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The distribution, immune complex trapping ability and morphology of follicular dendritic cells in popliteal lymph nodes of aged rats

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Summary. Immune system function declines with age, and lymph nodes involute. The aims of the study were to describe the distribution of follicular dendritic cells (FDCs) in the lymphoid follicles of aged rats, and to determine whether these cells have reduced ability to trap immune complexes (ICs). Popliteal lymph nodes of rats aged 24-28 months were immunostained for S-100 protein as a marker of FDCs. Some rats were pretreated with peroxidase-anti-peroxidase complex (PAP) as an IC. FDCs were densely distributed in lymphoid follicles, which contained many primary follicles and a few secondary follicles. FDCs in primary follicles stained weakly for S-100 protein, and showed weak trapping, while those in secondary follicles stained strongly for both S-100 protein and trapping. Ultrastructurally, in involuted lymphoid follicles FDCs were atrophic. We conclude that FDCs in aged rats are densely distributed in involuted follicles and show reduced trapping ability and atrophic morphology. This seems to reflect the long life of FDCs and the reduced numbers of lymphoid cells in these follicles. We suggest that FDCs in aged rats may show some of their normal functions if fully developed germinal centers are induced, and may not play an important role in the process of involution of the follicles.

Key words: Follicular dendritic cells, Aging, Nerve tissue protein S100, Antigen-antibody complex

Introduction

The function of the immune system peaks approximately at puberty, and thereafter gradually declines (for review, see Hirokawa et al., 1992). In old age, lymph nodes are usually atrophic, and show indistinct compartmentalization into the medulla, the paracortex, and the peripheral cortex, in which lymphoid follicles involute to contain few or no germinal centers (GCs) (Luscieti et al., 1980; Pahlavani et al., 1987). Follicular dendritic cells (FDCs) are distributed within lymphoid follicles; these cells trap antigens in the form of immune complexes (ICs), retain them on their surfaces for long periods, and interact with B cells, leading to somatic hypermutation, affinity maturation, and production of memory B cells (for review, see Heinen et al., 1995). Several studies on age-related changes in murine FDCs have been reported: in contrast to FDCs in young mice, those in aged mice have reduced trapping ability, and a smaller and looser network staining pattern reflecting the IC-trapping regions of lymphoid follicles (Legge and Austin, 1968; Hanna et al., 1971; Holmes et al., 1984; Szakal et al., 1988, 1990); moreover, they show atrophic morphology with flattened cell bodies, shrunken nuclei, and poorly developed cytoplasmic dendritic processes (Szakal et al., 1988). However, little is known about the distribution of FDCs in lymphoid follicles in old age, for example whether FDCs are sparsely distributed in lymphoid follicles, particularly when showing reduced IC-trapping. Furthermore, to our knowledge, no information is available concerning the trapping ability and morphology of FDCs in aged rats.

The aims of the present study were: 1) to describe the distribution of FDCs in aged rats; 2) to determine whether these cells show reduced trapping ability; 3) if so, to determine whether they are more sparsely distributed in the lymphoid follicles showing markedly reduced IC-trapping compared with those not showing it; and 4) to determine whether they show atrophic morphology.

Many immunohistochemical markers for human, rat, and murine FDCs have been reported. Most of these markers recognize the cytoplasmic membranes of FDCs, and immunohistochemical studies reveal a dense meshwork staining pattern, formed predominantly by the staining of cytoplasmic dendritic processes of FDCs within lymphoid follicles. Mature FDCs have intricate

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and well developed dendritic processes (Szakal et al., 1983), and it is difficult to distinguish individual FDCs from the meshwork staining using these markers. Furthermore, these markers, which include monoclonal antibodies such as OX2, ED5 and Ki-M4R, are not necessarily specific, particularly for rat and murine FDCs, as they cross-react with some other cell types such as sinus lining cells and B cells (for review, see Maeda et al., 1995). However, S-100 protein (for review, see Kligman and Hilt, 1988) recognizes the nuclei and cytoplasm of rat FDCs in all regions of lymphoid follicles. Although S-100 protein recognizes some other types of cells in rat tissues such as fat cells and nerve fibers, within lymphoid follicles it is specific only for FDCs (Cocchia et al., 1983; Sato and Dobashi, 1995; Schwaeble et al., 1995). Thus, S-100 protein is useful in identifying individual FDCs. We have found that FDC immunostaining for S-100 protein increases during the development of lymphoid follicles, indicating that S-100 protein expression by FDCs reflects the development of these cells (Sato and Dobashi, 1995). In the present study, we therefore used S-100 protein immunoreactivity to examine the distribution of rat FDCs. We have also detected ICs trapped by FDCs on paraffin sections, using a previously described indirect immunoperoxidase method (Sato et al., 1996).

To address the above four issues, we immunostained the popliteal lymph nodes of 24- to 28-month old rats for S-100 protein as a marker of FDCs. In addition, some rats were injected with peroxidase-anti-peroxidase complex (PAP) as an IC, and lymph nodes were then immunostained for S-100 protein or for trapped PAP on paraffin sections: the localization of these two markers in the same region was estimated using the mirror- or serial-sectioning technique. Finally, the cells were studied ultrastructurally with the two markers. Here the term "FDC" is defined in a broad sense as all dendritic-shaped non-lymphoid cells in lymphoid follicles (Stein et al., 1982; Rademakers, 1992; Sato and Dobashi, 1995).

Materials and methods

1. Animals and tissue sampling procedures

Elderly male and female Wistar rats (Charles River Japan Inc., Atsugi, Japan) aged 24-28 months were kept under specific pathogen-free conditions. 10-14-week-old rats were used as young controls. Details of the rats used are presented (Table 1). Some rats received hind footpad injections of 0.2 ml of sheep red blood cells (SRBCs) or lipopolysaccharide (LPS), diluted with saline: SRBCs were injected at a concentration of $1x10^9$ cells/ml, and LPS (*E.Coli* lipopolysaccharide B, lot 0111, B4; List Biological Lab, Inc., Campbell, CA) at a dose of 100 μg. After anesthesia with an overdose of diethyl ether, the stimulated and unstimulated rats were killed and their popliteal lymph nodes were harvested. For detection of the preadministered PAP, some rats (shown in Table 1) received 0.2 ml of rabbit soluble PAP (Dakopatts, Glostrup, Denmark) diluted 1:5 with saline in the rear footpads, 24 h before removal of the lymph nodes.

