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The density and distribution of follicular dendritic cells in rat popliteal lymph nodes: a decrease in their density and a change in their distribution after stimulation

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Summary. To determine whether follicular dendritic cells (FDCs) increase after stimulation, and also to confirm the widely accepted finding that the density of FDCs in the light zone is higher than that in the dark zone, we examined the density of FDCs in lymph follicles of rat popliteal lymph nodes. Rats aged five weeks were stimulated by injection of sheep erythrocytes, and then examined 10 days later. Unstimulated rats were also examined. After embedding in paraffin, the removed lymph nodes were immunostained with anti-S-100 protein antibody as a marker of rat FDCs. The density of FDCs was determined by measuring the area of the lymph follicle and counting the number of S-100 protein-positive cells within it. The density of FDCs in the lymph follicles of stimulated rats was found to be significantly lower than in 5-week-old and unstimulated rats. The density of FDCs in the light zone was similar to that in the dark zone when germinal center bordering cells (GCBCs), distributed at the border between the dark zone and the adjacent corona, were counted as FDCs. We conclude that the density of FDCs in lymph follicles decreases after stimulation, and suggest that FDCs have no or only very slight proliferative activity under normal conditions. Investigators may need to consider GCBCs in order to understand how FDCs differentiate.

Key words: Follicular dendritic cells, Rat lymph node, S-100 protein, Proliferative activity

Introduction

Follicular dendritic cells (FDCs) are non-lymphoid cells found only within lymph follicles. Their typical morphological features are euchromatic nuclei and scanty cytoplasm with intricate dendritic cytoplasmic processes (Heusermann et al., 1980; Imai et al., 1986; Rademakers, 1992). Some investigators have used the term «FDCs» to mean only cells within lymph follicles having the ability to trap immune complexes and/or showing the typical morphological features, particularly those in the light zone (Heusermann et al., 1980; Imai et al., 1986). Others have used it to mean all dendritic-shaped non-lymphoid cells existing within lymph follicles, regardless of trapping ability. If the term «FDCs» is used in the latter sense, it is widely accepted that many such cells are distributed in the light zone, whereas only a few exist in the dark zone (Stein et al., 1982; Rademakers, 1992).

Although many studies have been done to determine the morphological and functional features of FDCs, few investigations have focused on their proliferative activity. Whereas there is a notion that the number of FDCs does not increase, there is a study that FDCs increase after stimulation (Heinen et al., 1985). Thus, examination of changes in the number of FDCs under various conditions is a useful approach for assessing the proliferative activity of FDCs.

There have been a number of studies on the numbers of FDCs. In human tissues, these studies have included reactive lymph nodes, follicular lymphomas (Peters et al., 1987) and lymph nodes infected by human immunodeficiency virus type-1 (Rademakers et al., 1992). On the other hand, there have been few animal studies on FDC numbers. Whereas one study of murine lymph nodes has indicated that the number of FDCs in germinal centers (GCs) increases upon stimulation (Heinen et al., 1985), the number of FDCs in rat lymph nodes remains to be investigated.

We believe that these previous studies have some difficulties in identifying FDCs by their morphological features in ultrathin sections or semithin Epon sections. One reason for the difficulty is that lymph follicles also contain a number of other cells that need to be distinguished from FDCs, including centroblasts, centrocytes, tingible body macrophages and endothelial cells (Peters et al., 1987). Furthermore, FDCs display diverse shapes (Rademakers, 1992). Other problems include the need for considerable labor in examining the

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specimens, and the wide range of specimen materials that need to be studied.

On the other hand, FDCs in rats express a strong immunoreaction for S-100 protein in their nuclei and cytoplasm both within GCs and lymphocytic coronas (Iwanaga et al., 1982; Cocchia et al., 1983), whereas in humans S-100 protein expression is restricted only to FDCs within GCs (Carbone et al., 1985). The expression of S-100 protein on the nuclei of FDCs in rats makes it possible to count the number of FDCs without the difficulty involved in identifying the cells morphologically.

