Histol Histopath (1993) 8: 491-499

Histology and Histopathology

Further histological observations on popliteal lymph nodes after interruption of the afferent lymphatic vessels

H. Hoshi, R. Takemoto, H. Nagata, K. Horie, G. Murakami and S. Suzuki
Department of Anatomy, Nihon University School of Medicine, Tokyo, Japan

Summary. Lymphatic vessels afferent to the popliteal nodes in young adult rats and guinea pigs were interrupted at the lowest edge of the popliteal fossa, and regressive changes in the nodes occurring 4 to 16 or 18 weeks after surgery were re-examined histologically. In the rat, some popliteal nodes were drained by lymphatic channel(s) from the tail. After surgery, the popliteal nodes without lymphatic channel(s) from the tail underwent regression of all their constituent deep cortical units and also the peripheral cortex comprising lymph follicles, until at 18 weeks, the lymphoid organization of the node reached a minimal level. On the other hand, in popliteal nodes having a lymphatic channel from the tail, some deep cortical units and the overlying peripheral cortex underwent hypotrophy, leaving the remaining units and their overlying peripheral cortex little affected. In the guinea pig, the treated nodes underwent progressive atrophy and ultimately became rudimentary, or even vanished, after 16 weeks. Treated rudimentary nodes were composed largely of stromal cells only, and these were replaced to a various extent by fat. The present results are discussed in relation to complete and incomplete inhibition of the afferent lymph flowing into the surgically treated nodes.

Key words: Lymph node, Afferent lymphatics, Deep cortical units, Lymph follicles, Rat, Guinea pig

Introduction

We have been carrying out a series of studies to examine the extent to which the persistence of normally established lymphoid structures in the lymph node is dependent on the afferent lymph carried into the node via the afferent lymphatic vessels. Previously, we performed surgical operations to interrupt the afferent lymphatics to the popliteal node at the lowest edge of the popliteal fossa in the rat. After surgery, the popliteal nodes became smaller, and their lymphoid structures, such as germinal centres, lymph follicles and deep cortical units, decreased in size and number, until the lymphoid organization of the nodes reached a minimal level at around 16 weeks (Hoshi et al., 1985). However, there was considerable variation in the degree of reduction of the nodal structures among the treated nodes. It has been reported that in the rat the popliteal node sometimes receives slender afferent lymphatic channel(s) from the tail (Miotti, 1965; Tilney, 1971), which might account for the variable degree of regression of the treated nodes observed in the previous experiments.

In the present study, therefore, we re-examined the popliteal node after interruption of the afferent lymphatics at the lowest edge of the popliteal fossa in the rat. Attention was paid to whether or not the treated node received lymphatic drainage from the tail. As our preliminary observations had indicated that in the guinea-pig, the popliteal nodes receive no lymphatic channel(s) from the tail, we also examined histological changes in the popliteal node of the guinea pig after interruption of the afferent lymphatics.

Offprint requests to: Dr. Hajime Hoshi, Department of Anatomy, Nihon University School of Medicine, Ohyaguchi, Itabashi-Ku, Tokyo 173, Japan

Materials and methods

Animals

Sixty male Wistar rats, aged 8 weeks, and 46 male Hartely guinea pigs, 350-450 g in weight, were used. All were maintained in a conventional environment.

Surgical operation

A surgical operation to interrupt the afferent lymphatics to the popliteal lymph node was performed on one side in clean but not sterile conditions under Nembutal anaesthesia. The methodological details have been described previously (Hoshi et al., 1981). Briefly, a longitudinal incision was made over the popliteal fossa. The lateral marginal vein and accompanying lymphatics

were ligated at the lowest edge of the popliteal fossa and the vessels and supporting fascia were cut. In each animal, the afferent lymphatics supplying the popliteal node of the left hind leg were interrupted, while the popliteal node on the right hind leg was left untouched.