For light microscopy, the specimens were fixed in B5 fixative (a mixture solution of formaldehyde solution and a solution that includes mercuric chloride; Jacobsen and Jacobsen, 1984) at room temperature for 3 h, and then embedded in paraffin wax. At least 50 sections, containing step, mirror, and serial sections, were prepared per lymph node. These specimens were also processed for hematoxylin-eosin (HE) and silver staining. For electron microscopy, the specimens were fixed at 4 °C for 4 h in periodate-lysine-paraformaldehyde (PLP) containing 4% paraformaldehyde, and then cut into 40 μm sections with a Vibratome.

2. Immunohistochemistry for S-100 protein

For light microscopy, the specimens were immunostained using the avidin-biotin complex (ABC) method. Endogenous peroxidase activity was blocked with 5 mM periodic acid at 4 °C for 15 min. The sections were then sequentially incubated with 2% skimmed milk (Yukijirushi, Sapporo, Japan) in phosphate-buffered saline for 15 min instead of using blocking serum as described by others (Duhamel and Johnson, 1985), rabbit anti-ox S-100 protein antibody (1/5000; prepared according to the method of Zuckerman et al., 1970) for 1 h, biotinylated goat anti-rabbit IgG antibody (1/200; Vector Labs., Burlingame, CA) for 30 min, and ABC Elite reagents (Vector Labs.) for 30 min at room temperature. As a chromogen, 0.03% 3,3'-diaminobenzidine (DAB) was applied. The sections were counterstained with methyl green. As a negative control, the primary antibody was omitted, or normal rabbit serum was used instead of the primary antibody.

For electron microscopy, sections were immunostained using the PAP method described by Cocchia et al. (1983). The endogenous peroxidase activity was

Table 1. The numbers and ages of rats used in the present study.

AGE/SEX	NUMBER OF RATS			
	Unstimulated	SRBCs (x1)	SRBCs (x2)	LPS
10 weeks				
male	5			14(3)
female	10			11(10)
11 weeks				
female	5	5		
14 weeks				
female	6		11	
24 to 28 months				
male	2(2)		1(1)	4(2)
female	2(1)	5(2)	5(1)	5(2)

The numbers of rats injected with PAP are given in parentheses. SRBCs (x1): rats stimulated with SRBCs were sacrificed between days 10 and 25; SRBCs (x2): rats stimulated with SRBCs were stimulated again on day 25, and then sacrificed on day 29; LPS: rats stimulated with LPS were sacrificed on day 10.

inhibited by incubation of the sections with 0.1% phenylhydrazine hydrochloride in 0.1M phosphate buffer at 37 °C for 1 h. After incubation in 5% swine serum for 15 min, the sections were sequentially incubated overnight at 4 °C with rabbit anti-ox S-100 protein antibody (1/1000), swine immunoglobulins anti-rabbit immunoglobulins (1/100; Dakopatts), and rabbit PAP reagent (1/100; Dakopatts). The labeled peroxidase was visualized with DAB in 0.05M Tris-HCl (pH 7.6) containing H_2O_2. After postfixation with 0.5% glutaraldehyde for 15 min and 1% OsO_4 for 1 h at 4 °C, sections were dehydrated and embedded in Epon columns. Ultrathin sections were examined with an electron microscope (HS-9; Hitachi, Tokyo, Japan). As a negative control, the primary antibody was omitted.

3. Detection of preadministered PAP

The rats given rabbit PAP were examined as shown in Table 1. Preadministered PAP was detected by light microscopy using a modification of the indirect immunoperoxidase method previously described (Sato et al., 1996). Endogenous peroxidase activity was blocked with 5mM periodic acid at 4 °C for 15 min. For proteolytic digestion, the sections were incubated with 0.4% pepsin (Sigma, St.Louis, MO) in 0.01 M HCl at 37 °C for 30 min. The sections were then sequentially incubated with 5% swine serum for 15 min and peroxidase-conjugated swine immunoglobulins anti-rabbit immunoglobulins (1/100; Dakopatts) for 1 h at room temperature. The subsequent procedures for visualization of labeled peroxidase were the same as described for the staining for S-100 protein. For specificity control, the peroxidase-conjugated anti-rabbit antibody was omitted, or PAP trapping was immunostained in the absence of proteolytic digestion with 0.4% pepsin

For electron microscopy, PAP was detected in sections using the DAB/H_2O_2 method, as described previously (Imai et al., 1986). The labeled peroxidase was visualized with DAB in Tris-HCl containing H_2O_2. The subsequent postfixation procedures were the same as described for the staining for S-100 protein.

4. Mirror- and serial-sectioning technique

To estimate the localization of S-100 protein-positive FDCs and PAP trapping in the same regions of lymphoid follicles, we immunostained the specimens of the rats given PAP using the mirror- or serial-sectioning technique, as described previously (Sato et al., 1996). Each of a pair of mirror or serial sections was stained for S-100 protein or for trapping using the methods described above. In addition, some sections were immunostained for S-100 protein after proteolytic digestion with 0.4% pepsin described for the detection of preadministered PAP.

Results

1. Light microscopy for HE and silver staining

In young rats, popliteal lymph nodes showed a clear demarcation between the peripheral cortex, paracortex and medulla. In rats stimulated with SRBCs or LPS, lymph nodes were larger, and had many well developed secondary follicles with few fibers seen in GCs on silver staining (Fig. 1a). Unstimulated rats had less well developed secondary follicles.

In contrast, aged rats had small popliteal lymph nodes with indistinct compartmentalization and decreased cellularity. These rats varied in the degree of development of lymphoid follicles, and well developed secondary follicles were absent or only few in number whether stimulated or not. Since most of the lymphoid follicles showed involution, and contained many primary follicles, some involuted lymphoid follicles with a few lymphoblasts, and a few involuted secondary follicles with small GCs, it was difficult to distinguish these follicles from surrounding tissues (Fig. 2a). There were pronounced intrafollicular fibers on silver staining (Fig. 2b).

