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Sites of lymph follicle formation in the draining popliteal lymph nodes of mice locally injected with antigenic and mitogenic substances

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Summary. Our previous studies showed that some antigenic and mitogenic substances, when locally injected into mice, efficiently produced new lymph follicles outside pre-exisiting follicles in draining lymph nodes, whereas others had virtually no effect. In the present experiments, young adult male mice were injected with several antigens and mitogens in the rear footpad, and the number and development sites of newly produced lymph follicles in the draining popliteal nodes were studied using serial sections of the nodes obtained between 5 and 21 days after injection.

In the unstimulated state, each popliteal node contained a limited number of lymph follicles which mostly lay in a portion of the peripheral cortex overlaying the deep cortex (this portion is referred to as the PCOU), whereas a portion of the peripheral cortex extending beyond the deep cortex (referred to as the PCBU) was underdeveloped with only occasional follicles. Mice treated with soluble PHA or fluid tetanus toxoid developed germinal centers in association with existing follicles but failed to produce new follicles. The PCBU of the draining nodes remained underdeveloped, and the number and distribution pattern of lymph follicles within a draining node were comparable to those in the control node. Animals treated with LPS (50 μg), Con A, alum-precipitated PHA or alum-precipitated tetanus toxoid produced significantly large numbers of new follicles outside pre-existing follicles in the draining nodes, the new follicles produced in the PCBU being generally more numerous than those in the PCOU. In these draining nodes, the peripheral cortex, comprising a number of follicles, was found to overlie the deep cortex and extend beyond the deep cortex towards the hilar region. In animals given a less effective stimulant, such as ferritin or a smaller dose of LPS (10 μg), the draining nodes produced a relatively small number of new follicles, most of which were formed in the PCBU.

The present results indicate that in the mouse popliteal node, the PCBU is morphologically underdeveloped under normal conditions, but develops lymph follicles in response to exogenous stimuli more readily than the PCOU, and that substances efficient in inducing follicle formation can be regarded as capable of stimulating the development of the peripheral cortex.

Key words: Lymph follicle, Germinal center, Antigen, Mitogen, Lymph node

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Introduction

We described earlier that in the mouse popliteal node, lymph follicles lay mostly in a portion of the peripheral cortex overlaying the deep cortex, whereas a portion of the peripheral cortex extending beyond the deep cortex was underdeveloped and poorly populated with lymphocytes, containing sparse high-endothelial venules and only occasional lymph follicles (Hoshi et al., 1981, 1984). Recent studies on lymph node morphology have revealed that the deep cortex is made up of one or more units, each unit being centered under the opening(s) of an afferent lymphatic vessel; a segment of peripheral cortex containing lymph follicles overlays each unit and sometimes extends beyond it, directly covering the medulla (Bélisle and Sainte-Marie, 1981a,b; Sainte-Marie et al., 1982; Aijima et al., 1986; Suzawa et al., 1987). On the basis of these and other findings, it has been proposed that some elements present in afferent lymph are responsible for the topography and development of the deep cortical unit and peripheral cortex, although the element(s) responsible for development of the unit may differ from those controlling development of the peripheral cortex, and that such elements would spread out in a decreasing concentration gradient from the lymphatic opening(s) into a limited circular area of the subcapsular sinus, thus stimulating the development of the underlying cortical

structures (Bélisle and Sainte-Marie, 1981b; Sainte-Marie et al., 1982).

We reported previously that, following local injection of endotoxin lipopolysaccharide into mice, the draining popliteal nodes developed germinal centers in association with pre-existing primary follicles and also produced new primary follicles, which soon developed germinal centers (Hoshi et al., 1984). Further observations on the induction of lymph follicles with various substances indicated that particulate or high-molecular weight antigens and mitogens were efficient in inducing lymph follicle formation, whereas soluble, poorly phagocytized antigens and mitogens were inefficient (Hoshi et al., 1986; Horie and Hoshi, 1989). Soluble stimulants were found to be effective if given in precipitated form. The thymus-dependence of the inducing agents appeared irrelevant. It was suggested that follicle formation was triggered by substances which stimulate either T- or B-lymphocytes and which concomitantly activate macrophages.

In draining popliteal nodes which showed an increase in the number of lymph follicles after treatment with effective stimulants, we sometimes encountered lymph follicles in a portion of the peripheral cortex extending beyond the deep cortex. It seems possible that although underdeveloped in normal conditions, the peripheral cortex extending beyond the deep cortex in the mouse popliteal node might be endowed with the capacity to develop lymph follicles in response to appropriate stimuli. If this is so, a number of questions are raised: 1) In which portion of the peripheral cortex do the draining popliteal nodes preferentially develop new lymph follicles when stimulated with effective agents; in a portion overlying the deep cortex or in a portion extending beyond it? 2) Do the substances capable of triggering lymph follicle formation belong to those which stimulate the development of the peripheral cortex? 3) Can lymph follicles appearing in the peripheral cortex extending beyond the deep cortex in stimulated nodes be regarded as newly formed follicles? Data on the latter aspect may be important for analysis of the early events taking place in lymph follicle formation, since, once established, newly formed follicles are hardly distinguishable from pre-existing follicles by their morphology alone. The present study was undertaken to answer these questions.

Materials and methods

Animals and injection of stimulants

Male C57B1/6 mice, aged 8-12 weeks, were used in this study. They were divided into groups and injected in the left rear footpad with a 40-μl volume of stimulant. The stimulants used were as follows:

1) Phytohemagglutinin-p (PHA, Difco). Each mouse received 50 μg of the compound.

2) Fluid tetanus toxoid (Denka Biochem. Co.). Each mouse was given 10 Lf of the toxoid.

3) Endotoxin lipopolysaccharide (LPS, *E. coli* lipopolysaccharide B, lot 0111, B4, Difco). A dose of 10 or 50 μg per mouse was injected.

4) Concanavalin A (Con A, Sigma). A dose of 100 μg of the compound per mouse was used.

5) Ferritin (from horse spleen, Sigma). Each mouse was given 100 μg of the agent.

PHA and Con A are known to be T-cell mitogen, whereas LPS, tetanus toxoid and ferritin belong to B-cell mitogen and/or thymus-independent antigen.

6) Alum-precipitated PHA.

7) Alum-precipitated tetanus toxoid.

PHA in solution and fluid tetanus toxoid were each absorbed onto gelatinous alumina, which was prepared from 1% potassium alum according to the method described by Williams and Chase (1967). Each mouse received a 40-μl volume of the alum-precipitated stimulant containing 40 μg dry weight of alumina and either 50 μg of PHA of 4 Lf of tetanus toxoid.

The animals were sacrificed at various intervals after injection. In each group, at least three mice were sacrificed at each stage.

Tissue processing

At autopsy, the popliteal nodes from both sides were removed, cleared of surrounding fat and weighed. The popliteal node from the right side served as a control. Popliteal nodes were also obtained from unstimulated mice and used as an additional control. The specimens were fixed for 3-5 h in Zenker-formol solution, dehydrated and embedded in JB-4 resin. Serial sections, cut at a thickness of 3 μm, were prepared and stained with May-Grünwald and Giemsa solutions.