When a solution of 0.4% Evans blue was injected into the root of the tail on the ipsilateral side 30 min before the animals were killed, treated nodes in rats which had lymphatic channel(s) from the tail were stained with the dye, whereas the other treated nodes in rats without this channel from the tail were unstained.

Blockage of the afferent lymph flow from the foot and footpad was verified by injecting 0.1 ml of colloidal carbon («Pelikan» Chinas ink diluted threefold with saline) into the left hind footpad 30 min before the animals were sacrificed. In 52 of 60 treated rats and 43 of 46 treated guinea pigs, the injected carbon did not reach the popliteal node on the treated side.

Histology

The animals were chloroformed at various intervals between 4 and 16 or 18 weeks after the operation. The popliteal nodes were removed from both sides. The nodes obtained from rats were cleared of surrounding fat and weighed. In the case of guinea pigs, surrounding fat was only roughly cleared from the nodes, because their capsule was very fragile, and they were not weighed. The lymph nodes on the right side served as the control. The specimens were fixed for 3-5 h in Zenker-formol solution, dehydrated, embedded in JB-4 resin and sectioned serially at a thickness of 3 µm. One of every two sections was mounted and stained with May-Grünwald and Giemsa solutions.

Examination of serial sections was carried out in order to determine the number of lymph follicles, germinal centres and deep cortical units (the term, «deep cortical units», was introduced by Bélisle and Sainte-Marie, 1981a,b) present in each node.

Histochemistry and immunohistochemistry

Treated rats and guinea pigs were sacrificed at 12 weeks after the operation, and the popliteal nodes were removed from both sides. Each node was frozen rapidly using a dry ice-acetone mixture.

Cryostat sections were fixed with acetone for 10 min. Cell surface IgM of rat lymphocytes was detected by the direct immunohistochemical method, using peroxidase-conjugated rabbit anti-rat IgM (Jackson IR Labs., diluted 1:50).

Acid phosphatase (AP) was demonstrated in cryostat sections using naphthol-AS-MX-phosphate as a substrate (Burstone, 1958) with hexazotized pararosaniline as the diazonium salt (Davis and Ornstein, 1959).

Results

Rats

Normal popliteal lymph node

In each popliteal lymph node of Wistar rats used in this study, the deep cortex consisted of 3-5 semi-spherical or semi-oval units, each unit being associated with an opening(s) of the afferent lymphatic vessel (Bélisle and Sainte-Marie, 1981a,b). In the peripheral cortex, the lymph follicles were readily distinguishable from the extrafollicular zone, being densely packed with

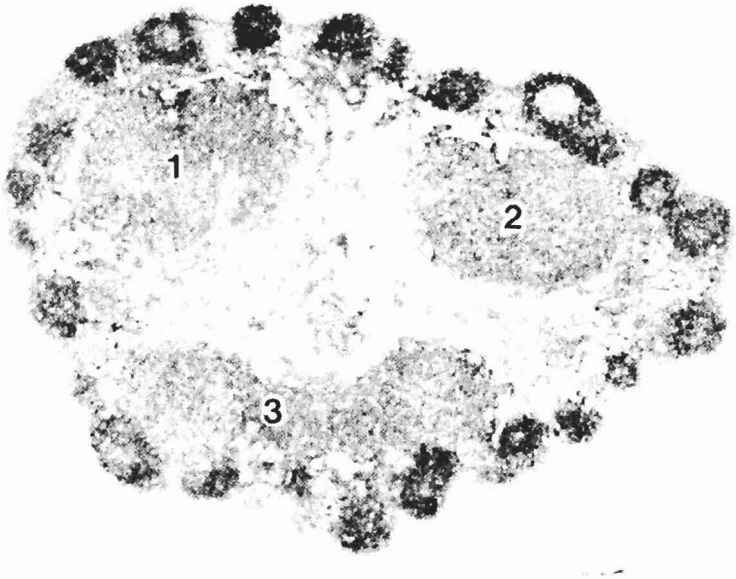


Fig. 1. Section through the middle of a rat popliteal node. Normal popliteal node. Three deep cortical units can be seen, each separated from the other by medullary tissue. May-Grünwald and Giemsa staining. x 30.

small lymphocytes (Fig. 1). Small lymphocytes composing a lymph follicle were mostly those bearing surface Ig-M (B lymphocytes) (Fig. 7). The mean number of lymph follicles per node was about 80, and some follicles contained germinal centres. Macrophages, appearing as AP (acid phosphatase-positive) cells in cryostat section, lined the subcapsular sinus (Fig. 9) and were frequently seen in the medullary region. Many plasma cells were present in the medullary cords.