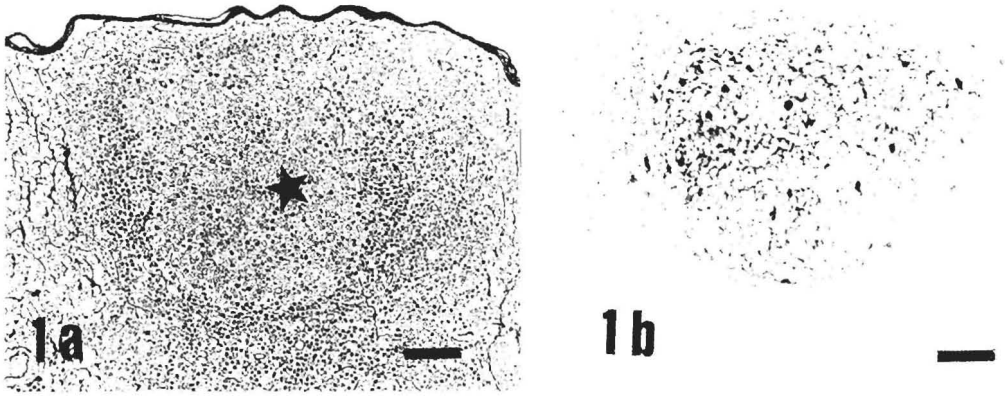


Fig. 1. Light micrographs of adjacent sections of a popliteal lymph node of a 14-week-old rat after secondary stimulation with SRBCs. **a.** Silver staining showing little impregnation by silver of the germinal center (asterisk) of a secondary follicle. **b.** Anti-S-100 protein labeling, counterstained with methyl green, ABC method. There is strong staining in a meshwork pattern in the follicle. x 145. Bar: 50 μm.

2. Immuno-light microscopy for S-100 protein

In young rats, as described previously (Sato and Dobashi, 1995), S-100 protein was strongly localized in the nuclei and cytoplasm of FDCs in lymphoid follicles (Figs. 1b, 3a), but only weakly in the antigen transporting cells (ATCs; Szakal et al., 1983) in the subsinus layer (not shown). FDCs were plump in the light zone and adjacent mantle zone, and spindle-shaped in other regions. The prominent cytoplasmic staining of FDC dendrites resulted in a meshwork staining pattern.

In aged rats, in contrast to the findings with HE (Fig. 2a) and silver (Fig. 2b) staining, it was easy to distinguish lymphoid follicles by S-100 protein staining, and S-100 protein-positive FDCs were densely distributed within the follicles. Within involuted primary follicles many spindle-shaped FDCs stained weakly for S-100 protein (Figs. 2c, 4a), while within secondary follicles spindle- or plump-shaped FDCs stained strongly. In secondary follicles, the meshwork staining pattern was seen, but it was often irregular and disrupted (Fig. 5a). ATCs also weakly stained for S-100 protein (not shown). Negative controls showed no reaction.

3. Immuno-light microscopy for trapped PAP

In young rats, there was a strong reaction for trapped PAP in lymphoid follicles, as described previously (Sato et al., 1996). The trapping regions were rounded in shape in primary follicles (not shown) and crescentic in the light zone and adjacent mantle zone of secondary follicles (Fig. 3b).

In contrast, in aged rats, the extent and intensity of trapping in lymphoid follicles varied. Most follicles showed a weak reaction. The trapping regions in involuted follicles tended to be small, and irregularly rounded, with a loose network, occasionally condensed or interrupted (Fig. 4b). In certain sections, a few follicles showed no trapping. However, the trapping regions in secondary follicles tended to show a strong reaction, and were often irregularly crescentic or half-rounded in the upper part of the follicles (Fig. 5b). Macrophages phagocytosing PAP were observed more frequently in sinuses and medullary regions than in young rats. Omission of peroxidase-conjugated anti-rabbit immunoglobulins antibody yielded no positive reaction. Moreover, staining for trapping without pepsin digestion showed little or no reaction.

4. Mirror- and serial-sectioning technique

S-100 protein-positive FDCs of young and aged rats were found both in regions trapping PAP and in those not trapping PAP (Figs. 3-5), as described previously (Sato et al., 1996). Moreover, these FDCs were densely distributed both in the lymphoid follicles showing