Reconstruction and three-dimensional analysis

In order to verify the presence of lymph follicles as well as to determine the location of each follicle within a node, all serial sections of a node were examined in the following manner. Each nodal section was magnified at x40 using a light microscope, and the image of the sectioned node was projected onto a sheet of white paper utilizing a drawing apparatus (Olympus, Tokyo) attached to the eyepiece of the microscope. Based on the projected image, the nodal components such as the peripheral cortex, deep cortex unit(s) and medulla, were outlined on the paper. If lymph follicles were encountered in the section, each follicle was also outlined on the drawing, identified with a reference number and traced three-dimensionally in the subsequent sections. The presence of germinal centers within a lymph follicle was examined at higher magnification. This systematic examination of drawings of a sectioned node enabled us to determine the total number of lymph follicles per node and the location and size of each follicle.

In the present study, the following structures were excluded from the category of lymph follicles: 1) follicle-like structures, which were sometimes found in the medullary cords or the marginal zone of the deep cortex

in draining popliteal nodes of animals treated with effective stimulants, and 2) small aggregations of lymphocytes, which were less than 0.05 mm in diameter.

Results

In C57B1/6 mice used in this study, each popliteal node was in many cases drained by a single afferent lymphatic vessel. The deep cortex of the node was composed of a single unit, and the afferent lymphatic vessel opened into the subcapsular sinus at the center of the underlying deep cortical unit (Figs. 4, 5). Some popliteal nodes received two afferent lymphatic vessels, and their deep cortex was either made up of a single mass or divided into two units. In the latter case, each unit was associated with one afferent lymphatic vessel (Fig. 7). In the former case, a single deep cortical mass was associated with two afferent lymphatic vessels (Fig. 6) and could be regarded as a unit complex that had formed as a result of fusion of two units (Bélisle and Sainte-Marie, 1981b). The schemata in Fig. 1 present the relationship between the afferent lymphatic vessel(s) and the deep cortex of the popliteal node, as demonstrated by the present three-dimensional examination of nodal sections.

The peripheral cortex overlay the unit(s) (this portion of the peripheral cortex is referred to as the PCOU) and also extended beyond it, covering the medulla directly (this portion of the peripheral cortex is referred to as the PCBU). In a single section, the popliteal node varied in appearance according to the angle as well as the level of sectioning. Fig. 2 shows schemata illustrating some of the different appearances of the peripheral cortex and its underlying components according to variations in the angle or level of sectioning. When a node was cut longitudinally, both the PCOU and PCBU were seen in a single section (Figs. 4-10). If a node was cut transversely, the peripheral cortex occupied the periphery of the sectioned node, but the component underlying the peripheral cortex varied according to the level: at some levels, the deep cortex was entirely encompassed by the peripheral cortex (Figs. 15B, 16B), whereas at other levels, the medulla underlay the peripheral cortex (Figs. 15C,D, 16C,D).

Lymph follicles were readily distinguishable from extrafollicular tissue in sections stained with May-Grünwald and Giemsa solutions, being densely packed with small lymphocytes. The present three-dimensional analysis of the nodal sections enabled us to determine the total number of lymph follicles detectable in each node and the location and size of each follicle. Some of these results are summarized in Table 1 and Fig. 3.

In the popliteal nodes of untreated mice and those on the contralateral side obtained from experimental animals, the number of lymph follicles per node was generally within the range of 9-11: 7 or 8 follicles were located in the PCOU (Figs. 4, 8) and the remaining 2 or 3 in the PCBU. Many of the follicles were small, having a diameter of less than 0.2 mm, but some were larger, with a diameter between 0.2 and 0.5 mm. Germinal centers were found in 2-4 larger follicles located in the

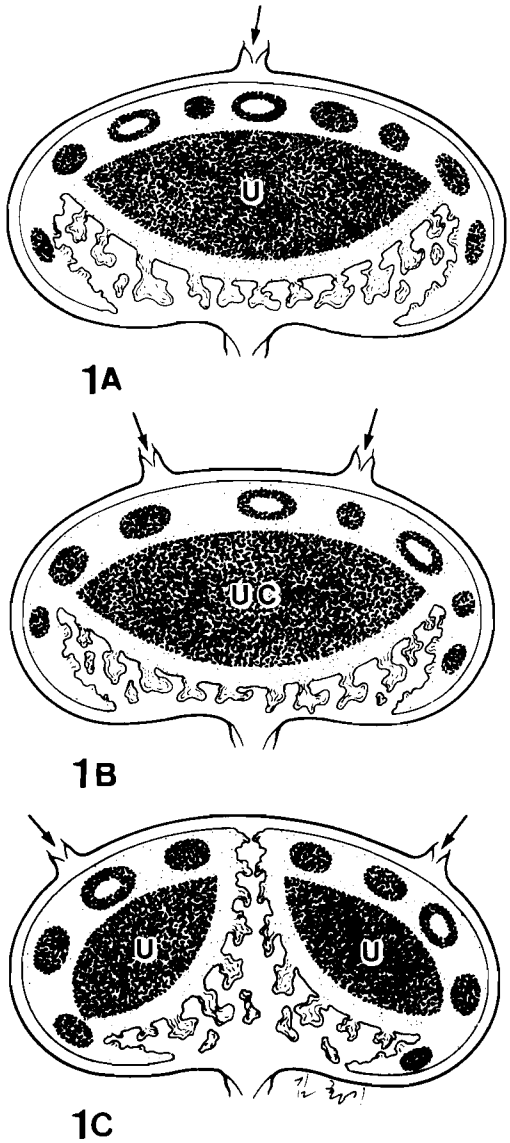


Fig. 1. Schematic illustrations of the topographical relationship between the deep cortical unit (U) and the opening of the afferent lymphatic vessel (arrow) draining into the mouse popliteal node. **A.** In some popliteal nodes, the deep cortex forms a single unit and one afferent lymphatic vessel opens into the subcapsular sinus at the center of the underlying unit. **B.** Some other popliteal nodes receive two afferent lymphatic vessels, which open into the subcapsular sinus in association with a single deep cortical mass or a unit complex (UC). **C.** In other popliteal nodes, the deep cortex is made up of two units, and each unit is centered under the opening of an afferent lymphatic vessel. Photographs illustrating some examples of these are shown in Figs. 4-7.

PCOU. Tall endothelial venules were frequently seen in the extrafollicular zone of the PCOU as well as in the deep cortex, but this type of venule seldom appeared in the PCBU. The extrafollicular zone of the PCOU was populated by small lymphocytes, whereas that of the PCBU was poorly populated by lymphoid elements.