The purposes of the present study were to determine whether the number of FDCs in lymph follicles increases upon stimulation, and to confirm the widely accepted notion that the density of FDCs in the light zone is higher than that in the dark zone (Stein et al., 1982; Rademakers, 1992). To address these issues, we first carried out immunohistochemical staining for S-100 protein in lymph follicles of rat popliteal lymph nodes after stimulation with sheep red blood cells (SRBCs), since little is known about changes in the distribution of S-100 protein-positive FDCs during GC development. We then determined the density of FDCs by measuring the areas of various regions in the lymph follicle and counting the number of FDCs stained for S-100 protein in these regions.

Materials and methods

Animals, experimental design and tissue preparation

A group of male Wistar rats (Charles River Japan Inc., Atsugi, Japan) aged five weeks, kept under specific pathogen-free conditions, were given an injection of 0.2 ml SRBCs at a concentration of $1x10^9$ cells/ml, in both hind footpads. The popliteal lymph nodes of the rats were removed on days 3, 5 and 10 after stimulation. Two groups without SRBC stimulation were also used: five-week-old rats (day 0); unstimulated rats at day 10. Groups examined at day 3 and day 5 contained three rats each; three other groups, on which quantitative studies were performed, contained five rats each. The lymph node specimens were fixed in Zamboni's solution at 4 ºC overnight, and then embedded in paraffin. More than 60 sections per group were prepared.

Immunohistochemistry

The sections were stained by the peroxidase-anti-peroxidase (PAP) method as follows. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol at 4 ºC for 30 min. The specimens were sequentially incubated with 5% swine serum for 20 min, rabbit anti-ox S-100 protein antibody (1/500; obtained according to Zuckerman et al., 1970) overnight at 4 ºC, swine anti-rabbit immunoglobulin antibody (1/100; DAKOPATTS, Glostrup, Denmark) for 1 h, and rabbit PAP reagent (1/100; DAKOPATTS) for 1 h at room temperature. The sections were incubated with 0.03% 3,3'-diaminobenzidine (Dojin Chemicals, Tokyo, Japan) in 0.05M Tris-HCl buffer (pH 7.6) containing 0.006% H_2O_2 and then counterstained with methylgreen. As a negative control, the primary antibody was omitted, or normal rabbit serum was used instead of the primary antibody.

Quantitative analysis

The density of FDCs was determined as follows. First, to examine the lymph follicles showing polarity of the light and dark zones, we selected transverse immunostained sections in which the peripheral cortex, paracortex and medulla coexisted in the same section. Then, 100 lymph follicles each in sections of the following three groups were photographed randomly at a final magnification of x360: the five-week-old group (day 0); the stimulated group (day 10 after stimulation with SRBCs); the unstimulated group (day 10 without SRBC stimulation).

Next, since it is difficult to identify polarity of the light and dark zones on immunostained sections, we examined substituted regions considered to represent these zones as follows. A straight line on a GC on a photograph was drawn at the center of the diameter of the GC on a vertical line from a straight line along the subcapsular sinus, thereby dividing the GC into two parts: 1) the upper half of the GC (subcapsular side), which was substituted for the light zone; 2) the lower half of the GC (basal side), which was done for the dark zone.

Third, to measure the areas of the lymph follicle, the GC and the upper half of the GC, we traced their contours on the photographs onto the digitizer of a computerized analysis system (DIANA98-III; Meiwa Shoji Co., Osaka, Japan). The measurements did not include the subsinus layer (Sainte-Marie and Peng, 1985). We obtained the area of the lower half of the GC by substracting the area of the upper half from the total area, and also the area of the lymphocytic corona by substracting the total area of the GC from the area of the lymph follicle. We use here the term «lymphocytic corona» to represent the region composed of primary follicles and coronas in secondary follicles. Consequently, the areas of the following regions were obtained: 1) lymph follicle; 2) lymphocytic corona; 3) GC; 4) upper half of the GC; 5) lower half of the GC. In each region, we recorded the number of S-100 protein-positive FDCs recognizable by the strong immunoreactions of their nuclei.