In rats, the popliteal node is sometimes drained by lymphatic channel(s) from the tail region. Therefore, when Evans blue solution is injected into the root of the tail, each such popliteal node together with a lymphatic channel is stained with the dye, and it is possible to trace a channel all the way from the root of the tail to the popliteal node by macroscopic dissection.

Treated popliteal lymph node

Twenty-five of 52 successfully treated popliteal nodes were each drained by an afferent lymphatic vessel(s) from the tail and stained with Evans blue solution injected into the root of the tail 30 min prior to necropsy. We will call this kind of treated node Eb(+). The other treated nodes had no lymphatic channel from the tail and were unstained with the dye. We will refer to these nodes as Eb(-)-treated nodes.

During this observation period, the weight of the popliteal nodes on the untreated side remained at around 8 mg. After the operation, all the treated nodes decreased in weight, and the decrease was more pronounced in Eb(-) than in Eb(+) ones. At 18 weeks after surgery, the weight of Eb(-)-treated nodes was less than 2 mg, whereas that of Eb(+) nodes was about 5 mg (Table 1).

Eight weeks after the operation, Eb(-)-treated nodes showed hypotrophy of the deep cortex and peripheral cortex. We recognized 2-4 deep cortical units in the deep cortex of each Eb(-) node, and all these units were much smaller than those in the contralateral node. Both subcapsular and medullary sinuses showed «sinus-lymphocytosis», that is, they were filled with small lymphocytes. At the 8- and 12-week stages, lymph follicles in the peripheral cortex were decreased in size and number (Fig. 8, Table 1). AP cells were not frequent in the medullary region, but were seen lining the subcapsular sinus and scattered in the cortical parenchyma (Fig. 10). In the following weeks, cortical structures underwent further hypotrophy. At 18 weeks, we were still able to observe 2-3 rudimentary deep cortical units in each Eb(-)-treated node, each unit associated with the opening(s) of a narrowed afferent lymphatic vessel (Figs. 3-5). The peripheral cortex was also hypotrophic but contained small lymph follicles,

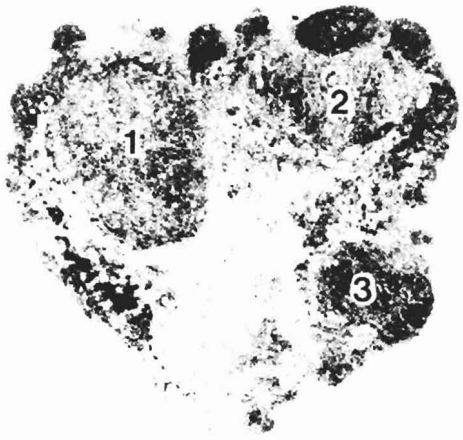
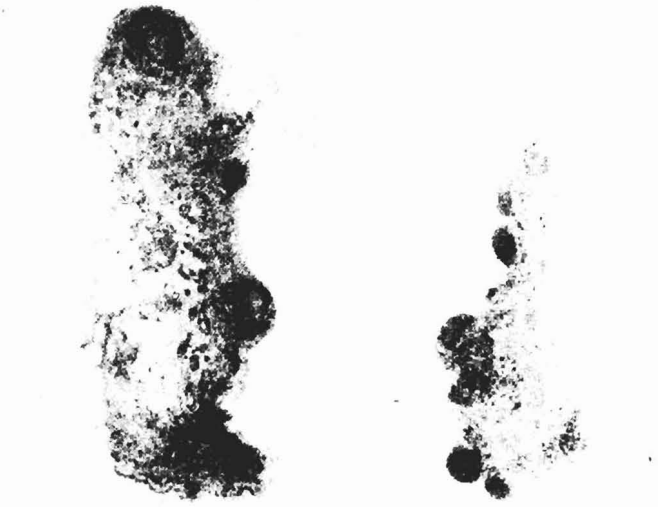