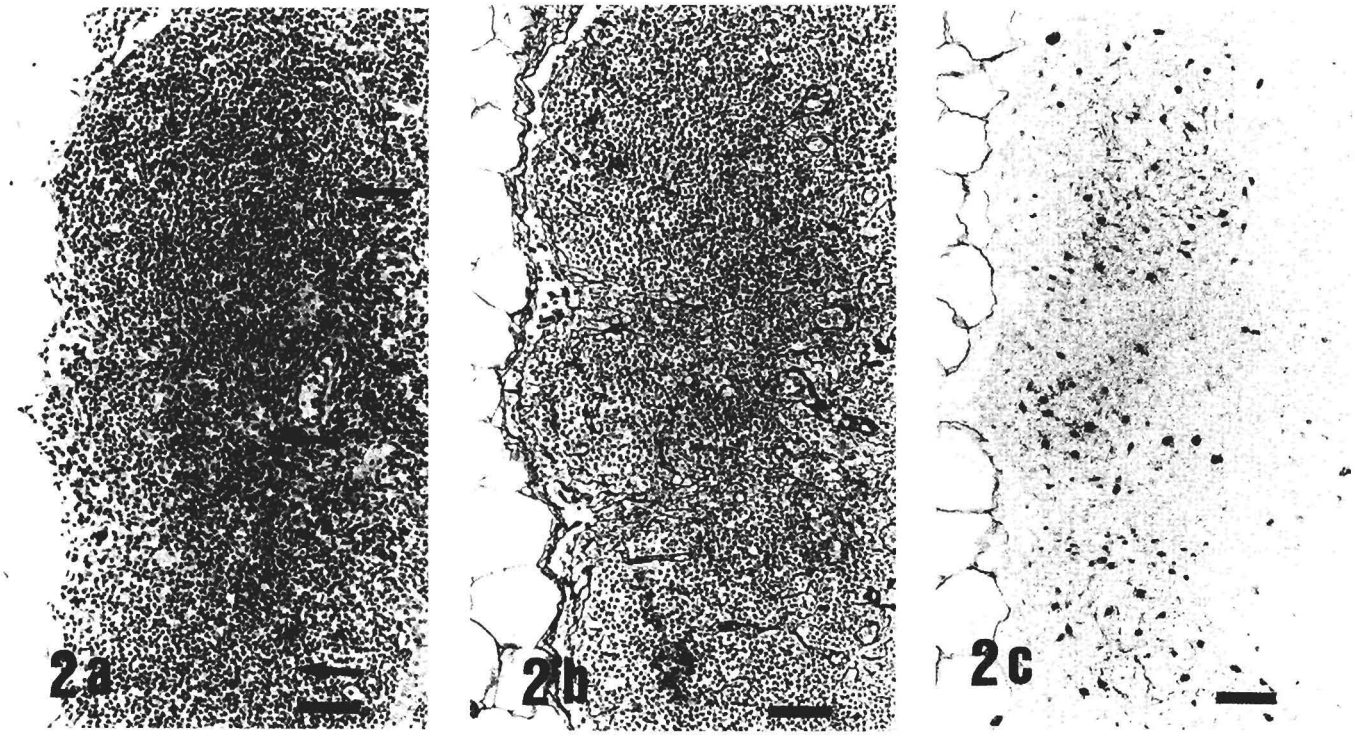


Fig. 2. Light micrographs of adjacent sections of a popliteal lymph node of a 25-month-old rat 25 days after SRBC stimulation. **a.** Hematoxylin-eosin staining, showing three primary follicles (arrows) with inconspicuous borders. **b.** Silver staining, showing the invasion of silver fibers of the follicles. **c.** Anti-S-100 protein labeling, counterstained with methyl green, ABC method. Many S-100 protein-positive spindle-shaped FDCs are distributed in the follicles, but their immunoreactions are weak. x 145. Bar: 50 μm.

markedly reduced trapping (Fig. 4) and in those not showing it (Fig. 5). On the other hand, FDC staining for S-100 protein after proteolytic digestion showed minimal or absent reaction for S-100 protein-specific staining, and a strong reaction for preadministered rabbit PAP detected by biotinylated anti-rabbit antibody and

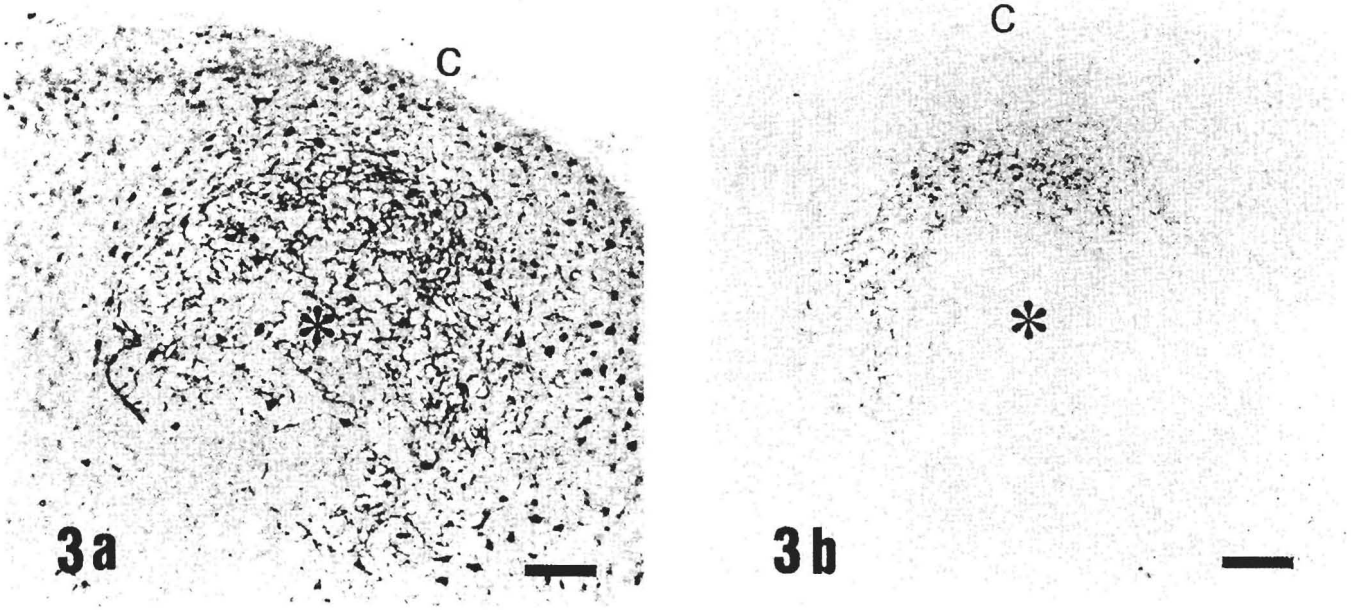


Fig. 3. Light micrographs of mirror sections of a popliteal lymph node of a 10-week-old rat after stimulation with LPS. **a.** Anti-S-100 protein labeling (ABC method). There is strong staining of a secondary follicle. **b.** Localization of preadministered PAP (indirect peroxidase method). There is strong staining of the trapping region in a crescentic shape in the light zone and adjacent mantle zone of the follicle. Asterisks indicate the germinal center of the secondary follicle.C: capsule. Counterstained with methyl green. x 175. Bar: 50 μm.