Finally, we calculated the density of FDCs in each region in the lymph follicle per 10^4 μm^2 by dividing the total number of S-100 protein-positive cells in the region by the area of the region. Statistical analysis of differences in the mean values was carried out using t-test.

Results

Immunohistochemistry for S-100 protein in rat popliteal lymph nodes

The immunostaining for S-100 protein showed an increase in the immunoreactivity of FDCs and a change in their distribution during development of GCs in the rat popliteal lymph nodes. S-100 protein-positive FDCs showed no mitotic figures in any of the specimens examined. Although rat tissues as well as human tissues expressed positive reactions for S-100 protein on nerve fibers (not shown) and fat cells, positive reactions outside lymph follicles were observed in other areas of rat lymph nodes such as the subsinus layer (Sainte-Marie and Peng, 1985).

In five-week-old rats (day 0), primary follicles were observed, but GCs were not conspicuous. Only a few lymph follicles showed accumulation of centroblasts in their central portion. A weak positive immunoreaction for S-100 protein was detected on spindle-shaped FDCs in lymph follicles. The reaction was recognized mainly in the nuclei, and partly in the cytoplasm of the FDCs (Fig. 1). The cytoplasmic staining was inconspicuous because of the scanty nature of the cytoplasm. On the other hand, outside the lymph follicles, spindle-shaped weakly immunoreactive cells were detected in the subsinus layer beneath the subcapsular sinus and were distributed not only in the region overlying the lymph follicles but also the region extending beyond them (Fig. 2). In addition, a slight reaction was detected in the paracortex. The positive cells showed mainly a spindle shape and seemed to be constitute a proportion of the fibroblastic reticulum cells (Fig. 3). These findings in the subsinus layer and paracortex were also observed in the other groups.

At day 3 after stimulation with SRBCs, accumulation of centroblasts was observed in the central portion of primary follicles. Many immature GCs were also recognized. FDCs showing a weak immunoreaction for S-100 protein were scattered in the primary follicles and the immature secondary follicles.

At day 5 after SRBC stimulation, large GCs were observed. FDCs showed a strong immunoreaction for S-100 protein in all portions of lymph follicles, particularly on the subcapsular side of GCs. In general, FDCs showed a comparatively plump shape on the subcapsular side of GCs, and a spindle shape in the corona and the

Fig. 1. Immunostaining of S-100 protein in a popliteal lymph node in a 5-week-old rat. Weak immunoreactions are scattered in two primary follicles (PF). SS: subcapsular sinus. Counterstained with methylgreen, PAP method. x 180

Fig. 2. Immunostaining of S-100 protein in a lymph node in a 5-week-old rat. Weak immunoreactions are distributed in a subsinus layer and a primary follicle (PF). Weak reactions are distributed in not only the region overlying the follicle (arrowheads) but also the region extending beyond the follicle (arrows). SS: subcapsular sinus. Counterstained with methylgreen, PAP method. x 300

Fig. 3. Immunostaining of S-100 protein in a lymph node in a 5-week-old rat. Slight positive reactions (arrowheads) in a paracortex. Counterstained with methylgreen, PAP method. x 300

basal side. In GCs, S-100 protein-positive FDCs were distributed not only in the subcapsular side of GCs but also the basal side (Fig. 4). In addition, a weak lace-like staining pattern was recognized on the subcapsular side of GCs in some secondary follicles, particularly in those that were well developed.

At day 10 after stimulation with SRBCs, well developed GCs with polarity of the light and dark zones appeared. In the light zone and adjacent corona, plump FDCs showed strong immunostaining for S-100 protein. In the above regions, the reaction product on the intricate cytoplasmic dendritic processes of FDCs displayed a lace-like pattern in the shape of a crescent. On the other hand, in the dark zone and corona, several spindle-shaped FDCs showed weak to moderate staining. In addition, at the border between the dark zone and adjacent corona, slender spindle cells also showed weak to moderate staining (Fig. 5): these spindle cells are referred to as germinal center bordering cells (GCBCs: Fossum, 1980). If the GCBCs were eliminated, S-100 protein-positive FDCs in the dark zone appeared to be distributed more sparsely than those in the light zone.