Fig. 2. Section through the middle of a rat popliteal node. An Eb(+)-treated node obtained 18 weeks after surgery. Note that units 1 and 2 are relatively large, while unit 3 is fairly small. The former units may have been associated with the lymphatic channel(s) from the tail, and the latter, with the interrupted lymphatic vessel. May-Grünwald and Giemsa staining. x 30.



Figs. 3 and 4. Sections through the middle of rat popliteal nodes. Eb(-)-treated nodes obtained 18 weeks after surgery. Note the marked reduction in size of the treated nodes, which contain highly atrophic deep cortical units. Small lymph follicles can be seen in the peripheral cortex. May-Grünwald and Giemsa staining. x 30.

Table 1. Number of cortical structures per popliteal node at different intervals after operation in rats.

POSTOPERATIVE INTERVAL	NUMBER OF LYMPH NODES EXAMINED	WEIGHT OF LYMPH NODES (mg)	NUMBER OF CORTICAL STRUCTURES	
			Follicles (Germinal centres)	Units (normal, small)
4 weeks				
Untreated	4	8.5	86.5±8.3* (51.3±27.3*)	3.5 (3.5, 0)
Treated				
Eb(+)	2	6.7	82.5 (46)	4.5 (4.5, 0)
Eb(-)	2	5.9	70.5 (38)	3 (3.0)
8 weeks				
Untreated	7	8.2	80.0±12.4 (37.3±14.5)	3.6 (3.6, 0)
Treated				
Eb(+)	4	5.4	61.8±1.7 (26.3±9.5)	4.1 (2.3, 1.8)
Eb(-)	3	2.7	47.3±16.3 (25.3±6.7)	2.7 (0.7, 2)
18 weeks				
Untreated	10	8.1	85.6±14.3 (18.8±19.0)	4.0 (3.7, 0.3)
Treated				
Eb(+)	4	5.0	67.3±18.3 (4.5±3.9)	3.0 (1.8, 2.0)
Eb(-)	6	1.6	37.5±8.0 (4.7±6.0)	2.5 (0, 2.5)

*: mean±SD

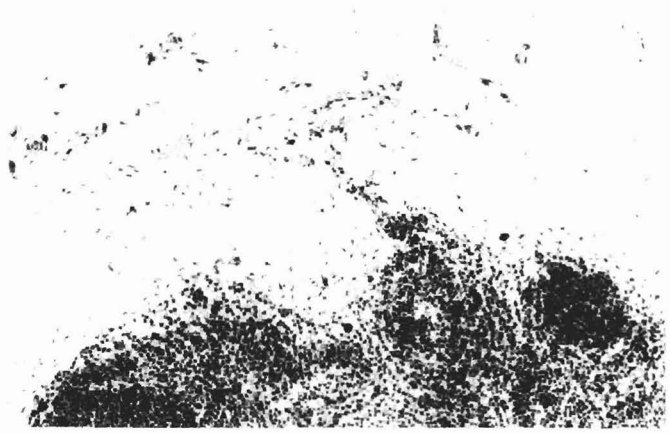


Fig. 5. Portion of the Eb(-)-treated node shown in Fig. 3 at higher magnification, showing an afferent lymphatic vessel opening into the subcapsular sinus. x 100

Fig. 6. Portion of the Eb(-)-treated node shown in Fig. 4. at higher magnification, showing the high-endothelial venules (arrow head) in the atrophic deep cortical unit. x 180

about 35 being present per treated node (Table 1). This means that about 45 lymph follicles had disappeared from the treated node during the 18 weeks after surgery.

