500

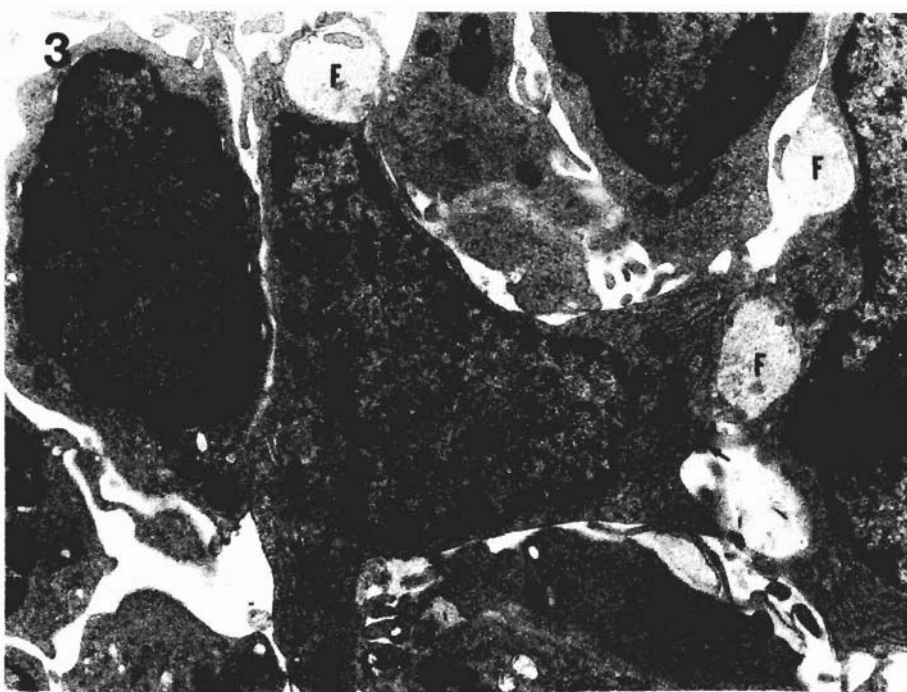


Fig. 3. Polygonal reticulum cell (RC) in peripheral cortex, 21st day after birth. Desmosomal junctions are present between the neighboring reticulum cell (arrow) and lymphocyte (double arrow). RC embraces bundles of collagen fibers (F) in the cytoplasm. x 8,000

3. Development of primary follicles

The progressive development of the peripheral cortex was remarkable, following completion of the paracortical area. The first primary follicle was recognized 28 days

4. Development of secondary follicles

Germinal centers were first recognized about five weeks after birth, although it varied in each individual. The early image of germinal centers was located in the central