Popliteal nodes after injection of soluble stimulants

Five days after local injection of PHA, some plasma cells were present in the medullary region and blast cells

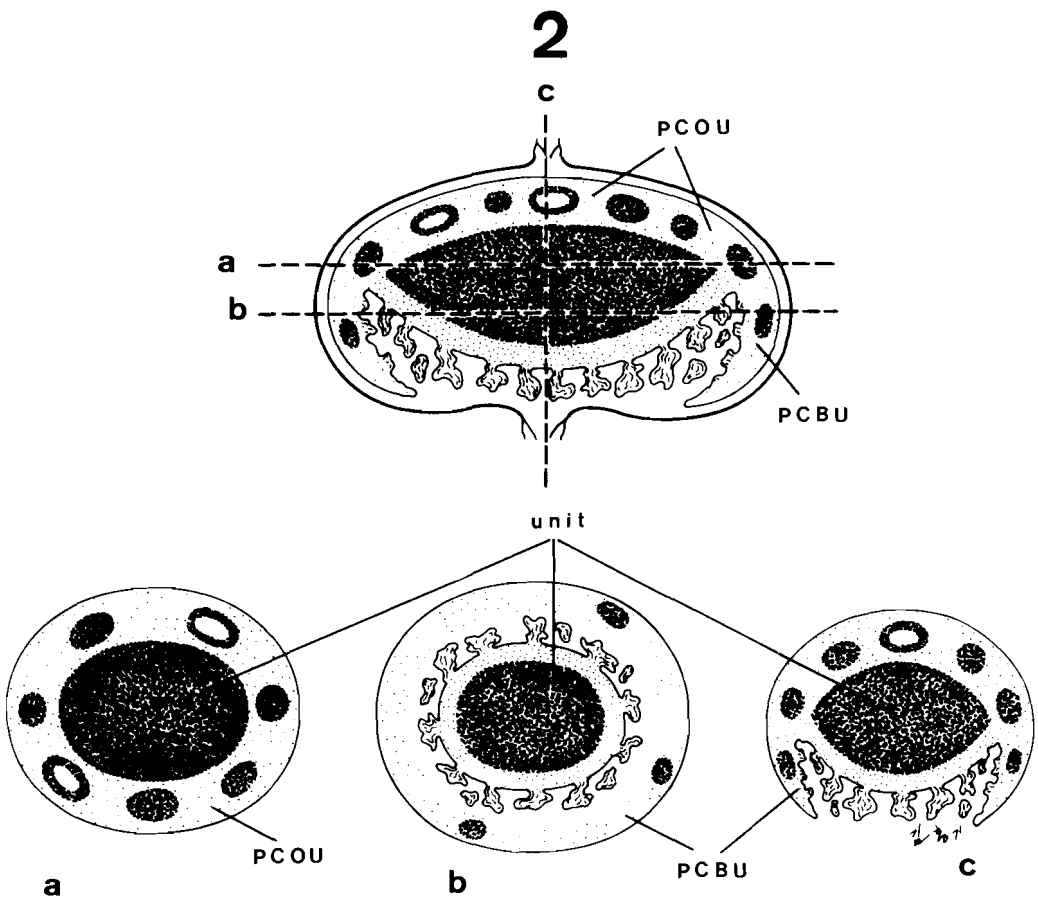


Fig. 2. Schematic illustrations of the different appearances of the components of a popliteal node according to the level and/or angle of sectioning.
a, b: transverse angles of sectioning.
c: longitudinal angle of sectioning. A deep cortical unit or mass appears rounded and, depending on the level of sectioning, is encompassed by cortical (**a**) or medullary (**b**) structures. Examples of **a** are shown in **Figs. 15B** and **16B** and those of **b** in **Figs. 15C, D** and **16C, D.** PCOU: the peripheral cortex overlying the deep cortical unit. PCBU: the peripheral cortex extending beyond the deep cortical unit.

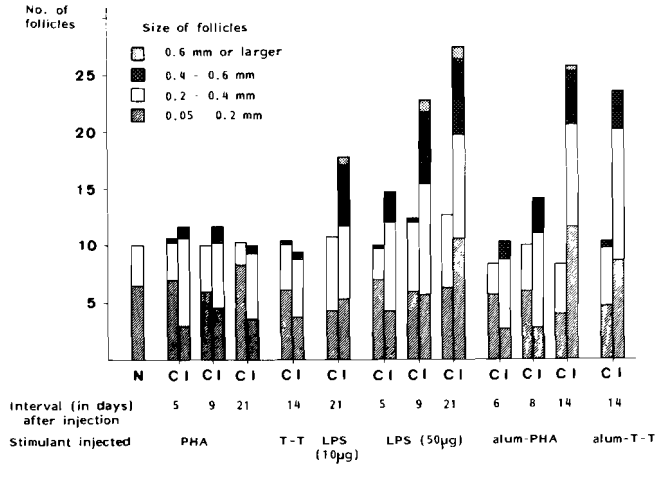


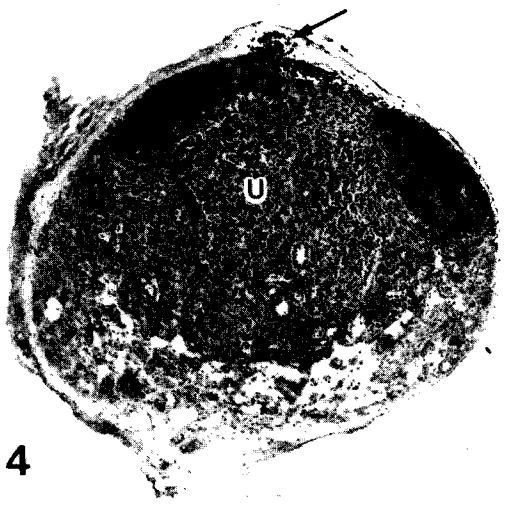
Fig. 3. Size distribution of lymph follicles in draining popliteal nodes at various intervals after local injection of soluble PHA, fluid tetanus toxoid (T-T), 10 µg LPS, 50 µg LPS, alum-precipitated PHA (Alum-PHA) or alum-precipitated tetanus toxoid (Alum-T-T). Three lymph nodes were examined at each interval. N: Node from an untreated normal mouse. C: Node on the contralateral side. I: Node on the ipsilateral side.

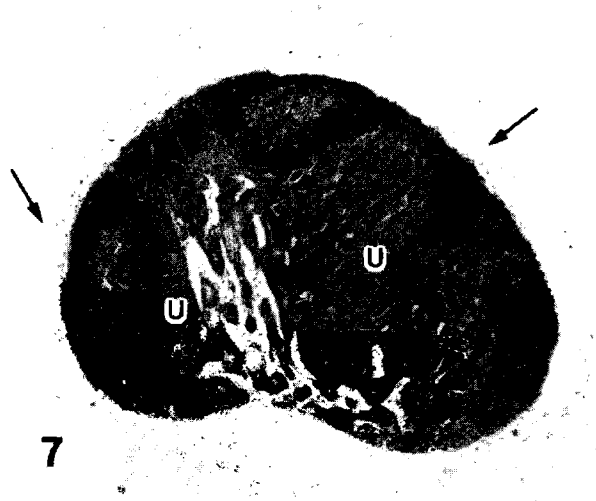
were scattered in the deep cortex in the ipsilateral nodes. Lymph follicles had expanded and some of them had developed germinal centers at the medullary pole. At 9 and 21 days, almost every follicle had expanded (Fig. 3) and contained a well developed germinal center (Fig. 9). Expansion of lymph follicles and development of germinal centers did not alter the number and distribution pattern of lymph follicles in the ipsilateral node: that is, 8 or 9 follicles were located in the PCOU and 2 or 3 follicles were in (Simpson, 1983). In fact, the apical ependyma was irregularly arranged and the limiting basal membrane often resulted incomplete (Alibardi and Sala, 1989).