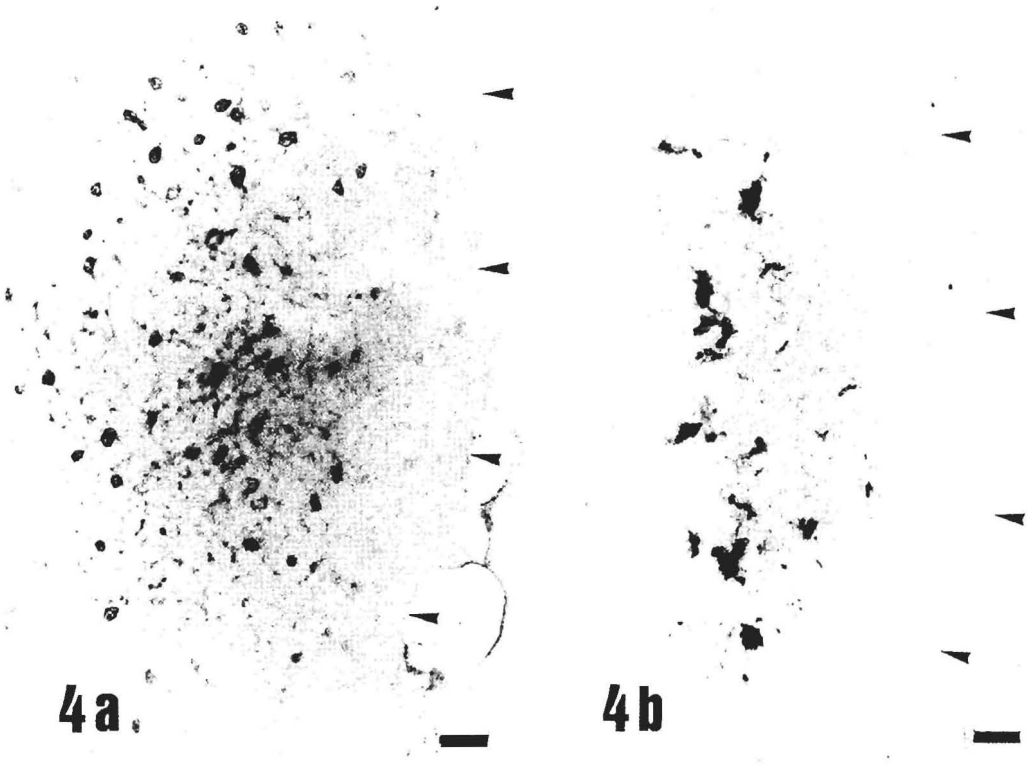


Fig. 4. Light micrographs of adjacent sections of a popliteal lymph node of a 25-month-old rat without stimulation. **a.** Anti-S-100 protein labeling (ABC method). Many weakly S-100 protein-immuno-reactive FDCs are distributed in a primary follicle. **b.** Localization of preadministered PAP (indirect peroxidase method). Note weak trapping of PAP in the follicle. The trapping region is small and disrupted. Arrowheads indicate the lymph node capsule. Counterstained with methyl green. x 230. Bar: 25 μm.

ABC reagents.

5. Immuno-electron microscopy for S-100 protein and for trapped PAP

In aged rats, only a few well developed FDCs were observed in developed GCs, whereas many FDCs showing atrophic morphology were observed in involuted follicles (Figs. 6-9). Although the degree of atrophy of individual FDCs varied, the following trends were observed: some FDCs showed poorly developed morphology, having spindle-shaped, dark heterochromatic nuclei and poorly developed dendritic processes in involuted follicles, particularly in the periphery of the follicles (Figs. 7, 9); others showed intermediate morphology between poorly- and well-developed FDCs, with plump, euchromatic or heterochromatic nuclei, and moderately developed dendritic processes, particularly in the center of lymphoid follicles and in GCs (Figs. 6, 8a). Moreover, these atrophic FDCs were often seen to adhere to bundles of reticulin fibers, and some appeared to have engulfed some bundles in their cytoplasm (Figs. 6-9), with a resulting similar appearance to fibroblastic reticulum cells (FRCs; Tykocinski et al., 1983). In addition, FDCs in aged rats often had small cytoplasmic lysosomal structures including a lamellar structure (Fig. 8b).

In young rats, S-100 protein-positive FDCs were distributed in lymphoid follicles, and well developed plump FDCs were frequently seen in the light zone. These cells had well developed dendritic processes, and were euchromatic (not shown), as described previously (Cocchia et al., 1983; Sato and Dobashi, 1996). On the other hand, in aged rats, S-100 protein recognized both relatively well developed (Fig. 6) and poorly developed FDCs (Fig.7).

Immunostaining for trapped PAP was seen on the cytoplasmic membranes of FDCs, as described previously (Imai et al., 1986). In young rats, there was a strong reaction for trapped PAP in the light zone and adjacent mantle zone in secondary follicles (not shown). In aged rats, some FDCs, particularly those that were relatively well developed, showed abundant PAP trapping, particularly in GCs (Fig. 8a), while others, particularly the poorly developed FDCs, showed absent or little trapping (Fig. 9). Even if trapping was relatively abundant, it was often detected only on a portion of the cytoplasmic membrane (Fig. 8a).