High-endothelial venules (HEV) were evident in the cortical parenchyma at all of the stages examined, although they became less frequent with time (Fig. 6). At 8 weeks, plasma cells were occasionally seen in the

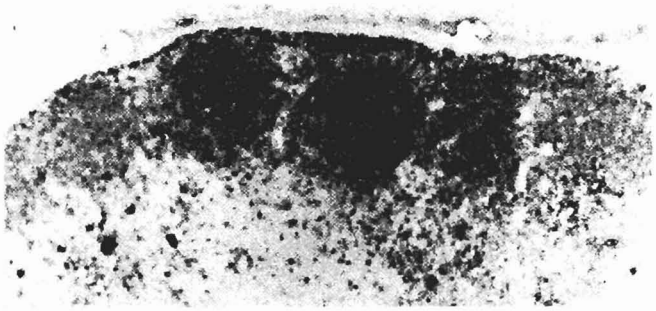


Fig. 7. Cryostat section of a rat popliteal node. Portion of a normal popliteal node, showing lymph follicles consisting of Ig-M bearing lymphocytes. x 80

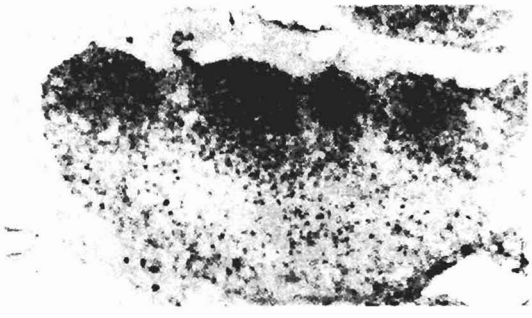


Fig. 8. Cryostat section of a rat popliteal node. Portion of an Eb(-)-treated node, showing small lymph follicles consisting of Ig-M-bearing lymphocytes. x 80

medullary region, but at 18 weeks these cells were virtually absent in the treated node.

The morphology of Eb(-)-treated nodes at 18 weeks after surgery suggested that they continued to collect small quantities of lymph from the surrounding tissue. In order to confirm this possibility, Fluoresbrite latex beads (YG latex, 0.5 μm in diameter, Polyscience Inc.) were injected into the fat tissue in the popliteal fossa on the treated side at 14 weeks after surgery and the popliteal node was harvested 3 days later. As expected, latex beads were seen scattered in the cortical parenchyma of the thus-treated Eb(-) node (Figs. 11, 12).

After surgery, Eb(+)-treated nodes underwent hypotrophy of the cortical structures, but not all the cortical units became hypotrophic. At 8 and 18 weeks, each Eb(+) node contained 4-5 units, 2-3 of which had become considerably reduced in size, while the other 1-2 units were little affected (Fig. 2). The portion of peripheral cortex overlying the diminished units was hypotrophic and contained a reduced number of small lymph follicles, whereas the portion of the peripheral cortex overlying little affected units remained well developed, containing a number of lymph follicles. At 18 weeks, the number of lymph follicles per treated node was about 60, which was less than that for the contralateral node, but more than that in an Eb(-)-treated node at the corresponding stage (Table 1). At any stage examined, Eb(+)-treated nodes showed no sinus-lymphocytosis, and only a small number of plasma cells were present in the medullary region.