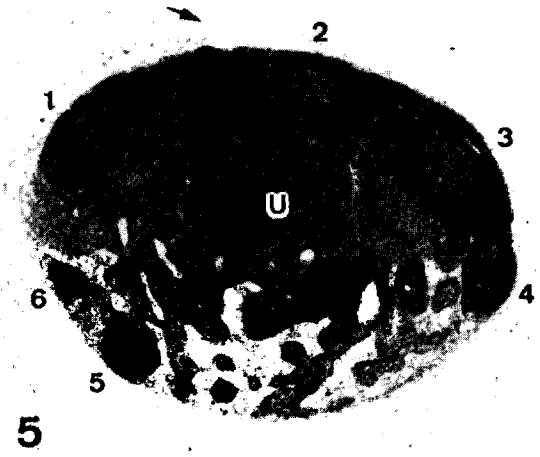
Trophic materials could be discharged from the apical aminegic-peptidergic terminals (Dellmann, 1973; Vigh et al., 1981) derived from growing nerves or from CSFCNs within regenerating SC (Alibardi and Sala, 1988; Alibardi and Meyer-Rochow, 1988; Alibardi et al., 1988). As observed in this study some of these dcv terminals contact the blastematic cells so that materials could be descharged.

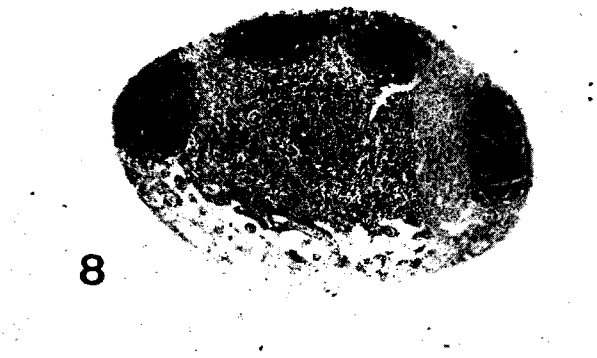
Previous studies (Simpson, 1968, 1983; Egar et al., 1970) also described dcv in regenerating axons of *Anolis* se in the number of lymph follicles in the draining popliteal node.

At 5 days after injection of LPS (50 µg), all ipsilateral nodes were enlarged. Blast cells were frequent in lymph









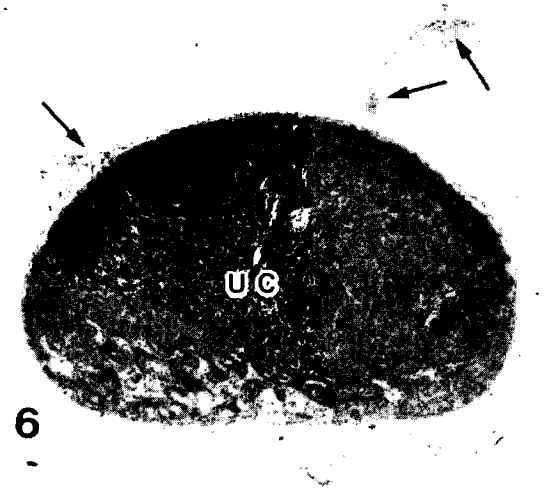


Fig. 4. A popliteal node from an untreated mouse, showing an afferent lymphatic vessel (arrow) opening into the subcapsular sinus at the center of an underlying unit (U) × 65

Fig. 5. A popliteal node on the injected side at 14 days after injection of alum-precipitated PHA, showing an afferent lymphatic vessel opening into the subcapsular sinus in association with an underlying unit (U). Six follicles, all numbered, can be seen in the periphery of the node. Follicles 1-3 are located in the PCOU, while follicles 4-6 are in the PCBU. × 45

Fig. 6. A popliteal node from an untreated mouse, showing a deep cortical mass or a unit complex (UC) associated with two afferent lymphatic vessels (arrows). Note that one afferent lymphatic vessel on the left side just opens into the subcapsular sinus. × 55

Fig. 7. A popliteal node on the injected side at 14 days after injection of soluble PHA, showing that the deep cortex is made up of two units (U). Note that each unit is associated with one afferent lymphatic vessel. Also note that the afferent lymphatic vessel on the right side just opens into the subcapsular sinus. × 30

Fig. 8. Node from an untreated mouse, showing four lymph follicles located in the PCOU. × 50

Fig. 9. Node on the injected side at 14 days after injection of soluble PHA. Three lymph follicles, each containing a prominent germinal center, can be seen in the PCOU. × 45

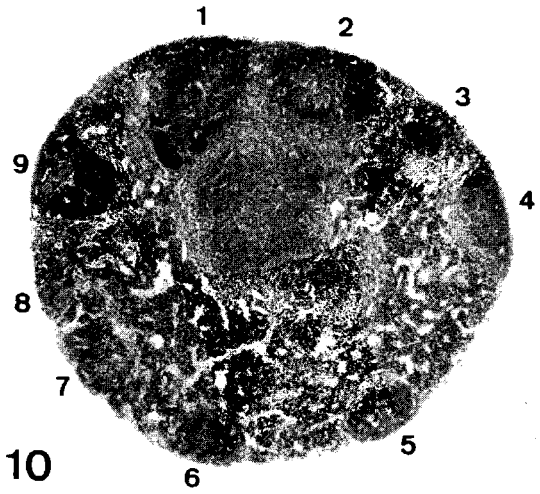


Fig. 10. Node on the injected side at 21 days after injection of 50 μg LPS. Nine numbered follicles can be seen in the peripheral cortex. Follicles 1 and 2 are present in the PCOU, while follicles 3-9 in the PCBU. × 30

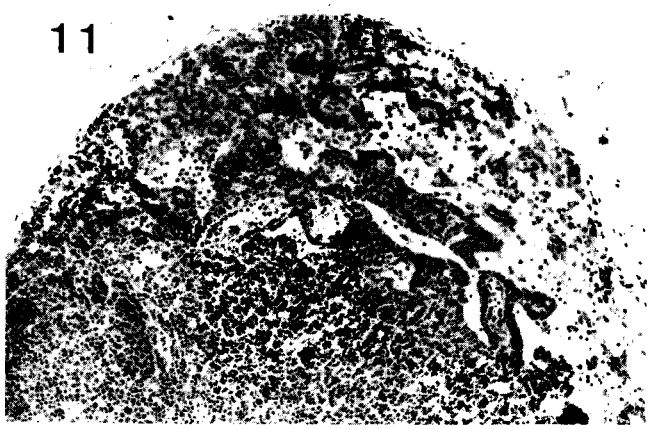


Fig. 11. A portion of the popliteal node shown in Fig. 9 at higher magnification, showing that the PCBU is underdeveloped with few lymphoid elements and no tall endothelial venules. × 110