Discussion

The current study demonstrates the following in the

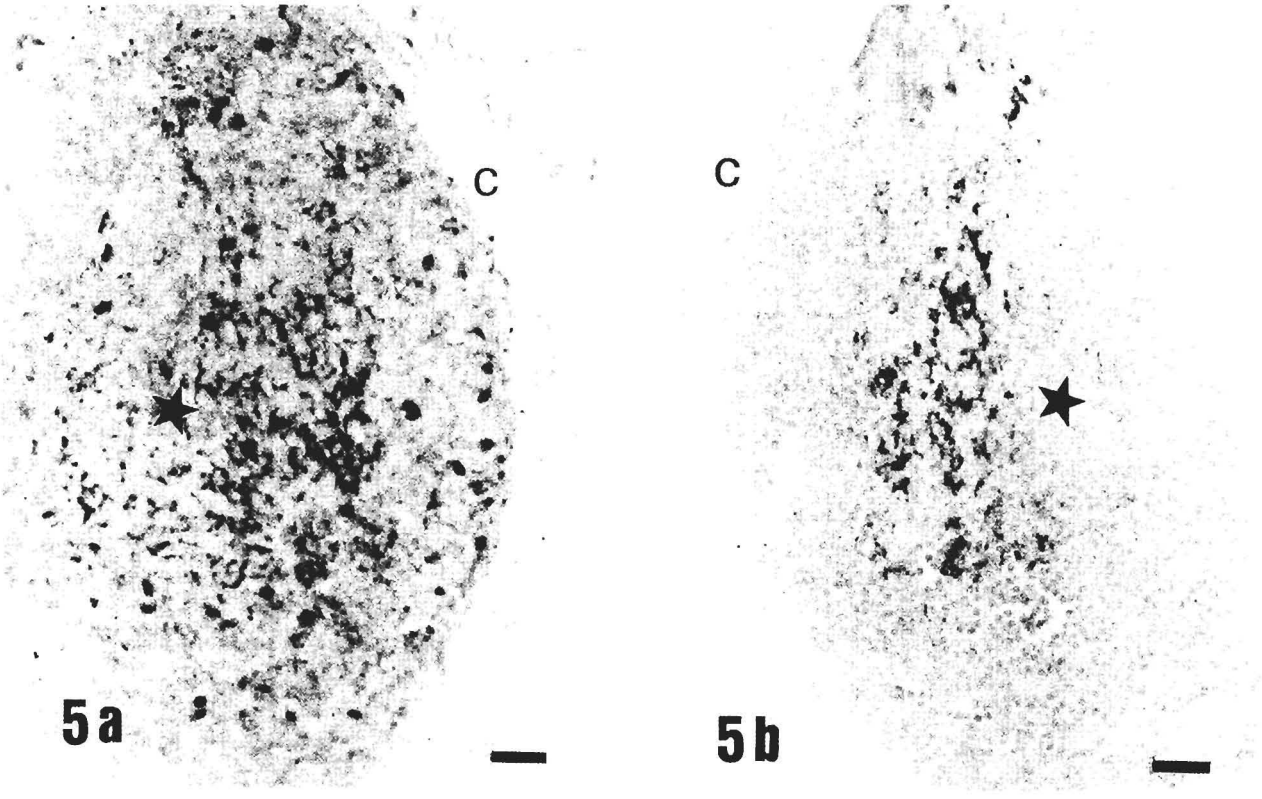
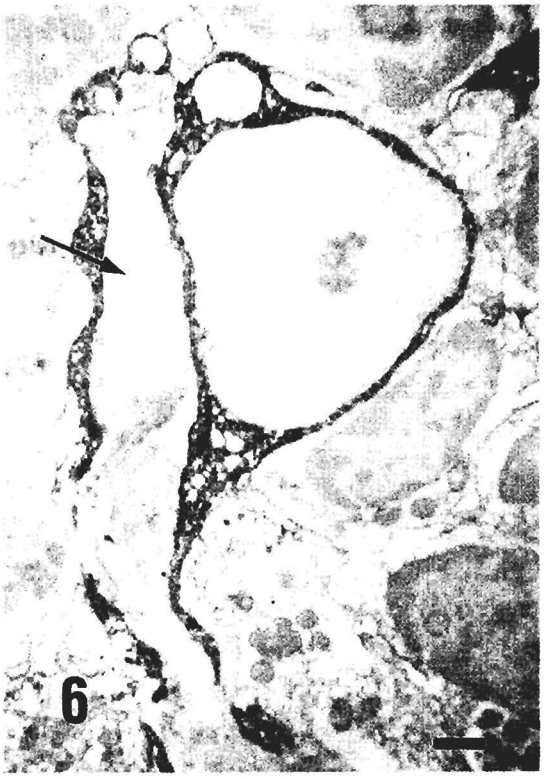


Fig. 5. Light micrographs of mirror sections of a popliteal lymph node of a 25-month-old rat after stimulation with LPS. **a.** Anti-S-100 protein labeling (ABC method). There is a strong irregular meshwork staining pattern in a secondary follicle. **b.** Localization of preadministered PAP (indirect peroxidase method). There is strong staining of the trapping region in a half-rounded shape in the upper part of the follicle. Asterisks indicate the germinal center of the follicle. C: capsule. Counterstained with methyl green. x 280. Bar: 25 μm.

lymph nodes of aged rats: 1) FDCs were densely distributed in lymphoid follicles; 2) FDCs showed reduced trapping ability; 3) FDCs were densely distributed both in lymphoid follicles showing markedly reduced trapping and in those not showing it; and 4) FDCs showed atrophic morphology in involuted lymphoid follicles. These results are helpful when considering the microenvironments in involuted follicles including FDCs and lymphoid cells.

Some investigators use the term "FDC" to refer to all dendritic-shaped non-lymphoid cells that are recognized by anti-FDC antibodies and/or those that have some morphological features of FDCs in lymphoid follicles (Stein et al., 1982; Rademakers, 1992; Sato and Dobashi,



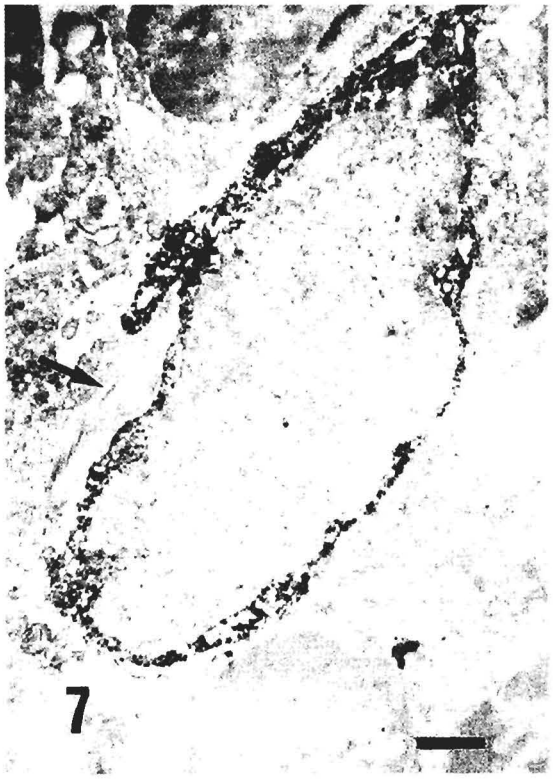


Fig. 6. Electron micrograph illustrating anti-S-100 protein labeling of a primary follicle in a popliteal lymph node of a 25-month-old rat without stimulation. An FDC with plump-shaped, euchromatic nucleus and nucleolus shows a positive cytoplasmic reaction, engulfing reticulin fibers (arrow). PAP method, not contrasted. x 6,500. Bar: 1 μm.

Fig. 7. Electron micrograph illustrating anti-S-100 protein labeling of a primary follicle in a popliteal lymph node of a 25-month-old rat without stimulation. A poorly developed FDC with spindle-shaped, dispersed chromatin and few dendritic processes shows a positive reaction in the cytoplasm, adhering to reticulin fibers (arrow). PAP method, not contrasted. x 8,500. Bar: 1 μm.