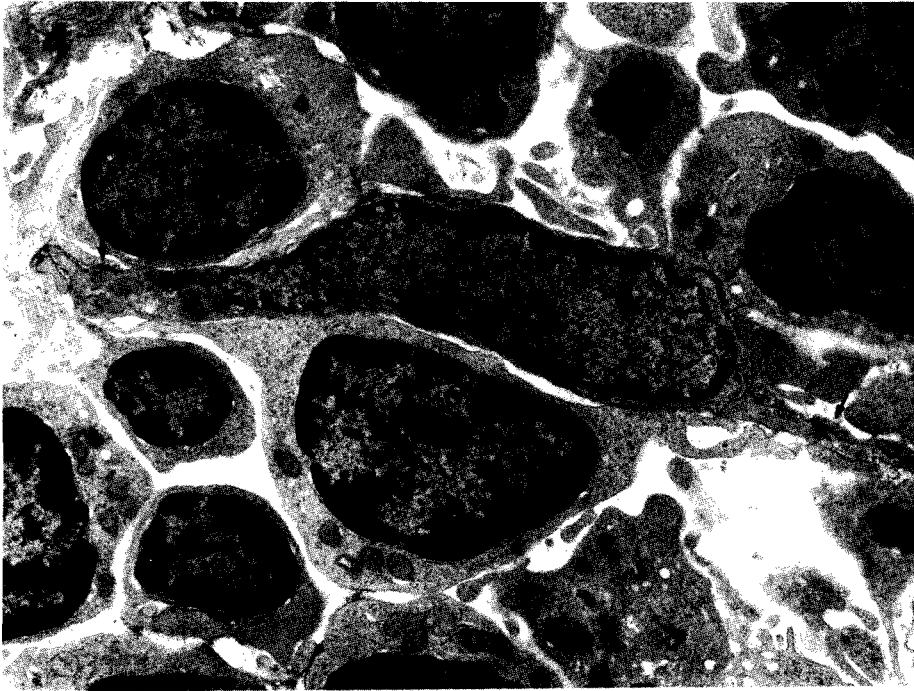
Origin and function of DRC

Fig. 4. Spindle-shaped reticulum cell (RC) in the peripheral part of primary follicle, four weeks after birth. Scarce PAP trapping is detected on a part of the cytoplasmic membrane (arrows). $\times 8,000$

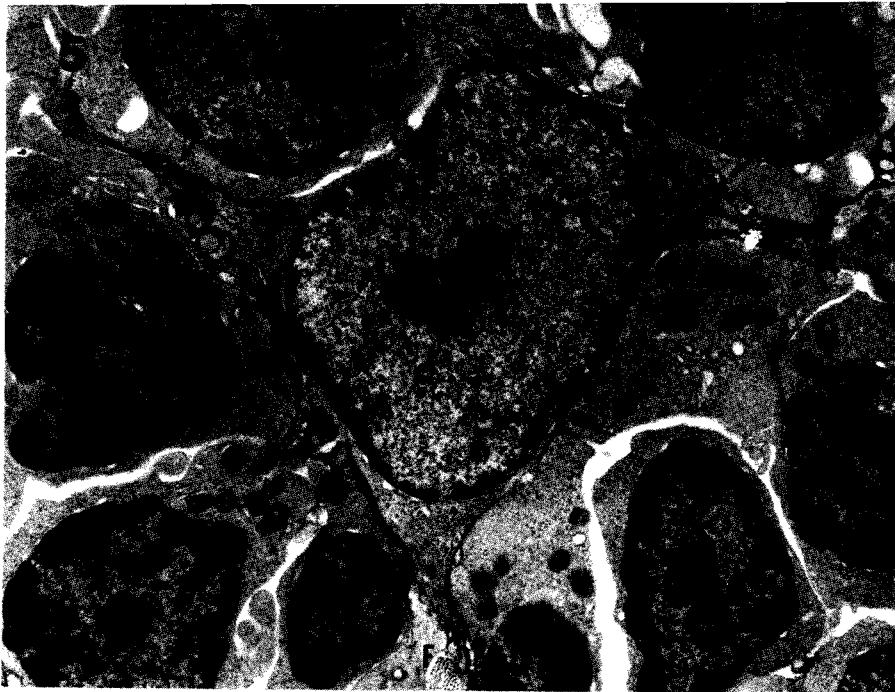


Fig. 5. Polygonal reticulum cell (RC) in the central portion of the same follicle as that of Fig. 4. It shows slightly complicated cytoplasmic processes and fairly abundant PAP trapping (arrow) as well as small bundles of collagen fibers (F). $\times 8,000$