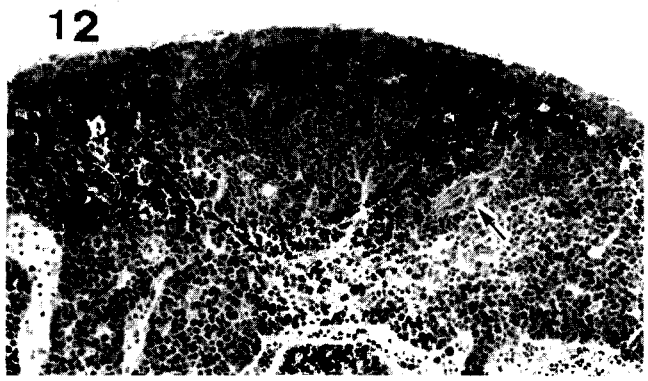


Fig. 12. A portion of a popliteal node on the injected side at 5 days after injection of 50 μg LPS, showing a lymph follicle developing in the PCBU. A tall endothelial venule (arrow) can be seen in the vicinity of the developing follicle. × 150

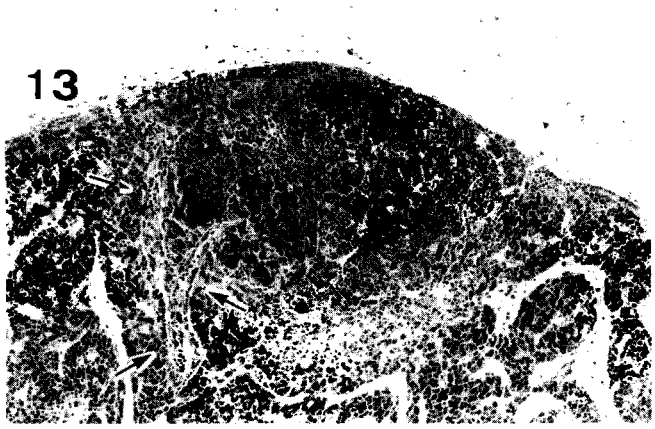


Fig. 13. A portion of a popliteal node on the injected side at 5 days after injection of 50 μg LPS, showing a lymph follicle containing a developing germinal center in the PCBU. Arrow points to a tall endothelial venule. × 100

follicles and the extrafollicular zone of the PCOU. Numerous cells of the plasma cell series populated the medullary cords and the extrafollicular zone of the PCBU. Lymph follicles had expanded, having a diameter of more than 0.2 mm (Fig. 3), and each contained a developing germinal center at the medullary pole. The secondary follicles corresponded to those follicles which had been present in the node before the treatment, and their number per draining node was comparable to the total number of lymph follicles in the control (Table 1). In addition, smaller primary follicles, representing newly formed follicles, were observed in the PCBU (Fig. 12) and occasionally in the PCOU outside expanded secondary follicles. Lymphocytic nodules resembling small lymph follicles were sometimes found in the medullary cords, but these structures were not included in the follicle count. Tall endothelial venules were evident in the PCBU in the vicinity of developing primary follicles (Fig. 12). At 9 days, lymph follicles had increased