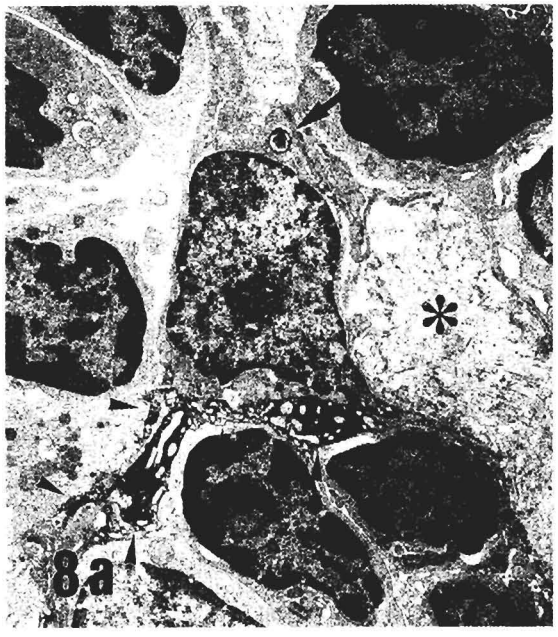


Fig. 8. Electron micrographs showing localization of preadministered PAP in a germinal center of a secondary follicle in a popliteal lymph node of a 26-month-old rat after secondary stimulation with SRBCs. Contrasted with uranyl acetate and lead citrate. **a.** Abundant PAP trapping (arrowheads) is seen only on a portion of the cytoplasmic membrane of an FDC with plump-shaped, heterochromatin and moderately developed dendritic processes. The FDC adheres to reticulin fibers (asterisk) and has a lamellar structure (arrow). x 5,500. Bar: 1 μm. **b.** A high-power view of the lamellar structure shown in Fig. 8a. N: nucleus. x 58,000. Bar: 0.1 μm.

1995). However, others use "FDC" to describe only those cells with trapping ability and/or typical morphology within the follicles, particularly in the light zone (Heusermann et al., 1980; Imai et al., 1986). Using the latter definition, dendritic-shaped cells in the mantle zone, including primary follicles, or those in the mantle and dark zones, are usually called reticulum cells or FRCs (Tykocinski et al., 1983; Gloghini et al., 1990). If this definition is used, the findings in the present study would be affected in the following ways: 1) in involuted follicles, FRCs and "the transitional forms" between FRCs and FDCs, described by Heusermann et al. (1980), which seem to correspond to "FDCs with intermediate morphology" in the present study (Figs. 6, 8a), were densely distributed, but FDCs were absent or sparsely distributed; 2) in primary follicles, FRCs and the transitional forms expressed weak reactions for S-100 protein and showed weak trapping; and 3) in secondary follicles, FDCs expressed strong reactions for both S-100 protein and trapping, but FRCs and the transitional forms expressed weak reactions for S-100 protein and showed weak trapping. However, the finding that dendritic-shaped non-lymphoid cells were densely distributed in these follicles would remain unchanged.

In the present study, S-100 protein-positive FDCs were densely distributed in lymphoid follicles of aged rats, even if the follicles were poorly developed (Figs. 2c, 4a). However, it seems unlikely that FDCs increased in number in these follicles, for the following reasons. First, most of these follicles showed involution and were of small size, suggesting a decline in immune function in aged rats. Second, it has been suggested that FDCs have a lack of or only very weak proliferative activity (Sato and Dobashi, 1995, 1996). On the other hand, it is well known that the number of lymphoid cells in lymphoid follicles decreases at the end of the GC reaction (Stein et al., 1982; Heinen et al., 1995). Moreover, it is known that FDCs are long-lived (Tsunoda et al., 1990; Heinen et al., 1995). Thus, the findings in the present study can be explained on the basis of the following hypothesis: within lymphoid follicles, lymphoid cells markedly decrease in number during involution of the follicles, but FDCs do not decrease significantly, so that FDCs appear to be densely concentrated. The findings in the present study therefore seem to reflect the long life of FDCs and their tendency to remain in the same region. However, whether individual FDCs appearing early on in the first formation of lymphoid follicles live until involution of the follicles remains to be investigated. In addition, what happens to the total number of FDCs remains unknown (Sato and Dobashi, 1996). Furthermore, whether FDCs in other aged species are densely distributed is also unknown. However, similar findings probably occur in aged mice, because FDCs in aged rats in the present study and those in aged mice (Szakal et al., 1988) had similar trapping ability and morphology.

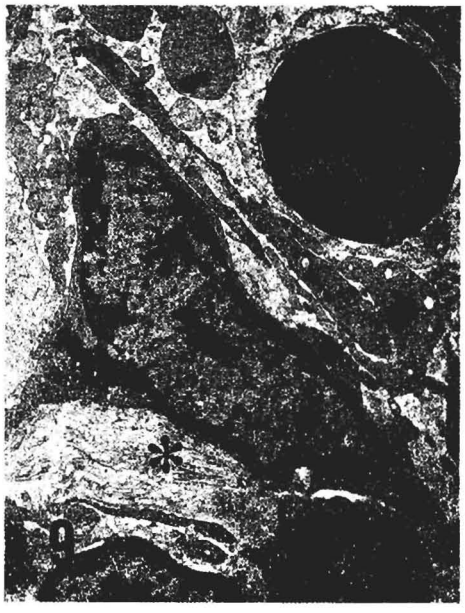


Fig. 9. Electron micrograph illustrating the localization of preadministered PAP in the mantle zone of the same secondary follicle shown in Fig. 8. No PAP trapping is detected in a poorly developed FDC with spindle-shaped, heterochromatin and a few dendritic processes. The FDC adheres to reticulin fibers (asterisk). Contrasted with uranyl acetate and lead citrate. x 6,600. Bar: 1 μm.

In the present study, FDCs in aged rats showed reduced trapping ability (Figs. 4b, 9), in accordance with previous data for aged mice (Legge and Austin, 1968; Hanna et al., 1971; Holmes et al., 1984; Szakal et al., 1988, 1990). On the other hand, in the present study a few involuted follicles showed no trapping in certain sections. However, there remains the possibility that sections included the periphery of these follicles and therefore did not show trapping, while the center of the follicles was missed in these sections but showed trapping in other sections, as described previously (Sato et al., 1996).