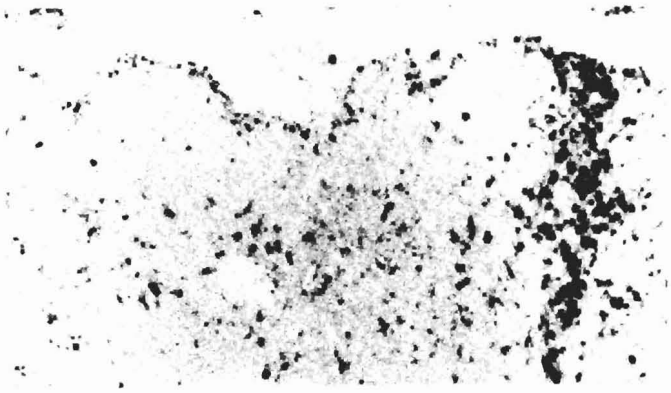


Fig. 9. Cryostat section of a rat popliteal node. Portion of a normal popliteal node, showing AP-positive macrophages lining the subcapsular sinus and scattered in the cortical parenchyma. x 100

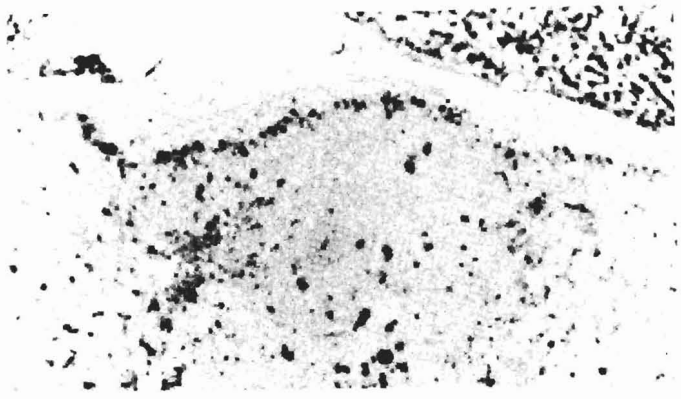


Fig. 10. Cryostat section of a rat popliteal node. Portion of an Eb(-)-treated node obtained at 12 weeks after surgery. Note that AP-positive macrophages are still present in the lining of the subcapsular sinus. x 100

Guinea pigs

Normal popliteal node

The deep cortex was made up of 1-3 units, each of which was associated with the opening(s) of an afferent lymphatic vessel (Fig. 13). The number of lymph follicles per popliteal node was about 100, and about 70 follicles contained germinal centres (Table 2). A small number of plasma cells were present in the medullary cords. AP cells, possibly macrophages, lined the

Fig. 11. Section of an Eb(-)-treated node from a rat which was injected with YG latex beads into the fat tissue of the popliteal fossa at 14 weeks after surgery and killed 3 days later. A deep cortical unit and overlying peripheral cortex comprising 4 lymph follicles can be seen. Haematoxyline staining. x 50

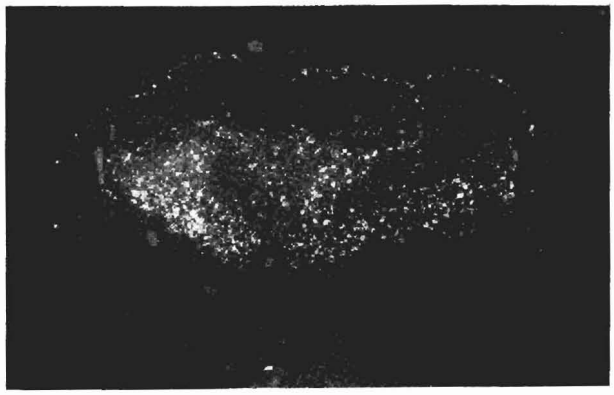


Fig. 12. Section of an Eb(-)-treated node from a rat which was injected with YG latex beads into the fat tissue of the popliteal fossa at 14 weeks after surgery and killed 3 days later. Unstained section neighbouring with the section shown in Fig. 11, photographed using a fluorescent microscope. Note that fluorescent YG latex beads are scattered in the lining of the subcapsular sinus and in the cortical parenchyma. x 50

Table 2. *Number of lymph follicles and germinal centres per popliteal node at different intervals after operation in guinea pigs.*

Postoperative interval	POPLITEAL NODES ON THE UNTREATED SITE			POPLITEAL NODES ON THE TREATED SIDE		
	Number of lymph nodes examined	Number of follicles*	Number of germinal centres*	Number of lymph nodes examined	Number of follicles*	Number of germinal centres*
4 weeks	4	101.8±24.3*	70.3±18.6*	4	52.5±11.7*	38.5±14.5*
8 weeks	4	120.3±17.9	87.5±10.0	4	41.3±18.0	25.8±9.3
16 weeks	4	108.5±31.0	65.5±28.9	24#	2.6±5.2	0

*: mean±SD; #: the number includes those popliteal nodes which we were unable to find in the popliteal fossa on the treated side at necropsy.