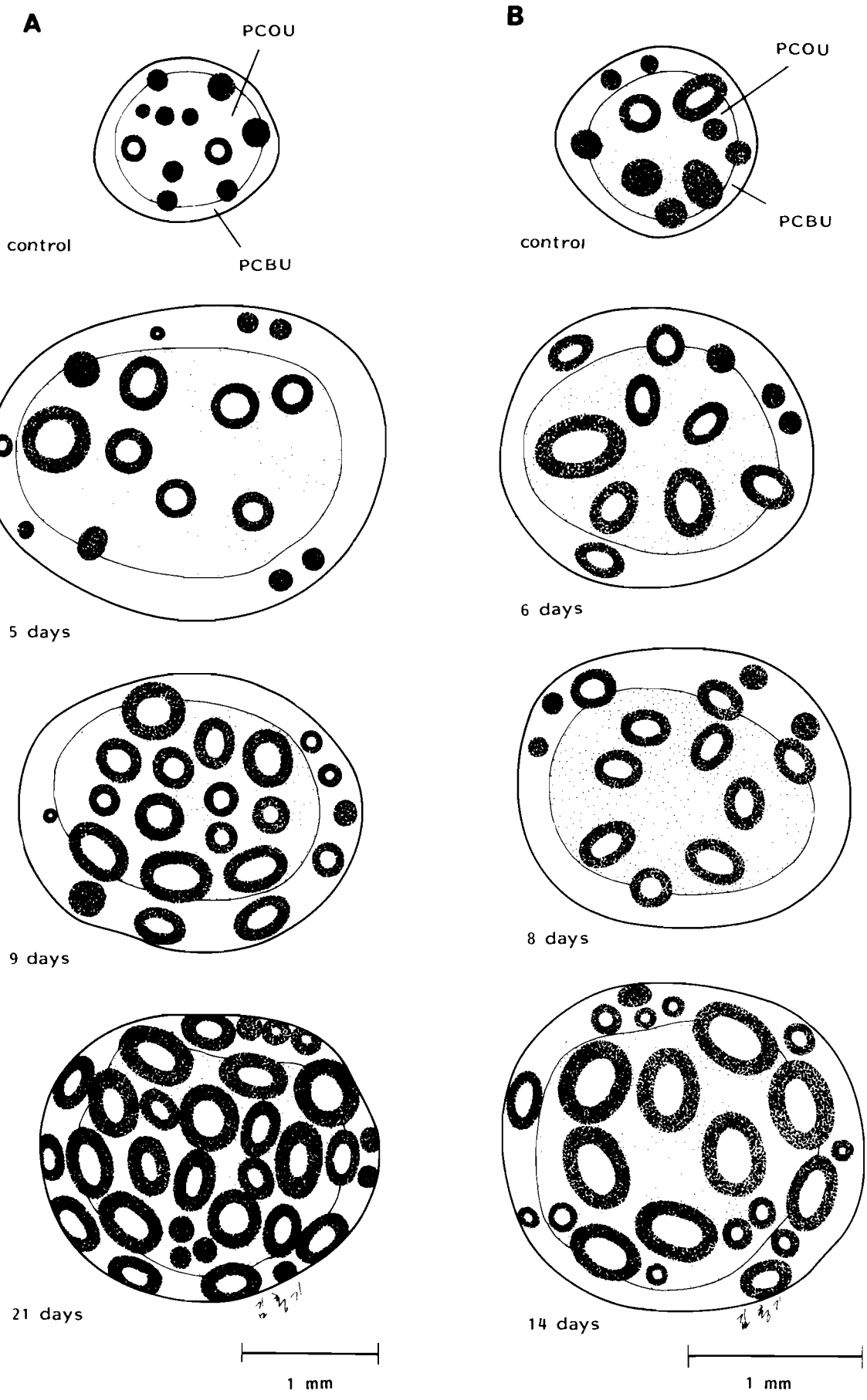
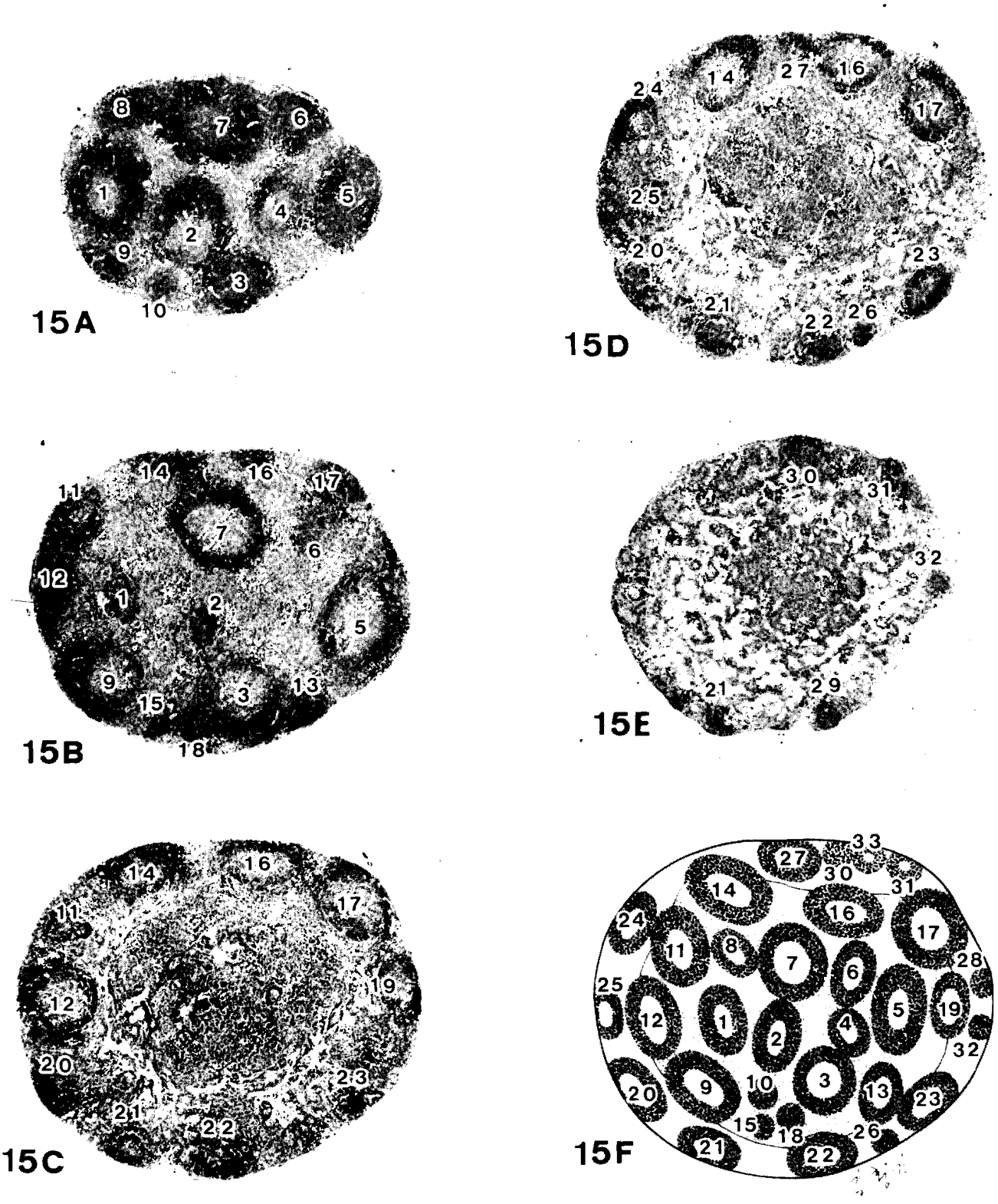
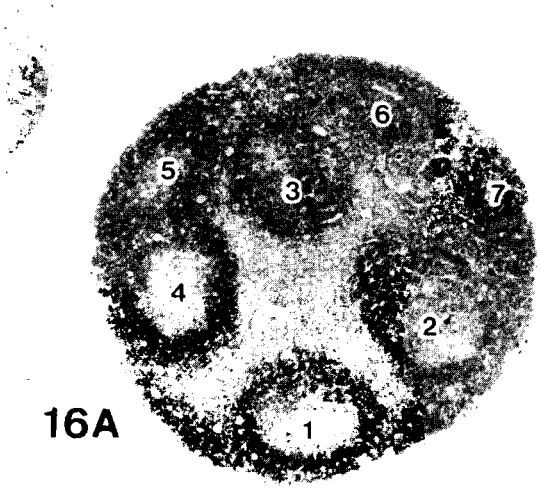


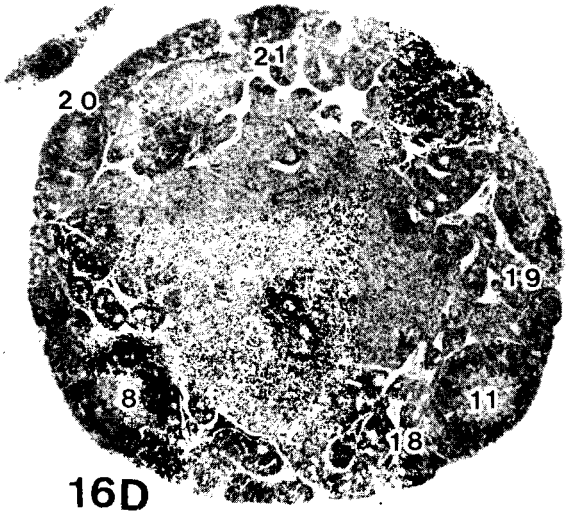
Fig. 14. Schematic illustrations of distribution of lymph follicles in the PCOU and PCBU of popliteal nodes on the injected side at various intervals after injection of 50 μg LPS (column A) or alum-precipitated PHA (column B). The distribution of lymph follicles in the schemata was reconstructed from serial sections of popliteal nodes.



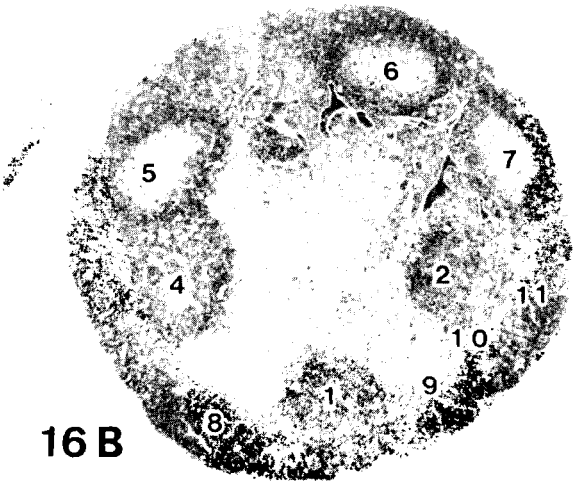
Figs. 15 and 16. Sections (A-E) from different levels of a popliteal node on the injected side at 21 days after injection of 50 μg LPS (**Fig. 15** × 35) and at 14 days after injection of alum-precipitated PHA (**Fig. 16.** × 30). Lymph follicles appearing in these sections are numbered and schematically illustrated in Fig. F.