In the present study, FDCs were densely distributed both in the lymphoid follicles showing markedly reduced trapping (Fig. 4) and in those not showing it (Fig. 5). In young rats, primary follicles include many FDCs (Sato and Dobashi, 1995), and show a strong reaction for trapped PAP (Sato et al., 1996). In contrast, in aged rats, lymphoid follicles included many FDCs, but there tended to be a weak reaction for trapped PAP. Since FDCs in aged rats were densely distributed regardless of the intensity of trapping (Figs. 4, 5), it seems unlikely that the reduced trapping ability of lymphoid follicles is due to significant depletion of FDCs. This reduced trapping ability may be due to an age-related defect in antigen transportation and atrophy of FDCs, described by Szakal et al. (1988, 1990).

Ultrastructurally, in the present study, most FDCs in involuted follicles showed atrophic morphology, often with poorly developed dendritic processes, heterochromatic nuclei (Figs. 7, 9), and absent or weak trapping ability (Fig. 9), in accordance with previous findings in aged mice (Szakal et al., 1988). As reported in human lymphoid tissues (Rademakers et al., 1992), the atrophic morphology (Figs. 7, 9) and lysosomal structures in FDCs (Fig. 8b) seem to be signs of regression. Furthermore, FDCs often adhered to bundles of reticulin fibers, and engulfed some bundles in their cytoplasm (Figs. 6-9), thereby showing some

morphological features of FRCs. These features have not been described in any previous study (Szakal et al., 1988), but this discrepancy may have been due to the differences in the degree of fibrous changes in the lymph nodes. On the other hand, atrophic FDCs showed a lack of or slight trapping ability (Fig. 9), in accordance with the previous finding of FRCs in lymphoid follicles of young rats (Imai et al., 1986). From the findings of regression of FDCs and their similar morphology to FRCs, we can speculate the following: in aged rats, FDCs that had differentiated from FRCs may have de-differentiated to FRCs again. This speculative view is supported by the hypothesis, proposed by Heinen et al. (1995), that fully developed FDCs, in shedding their surface membranes, may regenerate or recover the characteristics of precursor cells. However, the origin of FDCs still remains unclear: they are considered to be derived from mesenchymal cells such as FRCs (Heusermann et al., 1980; Heinen et al., 1995) or bone marrow cells (Szakal et al., 1995). Further investigations are required.

It has been reported that immune complex-coated bodies (iccosomes) can be observed ultrastructurally in young mice, while they cannot be seen in aged ones (Szakal et al., 1988). However, in the present study iccosomes were not found in either young or aged rats. This discrepancy may be due to differences in experimental design, such as the species used or the method of stimulation employed, as discussed previously (Sato and Dobashi, 1996).

In the present study, within primary follicles, FDCs in aged rats expressed weak reactions for S-100 protein and weak trapping ability (Fig. 4), whereas within secondary follicles they expressed strong reactions for both S-100 protein and trapping (Fig. 5). These different findings in primary and secondary follicles suggest that the degree of development of lymphoid follicles is closely related to S-100 protein expression by FDCs and their function (at least their trapping ability). Moreover, the present ultrastructural study demonstrated that relatively developed FDCs showed relatively abundant PAP trapping (Fig. 8a), while poorly developed FDCs showed an absence of or little trapping (Fig. 9), suggesting that the morphology of FDCs is related to their trapping ability. In addition, involuted follicles had FDCs with atrophic morphology (Figs. 6-9). Thus, the degree of development of lymphoid follicles may be related not only to trapping ability but also to the morphology of FDCs.

In this connection, it is well known that lymphoid cells are important for FDC development. For example, mature FDCs are absent in the lymph nodes of B cell-depleted mice (Cerny et al., 1988), and B-T cell collaboration is important for the differentiation of murine FDCs (Kapasi et al., 1993). In addition, a recent study suggests that the aging of helper T cells plays a pivotal role in changes in the antibody repertoire and decreased somatic hypermutation of mice (Yang et al., 1996). Thus, in the present study, a decrease in the number or function of lymphoid cells or both within lymphoid follicles may affect FDCs, resulting in their atrophy. On the other hand, it is widely accepted that FDCs are capable of influencing lymphoid cells in various ways such as the proliferation and survival of B cells (for review, see Heinen et al., 1995). Thus, there remains a possibility that atrophy of FDCs may have an affect on lymphoid cells, and causing involution of lymphoid follicles.

Not all FDCs in aged rats showed poorly developed atrophic morphology, because some FDCs showed the intermediate morphology between FRCs and mature FDCs (Figs. 6, 8a). Furthermore, within secondary follicles FDCs in aged rats tended to show strong reactions for both S-100 protein and trapping (Fig. 5). The effect of stimulation in the present study, as in previous studies (Szakal et al., 1988, 1990), was unclear, resulting in the development of only a few secondary follicles. However, it has been reported that sonication of FDCs from aged mice restores the antibody response (Burton et al., 1991), suggesting that aged animals may under certain circumstances be able to mount a normal immune response. Moreover, it has been reported that treatment with dehydroepiandrosterone (DHEA), which affects the immune system through a DHEA-binding receptor complex on T cells, enhances the GC response in aged mice (Caffrey et al., 1994), suggesting that well developed GCs may be induced in aged animals under certain circumstance. These reports and the finding of the present study (Fig. 5) lead to the hypothesis that if fully developed GCs are induced, FDCs in aged rats may show normal function, at least normal IC trapping and S-100 protein expression, and might show other normal characteristics including normal expression of other FDC markers. This hypothesis indicates that even if atrophy of FDCs may have affected lymphoid cells, FDCs in aged rats may not have a decisive affect on GC reaction, and may not play an important role in the progression and maintenance of the involution of the follicles. Thus, at least in aged rats, it seems likely that FDCs are passively affected by other components such as lymphoid cells rather than FDCs actively affect the others, although there is the possibility that FDCs may have a weak influence.

In conclusion, we have shown that FDCs in aged rats are densely distributed in involuted follicles though they show reduced trapping ability and atrophic morphology, and that these cells demonstrate strong trapping ability in secondary follicles.

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