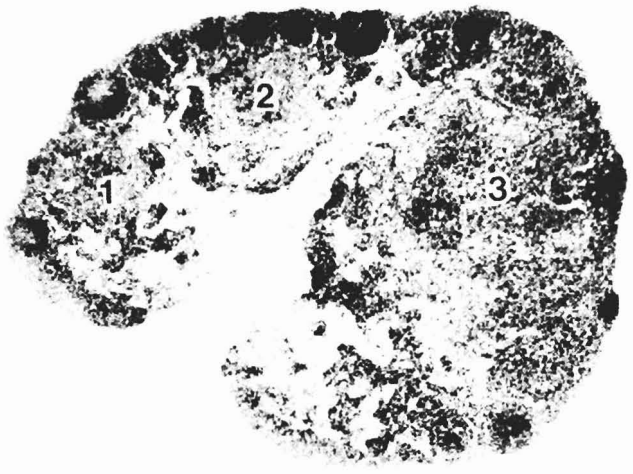


Fig. 13. Section through the middle of a guinea-pig popliteal node. Normal popliteal node. Three deep cortical units can be seen in the deep cortex. x 35

Fig. 14. Section through the middle of a guinea-pig popliteal node. A treated node obtained at 16 weeks after surgery. Note that the central portion of the node is largely occupied by fat. A thin rim of nodal parenchyma underlying the subcapsular sinus is populated with some small lymphocytes, but exhibits no lymph follicles. x 55

subcapsular sinus (Fig. 17) and were frequent in number in the medullary region; such cells were also scattered in the deep cortex and extrafollicular zone of the peripheral cortex.

Treated popliteal node

During the 8 weeks after surgery, the nodal structures

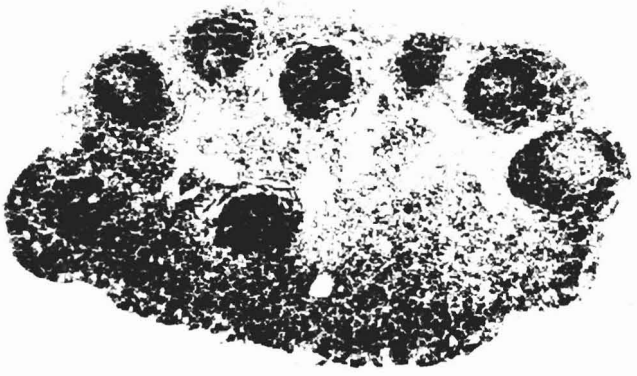


Fig. 15. Section through the middle of a guinea-pig popliteal node. A treated node obtained at 8 weeks after surgery, showing larger follicles containing germinal centres, with smaller ones lacking germinal centres. x 100

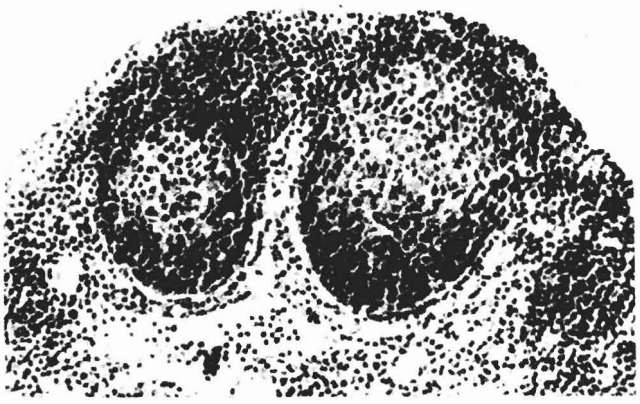


Fig. 16. Portion of the treated node shown in Fig. 15 at higher magnification, showing two lymph follicles containing active germinal centres. x 200