In the unstimulated group (at day 10 without stimulation), primary follicles and some secondary follicles were observed. A positive reaction in the GCs similar to that in the stimulated group was recognized, but the reaction tended to be weak. On the other hand, the negative control showed no positive staining.

Quantitative analysis of the FDC density in the lymph follicles of rat popliteal lymph nodes

Contrary to our expectation that the FDCs in the lymph follicles of the stimulated group would show the highest density, there was no evidence for any increase in the density of FDCs in the lymph follicle after stimulation with SRBCs (Fig. 6). The density of S-100 protein-positive cells in the lymph follicles of the

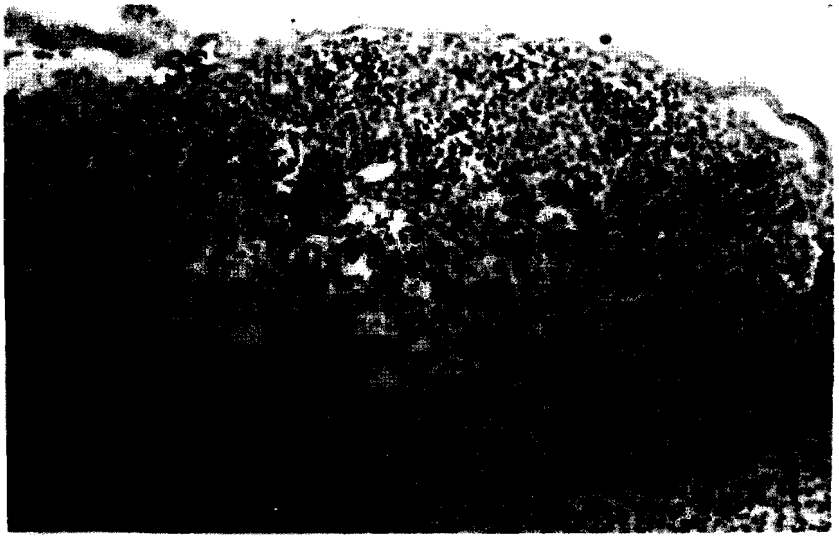

Fig. 4. Immunostaining of S-100 protein in a lymph node at day 5 after stimulation. Note a staining in a lymph follicle with a germinal center (GC). Positive cells are distributed in not only the apical side of the GC but also in the basal side of the GC. SS: subcapsular sinus. Counterstained with methylgreen, PAP method. x 180

Fig. 5. Immunostaining of S-100 protein in a lymph node at day 10 after stimulation. Note a strong staining in a well developed secondary follicle. A lace-like staining pattern is located in a light zone (LZ) and adjacent corona. A positive reaction (arrowheads) is also recognized in spindle-shaped cells on the border between a dark zone (DZ) and adjacent corona. Fat cells (F) show positive reactions. SS: subcapsular sinus. Counterstained with methylgreen, PAP method. x 180

stimulated group was significantly lower than those in the 5-week-old and unstimulated groups ($p<0.01$).

However, there was no significant difference between the density of S-100 protein-positive cells in the lymph follicles of the unstimulated group and that in the 5-week-old group (Fig. 6). Furthermore, the measured lymph follicles in the unstimulated group were larger than those in the 5-week-old group (Mean values ± SD of the areas of the measured lymph follicles in the stimulated, unstimulated and 5-week-old groups were 70174±26618, 41855±18465 and 26100±12574 μm^2, respectively, the differences among the values being significant ($p<0.01$)). These two findings indicated that the number of S-100 protein-positive cells in the lymph follicles of the unstimulated group increased during 10 days in comparison with the 5-week-old group.

In contrast to the previous studies (Stein et al., 1982; Rademakers, 1992), there was no evidence that the density of FDCs in the light zone was higher than that in the dark zone (Fig. 7). In the stimulated and unstimulated groups, there was no significant difference between the density of S-100 protein-positive cells in the upper half of the GC (substituted for the light zone) and that in the lower half (substituted for the dark zone). Although some lymph follicles in the 5-week-old group were recognized as immature small GCs on the photomicrographs, this group was excluded from

Table 1. The densities of S-100 protein positive cells in various regions of the lymph follicle in three groups (/(100 μm)²). Mean ± SD.