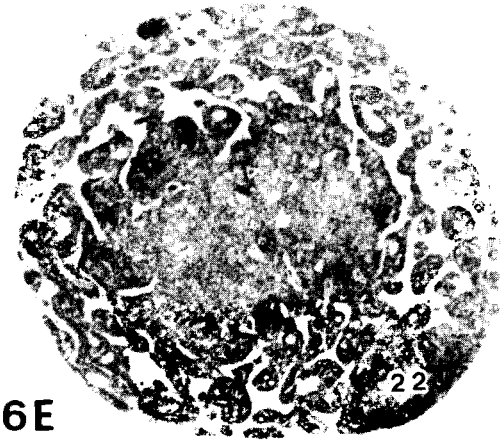
16A



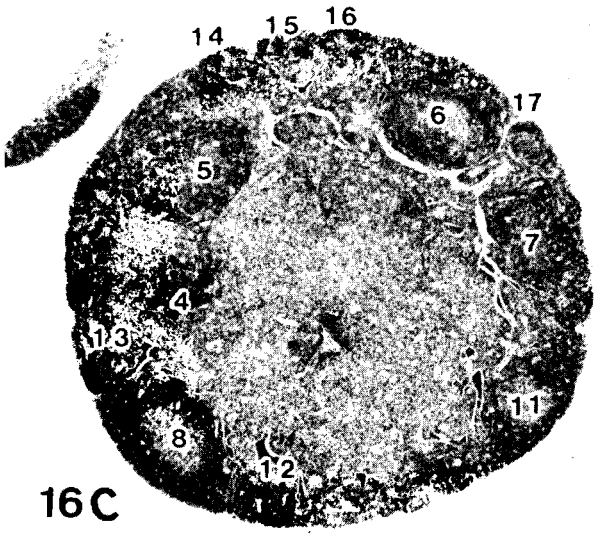
16D



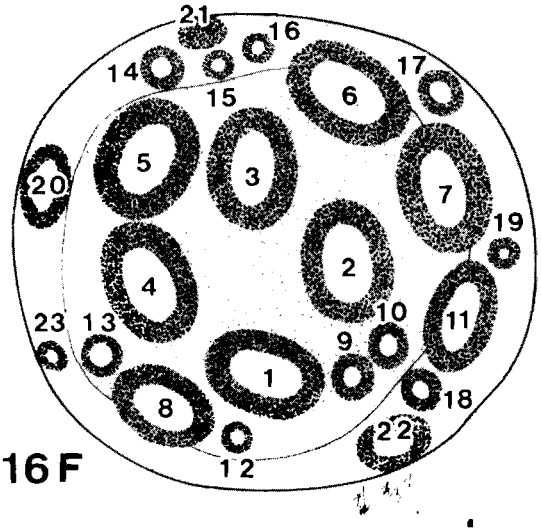
16B



16E



16C



16F

Table 1. Distribution of lymph follicles in draining popliteal nodes after injection of various stimulants.

Stimulants and intervals	Number of follicles in the PCOU[1]	Number of follicles in the PCBU[2]	Total number of follicles per node
Control[3]	7.9 (2.0)[4]	2.5 (0.1)	10.4 ± 1.8[5] (2.1)
PHA			
5 d.[6]	8.6 (5.3)	3.0 (0.3)	11.6 ± 0.9 (5.6)
9 d.	9.7 (9.7)	2.3 (2.3)	12.0 ± 1.0 (*12.0)
21 d.	8.3 (8.3)	1.7 (1.7)	10.0 ± (*10.0)
Tetanus toxoid			
14 d.	8.3 (7.6)	1.0 (0.7)	9.3 ± 1.2 (*8.3)
LPS (10 μg)			
21 d.	9.3 (8.7)	8.3 (7.3)	*17.6 ± 3.2 (*16.0)
LPS (50 μg)			
5 d.	8.0 (7.0)	6.0 (3.6)	*14.0 ± 0.8 (*11.6)
9 d.	12.6 (12.6)	10.0 (8.3)	*22.6 ± 1.8 (*20.9)
21 d.	13.6 (11.3)	13.6 (11.3)	*27.2 ± 7.5 (*22.6)
Con A			
21 d.	13.0 (12.0)	17.0 (15.3)	*30.0 ± 11.3 (*25.3)
Ferritin			
14 d.	7.2 (5.7)	7.2 (2.0)	*14.7 ± 0.6 (*7.7)
Alum-PHA			
6 d.	6.3 (6.3)	4.0 (2.3)	10.3 ± 1.6 (*8.6)
8 d.	8.0 (8.0)	6.0 (3.6)	*14.0 ± 0.8 (*11.6)
14 d.	15.6 (15.6)	10.0 (9.6)	*25.6 ± 2.6 (*25.2)
Alum-tetanus toxoid			
14 d.	14.3 (12.3)	9.0 (8.0)	*23.3 ± 0.4 (*20.3)

[1] Peripheral cortex overlying the deep cortical unit(s).
[2] Peripheral cortex extending beyond the unit(s).
[3] Normal and untreated contralateral lymph nodes, and thirty lymph nodes were examined.
[4] The number in parentheses indicates the mean number of lymph follicles containing germinal centers per lymph node.
[5] Values are mean ± S.D.
[6] Three lymph nodes were examined.
*: The number of lymph follicles per node on the injected side was significantly different ($P < 0.05$: t test) from that on the contralateral side.

in number in both the PCOU and PCBU. Those follicles which had been formed in the earlier stages increased in size and developed germinal centers (Fig. 13), and as a consequence, the number of larger secondary follicles was increased. In the following days, new follicles were formed in the PCOU and PCBU (Fig. 15), and at 21 days the mean number of follicles in both areas became 13.5. This indicated that each draining popliteal node had developed about 6 follicles in the PCOU and about 11 follicles in the PCBU during the 21-day period after treatment. Fig. 14 schematically presents examples of the distribution of lymph follicles in the PCOU and PCBU of the popliteal nodes on the injected side obtained at 5, 9 and 21 days after LPS injection, as demonstrated by three-dimensional observation.

In animals treated with Con A, the draining nodes showed increased numbers of follicles in the peripheral cortex at 21 days after the treatment: the number of follicles in the PCOU was 13 and that in the PCBU, 17, indicating that each draining node had developed about 5 new follicles in the PCOU and 15 follicles in the PCBU during the 21-day period. Regardless of whether a lymph follicle was located in the PCBU or PCOU, tall endothelial venules were observed in the vicinity of the follicles.

At 6 days after injection of precipitated PHA, blast cells were scattered in the deep cortex and many plasma cells were present in the medullary cords. Lymph follicles had expanded and their germinal centers had started to develop at the medullary pole. At 8 days, expanded follicles contained well developed germinal centers and the number of these secondary follicles was comparable to the total number of follicles in the control node. In addition, smaller primary follicles, representing the newly formed ones, were usually present in the PCBU and occasionally in the PCOU between expanded follicles. During the following days, lymph follicles increased in number in both the PCOU and PCBU (Fig. 5). The mean number of follicles located in the PCOU was 15.6 and that in the PCBU was 10, indicating that each ipsilateral node had developed about 8 new follicles in the PCOU and about 8 follicles in the PCBU 14 days after treatment (Figs. 14, 16).