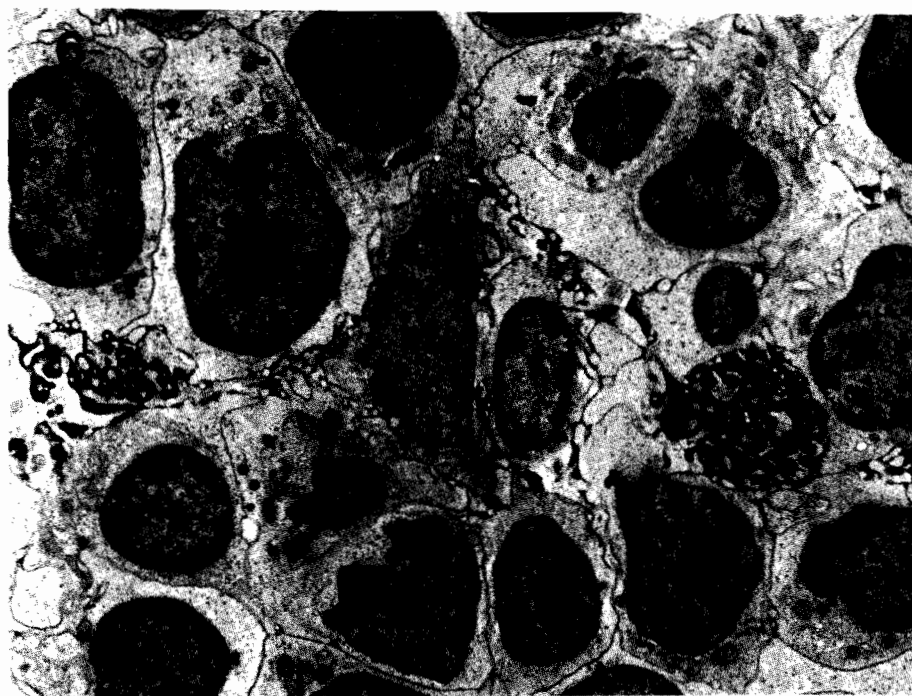


Fig. 6. Dendritic reticulum cell (DRC) in the cap area of a well-developed secondary follicle, seven weeks after birth. A large quantity of PAP is trapped on the labyrinth structures (LS). $\times 6,000$

2. Development of cortical area

During this stage, a distinct differentiation was observed in the cortical and medullary areas. In the cortical area, the peripheral cortex and paracortex became distinguishable, although the development of the former was preceded by that of the latter. About 14 days after birth, development of the paracortical area was nearly completed. It was made up of one or a few deep cortex units (DCU) with densely populated lymphocytes. In this area, non-lymphoid cells were principally IDC with electron-lucent cytoplasm. Following the last stage in the DCU development, a progressive differentiation of the peripheral cortex was present. It was characterized by the accumulation of small lymphocytes in this area. The increase of lymphocytes led to the broad expansion of the peripheral cortex. The lymphocytes were situated in the framework of the reticulum cells. Ultrastructurally the reticulum cells showed various morphological features, exhibiting spindle-shaped, round or oval and polygonal profiles. Nevertheless, we could easily trace their transformation from fibroblastic reticulum cells into ordinary reticulum cells. Generally, the polygonal reticulum cells displayed a well-developed rER, bundles of reticulin fibers and desmosomal junctions (Fig. 3).

3. Development of primary follicles

The progressive development of the peripheral cortex was remarkable, following completion of the paracortical area. The first primary follicle was recognized 28 days

after birth, being observed in some portion of the peripheral cortex, as a nodular accumulation of small lymphocyte mass. At the same time, PAP was first detected in the central portion of the newly formed primary follicles. The reticulum cells of the primary follicles showed some differences with regard to the ultrastructural features and the distribution pattern of PAP. In the marginal region of the primary follicles, the reticulum cells usually had spindle-shaped appearance. A small amount of PAP was found on the tip portion of the cell membrane (Fig. 4). On the other hand, the reticulum cells in the central area of the follicles were polygonal, being characterized by an euchromatic round nucleus, elongated cytoplasmic processes extending in all directions, and absence of labyrinth structures. More PAP could be observed on the distal portion of the central reticulum cells processes than in the marginal region reticulum cells (Fig. 5). At this stage, we could detect the PAP trapping of reticulum cells only in the inside of the primary follicles. Sometimes polygonal reticulum cells were present outside the follicles. They showed similar ultrastructural features to those of the reticulum cells inside the follicles. However, we could never find the PAP trapping ability on the cytoplasmic membrane of reticulum cells outside the follicles. In addition, neither lymphocyte nor macrophage showed the PAP trapping ability.