GROUP	CORONA	GERMINAL CENTER	LYMPH FOLLICLE
5-week-old	7.0±3.8*	11.3±4.6**	7.2±3.6*, ***
Unstimulated	5.6±3.3	11.2±4.7**	7.0±3.6***
Stimulated	3.7±2.3	6.6±3.6	4.5±2.2

Three groups contain 100 lymph follicles each. The 5-week-old group contains 18 immature germinal centers. The unstimulated group contains 57 germinal centers. The stimulated group contains 88 germinal centers. The "corona" consists of the regions of primary follicles and coronas in secondary follicles. Corresponding asterisks (*, ** or ***) indicate no significant differences. In the same row or column, there are significant differences between the values for all the other data ($p<0.01$). The density of S-100 protein positive cells in the lymph follicle in the 5-week-old group is significantly higher than those in the corona in the stimulated and unstimulated ones ($p<0.01$).

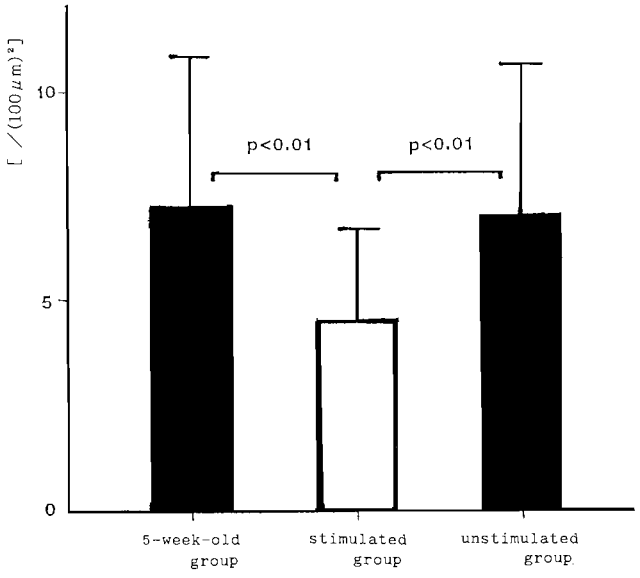

Fig. 6. The densities of S-100 protein positive cells in the lymph follicle among three groups (/(100 μm)²). The density of S-100 protein positive cells in the lymph follicle in the stimulated group is statistically lower than those in the 5-week-old and unstimulated ones ($p<0.01$). However, there is no significant difference between the density of S-100 protein positive cells in the lymph follicle in the unstimulated group and that in the 5-week-old one. Mean ± SD. Three groups contain 100 lymph follicles each.