In animals treated with precipitated tetanus toxoid, the ipsilateral nodes showed an increase in the number of follicles in the PCOU and PCBU at 14 days after treatment: the mean numbers of new follicles produced in the PCOU and in the PCBU were estimated to be about 5 and 6.5, respectively.

Mice treated with less effective stimulants, such as ferritin and a smaller dose (10 μg) of LPS, produced statistically significant but relatively small numbers of new follicles in draining nodes. Animals treated with ferritin were considered to have developed no new lymph follicles in the PCOU and about 5 new follicles in the PCBU during the 14-day period after treatment. In animals injected with 10 μg of LPS, the mean numbers of new follicles produced in the PCOU and PCBU were about 2 and 5, respectively, when examined at 21 days after injection.

Discussion

In C57B1/6 mice used in this study, the popliteal nodes were found to receive one or two afferent lymphatic vessels, and their deep cortex was composed of either one or two units according to the number of afferent lymphatic vessels draining into the node. The present observation is consistent with the previous findings on the rat lymph node by Bélisle and Sainte-Marie (1981a,b) that the deep cortex is made up of one or more units, each unit being centered under the opening(s) of an afferent lymphatic vessel. Underdevelopment of the PCBU of the popliteal node in the unstimulated state may be due to the fact that the element(s) responsible for normal development of the peripheral cortex, entering the node via the afferent lymphatic vessel(s), hardly reaches the peripheral region of the node where the PCBU lies, a large part of the element(s) being distributed in an area restricted to the central region of the node where the PCOU overlies the unit(s).

As shown previously (Hoshi et al., 1986; Horie and Hoshi, 1989), and confirmed in this study, local injection of a soluble substance such as PHA or fluid tetanus toxid stimulated germinal center development in association with existing primary follicles, but did not produce new follicles in draining nodes, the number of lymph follicles per node remaining unchanged. The injected stimulant may have been carried into the draining node with afferent lymph and distributed in the peripheral as well as the central region of the node, and indeed, germinal centers were formed within lymph follicles located in the PCOU and also within those in the PCBU. The development of the germinal center within a lymph follicle was usually accompanied by expansion of a lymph follicle, but this change did not cause any shift in the location of a lymph follicle from one portion of the peripheral cortex to another. It is noteworthy that after treatment with soluble stimulants, the PCBU showed no increase in the lymphocyte population, no increased appearance of tall endothelial venules and no formation of new lymph follicles. This observation suggests that soluble stimulants which are ineffective in inducing formation of lymph follicles are unable to stimulate the development of the peripheral cortex.

The present study confirmed our previous observations that when effective stimulants were locally injected, the draining nodes developed new lymph follicles not only in the PCOU but also in the PCBU. Effective stimulants used in this study included LPS, Con A, precipitated PHA and precipitated tetanus toxoid. Clearly, even though morphologically underdeveloped in the normal state, the PCBU has the capacity to develop lymph follicles in response to exogenous stimuli, although actual formation of lymph follicles in the PCBU may require the arrival of appropriate stimuli. Local injection of an effective stimulant may have caused an increase in the amount of afferent lymph flowing into the node, and thereby the injected stimulant contained in the afferent lymph may have been distributed as far as the peripheral region of the draining node, resulting in stimulation of follicle formation in the PCBU. Interestingly, the number of new lymph follicles produced in the PCBU was generally higher than that in the PCOU. Under normal conditions, the PCOU comprising a certain number of normally developed lymph follicles may be constantly stimulated with afferent lymph entering the node, whereas the PCBU is left unstimulated. Thus, once an appropriate stimulus has evenly spread throughout the node, the PCBU may more readily produce lymph follicles than the PCOU. That fact that the PCBU produces lymph follicles more readily than the PCOU is also indicated by the present observation that when a less effective stimulant, such as ferritin or a smaller dose of LPS, was locally administered, the draining nodes developed relatively small numbers of new lymph follicles, the majority being formed in the PCBU. If we look at the draining nodes in sections where increased numbers of lymph follicles were observed as a result of formation of new follicles after treatment with effective stimulants, it can be seen that a well developed peripheral cortex containing a number of follicles overlies the deep cortex and also extends beyond the unit(s) towards the hilar region. Therefore, it may be said that substances capable of inducing formation of lymph follicles belong to those which can stimulate the development of the peripheral cortex.

The lymph follicle is known to consist preferentially of B-lymphocytes (Gutman and Weissman, 1972; Howad et al., 1972; Sprent, 1973; De Sousa.et al., 1974; Hoshi et al., 1984) and to contain follicular dendritic cells (FDCs) as a stromal elements (Mitchel and Abbot, 1965; Hoefsmit, 1975; Chen et al., 1978). Ontogenic development of FDCs within normally developing primary follicles has been described (Veerman, 1975; Groscurth, 1980; Villena et al., 1983; Dijkstra, 1984; Imai et al., 1986). In this study, after treatment with an effective stimulant, new lymph follicles appeared in the draining node, beginning at around 5 days and continuing subsequently for several days. Thus, the formation of a lymph follicle requires a lag period of several days, and during the lag period, the local reticular tissue may undergo development of follicular dendritic cells and acquire the capacity to collect B-lymphocytes. Furthermore, the appearance of tall endothelial venules in the vicinity of developing new lymph follicles was always observed. Hence, the appearance of tall endothelial venules may be a related phenomenon, and

B-lymphocytes migrating from the circulation via the tall endothelial venules may be collected in developing lymph follicles. Since the present and previous studies on normal and athymic mice have shown that lymph follicle formation is triggered by substances which stimulate T- or B-lymphocytes and concomitantly activate macrophages (Hoshi et al., 1984, 1986, 1989; Horie and Hoshi, 1989), it seems quite possible that activated lymphocytes and macrophages may participate in the early events of lymph follicle formation. In this connection, it has been described that in the rat lymph node, macrophages form an almost continuous layer immediately below the subcapsular sinus (Hendriks et al., 1980; Sainte-Marie and Peng, 1985) and a similarly continuous layer of macrophages under the subcapsular sinus at the portion of both PCOU and PCBU in normal mouse popliteal nodes was also seen by us (unpublished observation). It is tempting to speculate that introduction of an effective stimulant into the draining node may be followed by localized activation of macrophages present under the subcapsular sinus together with stimulation of nearby populating lymphocytes, which may in some way cause modification of the local reticular tissue and blood vessels, resulting in the formation of lymph follicles.

Once established, new lymph follicles are hardly distinguishable from pre-existing follicles by their morphology alone, but the present results showed that most, if not all, of the follicles occurring in the PCBU in the mouse popliteal node following exogenous antigen stimulation can be regarded as new follicles. This information may be useful for those who wish to analyse the cellular events taking place in the formation of lymph follicles.

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