4. Development of secondary follicles

Germinal centers were first recognized about five weeks after birth, although it varied in each individual. The early image of germinal centers was located in the central

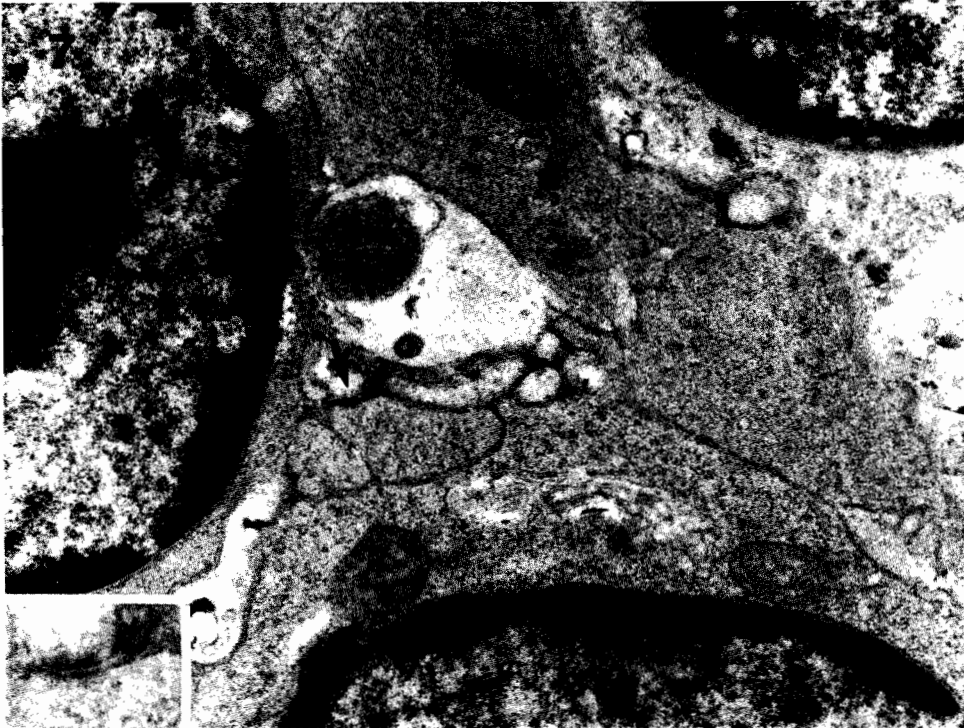


Fig. 7. Desmosomal junction (arrow and inset) between the cytoplasmic process of a dendritic reticulum cell and that of a lymphocyte. $\times 10,000$

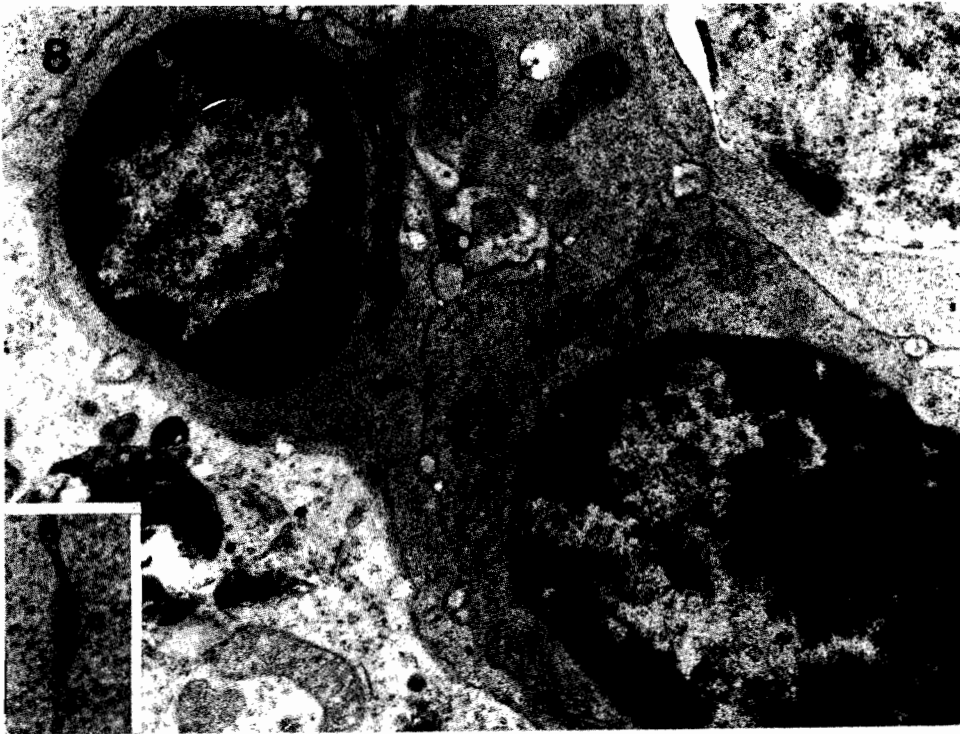


Fig. 8. Desmosomal junction (arrow and inset) between the opposing lymphocytes in the cap area. $\times 10,000$