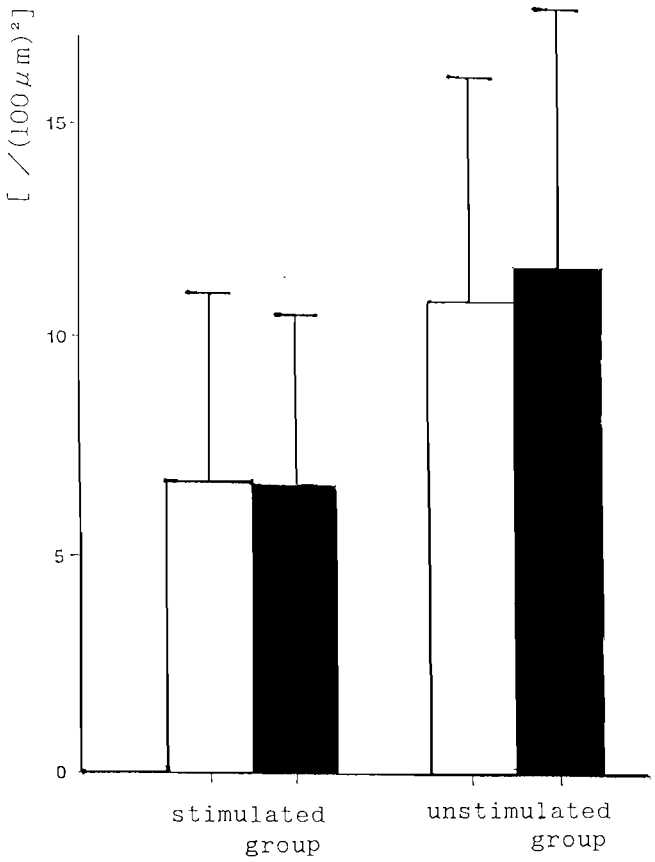

Fig. 7. The densities of S-100 protein positive cells in the substituted region for the light zone and the substituted region for the dark zone, in the stimulated and ustimulated groups. (/(100μm)²). Open bars: the upper half of the germinal center (the substituted region for the light zone); black bars: the lower half of the germinal center (the substituted region for the dark zone). There is no significant difference between the density of S-100 protein positive cells in the upper half of the germinal center and that in the lower half of the germinal center, within each group. However, the density of S-100 protein positive cells in one of the two regions of the germinal center in the stimulated group is statistically lower than that in one of the two regions of the germinal center in the unstimulated one ($p<0.01$). Mean ± SD. The unstimulated group contains 57 germinal centers. The stimulated group contains 88 germinal centers.

quantitative analysis because of their very small size and lack of polarization.

As expected, the lowest density of S-100 protein-positive cells was observed in the corona, followed in order by the lymph follicle and the GC in each group (Table 1). Furthermore, the density of S-100 protein-positive cells in the corona or lymph follicle in the 5-week-old group was significantly higher than that in the corona in stimulated and unstimulated groups ($p<0.01$).

Discussion

This study showed that the density of FDCs in the lymph follicle decreased after stimulation with SRBCs, and that the density of FDCs in the light zone was similar to that in the dark zone. Contrary to expectation, the density of S-100 protein-positive cells, i.e. FDCs, in the lymph follicle in the stimulated group was lowest of all the groups (Fig. 6). We thus obtained no evidence that the number of FDCs increased after stimulation. However, in the previous study it has been concluded that the number of FDCs in GCs at various sites within lymph nodes of young adult mice increased after secondary stimulation with SRBCs, which were injected intraperitoneally (Heinen et al., 1985). Our present findings differ from those of the previous study.

One reason for this discrepancy might have been the differences in experimental design such as the species used, the sites of the lymph nodes studied, and the method of SRBC injection.

The second reason for this discrepancy may have been differences in the ages of the animals and the number of stimulations with SRBCs: in the present study, young animals were injected with SRBCs only once. It seems likely that immature FDCs in young animals increase more readily than mature FDCs in older animals. However, there is a possibility that FDCs in developed GCs increase in number after secondary stimulation with SRBCs.

The third reason for this discrepancy may have been the differences in the cells which were counted as FDCs. In the previous study, FDCs were identified by their morphological features in semithin Epon sections. Thus, it would have been easy to count well developed FDCs showing typical morphological features in the light zone, but difficult to count poorly developed FDCs, which are distributed mainly in the dark zone and corona. As a result, the findings of the previous study may have reflected an increase in the number of well developed FDCs after secondary stimulation, rather than an increase in total FDCs. On the other hand, in the present study, it was easy to identify FDCs and to count their total number. Therefore, we conclude that our method is useful for examining the number of FDCs.

There is a possibility that rapid enlargement of lymph follicles after stimulation with SRBCs masked any increase in the number of FDCs. However, we found that the density of FDCs in the lymph follicle decreased after stimulation (Fig. 6). Furthermore, we found no mitotic figures in S-100 protein-positive FDCs in any of the specimens examined. Thus, it seems highly unlikely that FDCs have any significant proliferative activity.