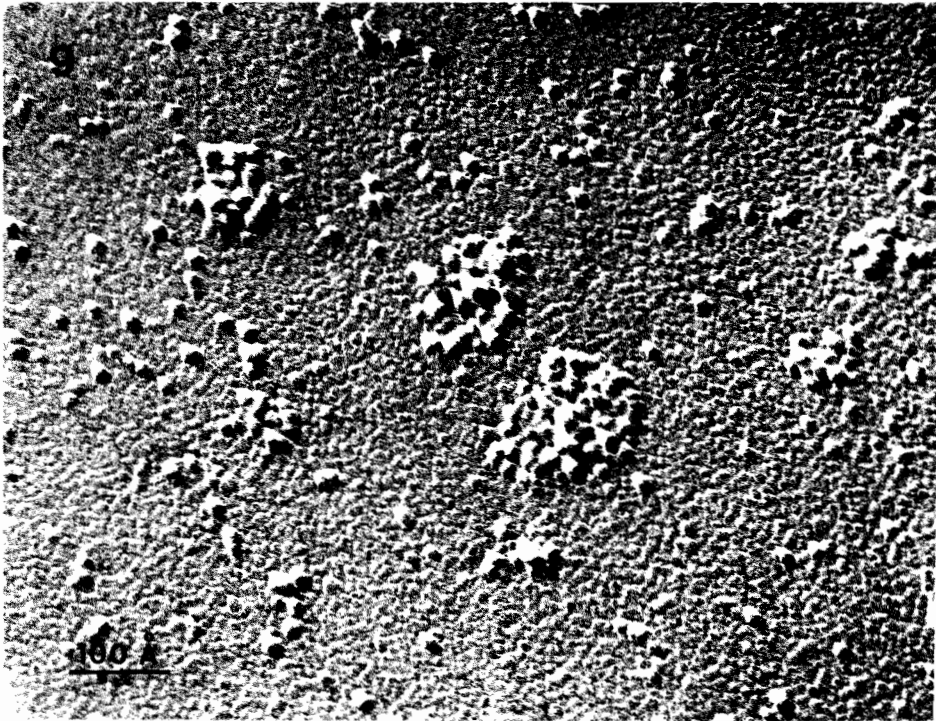


Fig. 9. Outer surface of the cytoplasmic membrane of the dendritic reticulum cell (freeze fracture technique). Note the aggregates of cytoplasmic membrane-granules in the intercellular junctions.

portion of primary follicles, which showed the characteristic deposition of PAP trapping. The first indication of newly formed germinal centers was an appearance of lymphoblasts and macrophages, easily recognizable as there was a distinct appearance in the homogeneous mass of densely populated small lymphocytes. With the development of germinal centers, the PAP trapping area progressively moved from the central portion of the germinal centers to the neighboring lymphocyte corona. The well-developed secondary follicles showed a clear polarization. The lymphocyte corona was broad in the outer portion, beneath the marginal sinus, but less narrow in the inner portion adjacent to the paracortex. The boundary between the germinal centers and the lymphocyte corona was obscure on the outer border though clear on the inner border. Fully-developed germinal centers showed a light zone and a dark zone situated in the upper and lower part of the germinal centers, respectively. In the present study, PAP trapping was clearly detected in the cap area, which was organized by the upper part of the light zone and the adjacent lymphocyte corona. The PAP trapping cells in the cap area were typical dendritic reticulum cells. Their elongated cytoplasm having a complicated labyrinth structure, formed dendritic processes in all directions. PAP trapping was especially remarkable on the labyrinth structure of the dendritic reticulum cells (Fig. 6). In the cap area, it was possible to detect PAP trapping on every type of reticulum cells. Outside the cap area, however, PAP trapping was absent or slight. At this stage, many ordinary reticulum cells were still present throughout the secondary

follicles as well as in the germinal centers.

The cytoplasmic processes of dendritic reticulum cells were frequently connected with those of neighboring dendritic reticulum cells by desmosomal junctions, these junctions being identified not only between the reticulum cells, but also between the reticulum cells and lymphocytes as well as between the lymphocytes themselves (Figs. 7, 8). The desmosomal junctions were shown as patch aggregates on the cytoplasmic membrane by means of freeze fracture technique (Fig. 9).

The dark zone consisted mainly of medium-sized or large lymphocytes. Macrophages were also scattered throughout the dark zone. In this area, PAP trapping was not remarkable. No PAP trapping could be observed on the cytoplasmic membranes of lymphocytes and macrophages.

Discussion

It has been known that DRC form the microenvironment of secondary follicles and exhibit the immune complex trapping ability. Antigen capture in lymphoid follicles was noted as early as 1950 by Kaplan et al. Mollors et al. (1962) described that injected complexes were trapped in the follicles. Nossal et al. (1971) minutely investigated the role of antigen in immunity and extensively reviewed studies in the early stage. At this time, Hanna et al. (1971) also reported the antigen retention in the spleen of mice. They agreed that the antigens were localized on the processes

Origin and function of DRC

of follicular dendritic cells and that antigen retention required the specific antibody. Klaus et al. (1980) and Mandel et al. (1980) reviewed the functional aspect of antigens on the follicular dendritic cells. In their observations, HRP was one of the excellent antigens used. In the present study, commercial PAP (Dakopatts) was used enabling many efficient photographs explaining follicular trapping to be taken.

Recent interest in ontogenical study of lymph nodes is focused on the origin and the functions of non-lymphoid cells. Concerning DRC, Villena et al. (1983) divided the postnatal development of rat popliteal lymph nodes into four stages and ascribed the origin of DRC to fibroblastic reticulum cells. Our present study supports their findings. The popliteal lymph nodes of newborn rat were immature in development and the basic framework of the cortex consisted of reticular cells. These reticular cells are called mesenchymal cells (Eikelenboom, 1978), primitive reticular cells (Groscurth, 1980) or fibroblastic reticulum cells (Villena et al., 1983). Terminology of antigen trapping cells in the lymphoid follicles is also confusing, being called antigen retaining cells, follicular dendritic cells, dendritic reticulum cells etc. Kojima (1976) named the peculiar cells with labyrinth structures and desmosomes in secondary follicles, "desmo-dendritic cells". In the present study, we have shown that the fibroblastic cells, constituting the framework of lymph nodes, were transformed into the dendritic reticulum cells in the germinal centers. Moreover, the ordinary reticulum cells in primary follicles had already gained the ability of immune complex trapping. However, a remarkable difference was observed between the ordinary reticulum cells and the dendritic reticulum cells. Imai et al. (1983) reported that the ordinary reticulum cells in primary follicles had no complicated labyrinth structure, but the dendritic reticulum cells in secondary follicles had abundant distinct labyrinth structures.

Recently, we investigated the postnatal development of white pulp in rat spleen and obtained findings similar to those of lymph nodes. Briefly, the accumulation of lymphocytes in the peripheral portion of white pulp induced the formation of nodular structure, about three weeks after birth. At the same time, PAP trapping appeared on the cytoplasmic membranes of reticulum cells in the nodules (unpublished data). Bélisle et al. (1981) studied the tridimensional structures of the deep cortex of rat lymph nodes and reported that the deep cortex consisted of deep cortex units (DCU). The development of DCU is related to the distribution of afferent lymphatic vessels. The clear polarization of well-developed secondary follicles and immune complex trapping functions in the cap area is quite helpful in immune response, since many antigens invade from the afferent lymphatic vessels.

It is not clear whether the appearance of the ability of immune complex trapping in lymph follicles originates from the differentiation of reticulum cells themselves or from the surrounding lymphocytes.

We often observed desmosomal junctions on the processes of reticulum cells, these junctions connecting the reticulum cells with neighboring reticulum cells and lymphocytes. These findings show the close relationship between the reticulum cells and lymphocytes; it being suggested that a transfer of information is carried through the desmosomal junctions.

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