Moreover, although one study has shown that human FDCs in culture can proliferate in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF; Clark et al., 1992), it has been reported that human FDCs show no proliferation in long-term culture (Tsunoda et al., 1990). In addition, there is only one report of mitotic figures in FDCs (Villena et al., 1983). Except for special culture conditions such as stimulation with substances such as GM-CSF, the above two findings and our present results indicate that, even if FDCs can proliferate, this is hardly detectable at least under normal conditions. Such conditions would include the postnatal development of lymph follicles, the GC reaction after stimulation, and culture conditions that are similar to physiological conditions. Although we found no evidence of FDC proliferation, we cannot exclude the possibility that FDCs have very slight proliferative activity, since there is a report of mitotic figures in FDCs, which have been described as fibroblastic reticulum cells (FRCs), in primary follicles (Villena et al., 1983). Therefore we suggest that FDCs have no or only very slight proliferative activity under normal conditions. Further studies using immunohistochemistry for cell proliferation antigen on FDCs and other methods are expected to elucidate the proliferative activity of FDCs.

On the other hand, although the origin of FDCs is still unsettled, they are considered to be derived from mesenchymal cells such as FRCs, or bone marrow cells (Heinen and Bosseloir, 1994). The possibility that FDCs have little or no proliferative activity is concordant with the hypothesis that FDCs are derived from mesenchymal cells, rather than bone marrow cells, because it seems unlikely that bone marrow-derived cells would have minimal proliferative activity. However, not all bone marrow-derived cells proliferate in lymph nodes (Fossum, 1989). In fact, one study has shown that Langerhans cells (LCs) and interdigitating cells (IDCs) in human lymph nodes with dermatopathic lymphadenopathy show only very slight proliferative activity (Osada et al., 1994), though it is clear that LCs and IDCs are derived from bone marrow cells (Thorbecke et al., 1980). Thus, our hypothesis seems valid irrespective of whether FDCs are derived from mesenchymal cells or bone marrow cells, even though the present study was unable to confirm their origin.

Although there was no evidence that the number of FDCs increased after stimulation, there was an increase of S-100 protein-positive cells in the lymph follicles of the unstimulated group (day 10 without stimulation) during a 10-day period, as confirmed by the following observations: 1) there was no significant difference between the density of S-100 protein-positive cells in lymph follicles of the unstimulated group and that in the 5-week-old group (Fig. 6). 2) The lymph follicles in the unstimulated group were larger than those in the 5-week-

old group. This increase in the number of S-100 protein-positive cells can be explained by an increase in immunoreactivity for S-100 protein during 10 days, because not all FDCs in the 5-week-old group were detected on the examined photos due to their weak positivity. However, there remains a possibility that few FDCs proliferated.

In contrast to the widely accepted notion that FDCs show a high density in the light zone (Stein et al., 1982; Rademakers, 1992), we found that the density of FDCs in the light zone was similar to that in the dark zone (Fig. 7). One reason for this discrepancy might be that the regions measured in the present study were substituted regions for the light and dark zones; the substituted regions may not have corresponded exactly to these zones. Another reason may be that germinal center bordering cells (GCBCs; Fossum, 1980), which were distributed at the border between the dark zone and adjacent corona, showed a positive reactions for S-100 protein (Fig. 5); GCBCs were counted as S-100 protein-positive within the lower half of the GC (substituted for the dark zone), and thus, the density of FDCs in the substituted region for the dark zone may have approached the value of that in the substituted region for the light zone. If GCBCs were eliminated from the FDC count, our findings were in accord with the widely accepted situation (data not shown). On the other hand, even if GCBCs were eliminated from the FDC count, the apparent decrease in the density of FDCs after stimulation (Fig. 6) remained unchanged, since GCBCs are particularly well distributed in well developed secondary follicles, and consequently the stimulated group among all groups includes the most GCBCs, which are to be eliminated. Thus, the elimination of GCBCs strengthens rather than weakens the validity of the result in Fig. 6.

Although it has been reported that GCBCs might act as a barrier in GCs (Fossum, 1980), these cells have seldom been described. We observed that the immunostaining for S-100 protein showed a change in the distribution of FDCs during GC development. From this finding, it seems likely that immature FDCs on the basal side of immature GCs at day 3 to day 5 (Fig. 4) mature and differentiate into GCBCs by day 10 (Fig. 5). Furthermore, both GCBCs and FDCs in the dark zone show a similar spindle-shaped morphology, display positive staining for S-100 protein and have no trapping ability. Thus, we speculate that GCBCs are a subtype of FDCs. However, there is a difference between GCBCs and FDCs in the dark zone, since GCBCs have basement membrane-like materials (Fossum, 1980). Thus, whether FDCs include GCBCs remains to be investigated.

On the other hand, an «outer zone» has recently been proposed as a compartment in the lymph follicles of human tonsils. The outer zone is assumed to be distributed not only in a region between the light zone and the corona but also in a narrow region around the dark zone, and may play a role as a traffic area of centrocytes when these cells differentiate (Hardie et al., 1993). Although it is still unclear if rat tissues have an outer zone, the region where GCBCs are distributed seems to correspond to part of the outer zone. Thus, there is a possibility that GCBCs play a role in the outer zone. Although little is known about the function of GCBCs, careful observation of these cells may contribute to a better understanding of how FDCs mature, differentiate, and function during the development of GCs, particularly in the dark zone.

As shown in Table 1, the lowest density of S-100 protein-positive cells was observed in the corona, followed in order by the lymph follicle, and the GC in each group. This finding is in accord with the widely known fact that many FDCs are distributed in the GC, whereas only a few lie in the corona (Stein et al., 1982). Furthermore, the density of FDCs in the corona or lymph follicle in the 5-week-old group was higher than that in the corona in the stimulated and unstimulated groups (Table 1). This indicates that primary follicles include many FDCs. FDCs in primary follicles display immature morphological features, but possess trapping ability (Imai et al., 1986). Although primary follicles and coronas in secondary follicles are considered to be immunohistochemically identical (Stein et al., 1982; Asano et al., 1993), the difference between these two regions may be that primary follicles are ready to induce GCs because they have many immature FDCs.

In this study, immunostaining for S-100 protein showed an increase in the immunoreactivity of FDCs and a change in the distribution of FDCs during GC development. This finding seems to be coincident with electron microscopic observations of postnatal development of FDCs and their trapping ability in rat popliteal lymph nodes (Imai et al., 1986). Thus we conclude that the expression of S-100 protein by FDCs reflects the development of these cells. Immunostaining for S-100 protein is useful for examining the development of rat FDCs, and may be applicable to studies of FDCs in various states such as pathological conditions and aging.

In this study, we extended the results of the previous work on the localization of S-100 protein-positive cells in rat lymph nodes (Iwanaga et al., 1982; Cocchia et al., 1983), and obtained the following two results. First, there was a weak immunoreaction in the subsinus layer (Fig. 2). The cells in this area have been described as antigen transporting cells (ATCs; Szakal et al., 1983) or penetrating cells (Fossum, 1980). Similarly, a monoclonal antibody OX2, which recognizes rat FDCs, is reactive with most cells in this region (Barclay, 1981). Thus, the expression of S-100 protein by ATCs is not surprising. FDCs and ATCs have been described as showing similar morphological features and to function in the transportation of immune complexes (Szakal et al., 1983). The expression of S-100 protein by ATCs may thus reflect this close relationship between FDCs and ACTs. Second, a slight reaction in the paracortex was detected. These positive cells may constitute a proportion of FRCs. This finding might be related to the

fact that both FRCs and some FDCs show a similar spindle-shaped morphological appearance, and also might support the hypothesis of the origin of FDCs, since FRCs are assumed to be candidate FDC precursors (Heusermann et al., 1980; Imai et al., 1986).

In summary, we conclude that the density of FDCs in the lymph follicle decreases after stimulation, and suggest that FDCs have little or no proliferative activity under normal conditions. Furthermore, it appears that the density of FDCs in the light zone is similar to that in the dark zone if GCBCs are counted as FDCs. Thus, investigators may need to pay attention to GCBCs for a better understanding of how FDCs differentiate. Moreover, we have confirmed that immunostaining for S-100 protein is useful for examining FDCs, and have extended the results of the previous work on the localization of S-100 protein-positive cells in rat lymph nodes.

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