of treated nodes had become markedly diminished. The number of lymph follicles per node was reduced to less than half that of the control (Table 2). At 4 and 8 weeks, most of the persisting follicles contained germinal centres, and some of these appeared to be active (Figs. 15, 16). All the deep cortical units were decreased in size. The lymphatic sinuses were populated with many lymphocytes. From 4 to 12 weeks, HEV were evident in the cortical parenchyma, although they became less frequent with time. At 12 weeks, macrophages (AP cells) were scarce in the lining of the subcapsular sinus (Fig. 18). In the following weeks, the treated nodes continued to undergo atrophy.

At 16 weeks, in 9 of 24 treated animals examined, we were unable to find the popliteal node in the popliteal fossa on the treated side. Presumably, in these animals the treated node had vanished, having been replaced by the surrounding fat tissue. The other 15 treated animals had a residual popliteal node on the treated side. Of 15 residual treated nodes obtained, two were «solid», having a minimized nodal parenchymal well populated with lymphocytes and exhibiting sinus-lymphocytosis. The remaining treated nodes were markedly depleted of lymphocytes and showed various degrees of replacement of the nodal tissue by fat; in some nodes, a large proportion of the nodal tissue was occupied by fat (Fig. 14); in others, fat cells were scattered solitarily or in small clusters in the atrophic cortical and medullary tissue. In 7 of the 15 residual treated nodes obtained, no lymph follicles were recognizable anywhere in the atrophic parenchyma, where HEV had completely disappeared. The other 8 treated nodes contained 3-15 small persisting follicles in the periphery of the remnant nodal structures, in which HEV was hardly observable.

At 12 and 16 weeks after surgery, Fluoresbrite beads were injected into the fat tissue of the popliteal fossa on the treated side, and the popliteal node was harvested 3 days later. No latex beads were evident in the interior of the thus-treated node.

Discussion

In the rat, the popliteal node sometimes receives a lymphatic channel from the tail, and this channel was not affected by the present surgical procedure, which interrupted the lymphatic vessels at the lowest edge of the popliteal fossa. Twenty-five of 52 successfully-treated popliteal nodes were drained by the lymphatic channel from the tail and stained with Evans blue dye injected into the root of the tail. These treated nodes were referred to as Eb(+)-treated nodes. The remainder of the treated nodes, having no lymphatic channel from the tail, were unstained with the dye, and were referred to as Eb(-) nodes. After the operation, the lymphoid structures of the treated nodes underwent hypotrophy, and as expected, this change was more pronounced in Eb(-) than in Eb(+) nodes.

Reduction of the lymphocyte population, depletion of macrophages and/or interdigitating cells and disappearance of postcapillary high-endothelial venules (HEV) in the rat popliteal node after interruption of the afferent lymphatics have been previously observed (Hendriks et al., 1980; Hendriks and Eestermans, 1983; Drayson and Ford, 1984; Hoshi et al., 1985). Lymph node macrophages and precursors of interdigitating cells are known to be lymph-borne (Kamperdijk et al., 1978; Kelly et al., 1978; Hendriks et al., 1980; Balfour et al., 1981; Delemarre et al., 1990). Interruption of the afferent lymphatics prevents the influx of new macrophages and precursors of interdigitating cells into the node, while pre-existing lymph node macrophages and interdigitating cells disappear with time, because of their limited life span. It has been proposed that depletion of lymph node macrophages and/or interdigitating cells following interruption of the afferent lymphatics is related to disappearance of HEV, which impairs immigration of recirculating lymphocytes, resulting in lymphocyte depletion (Gowans and Knight, 1964; Hendriks et al., 1980, 1987; Hendriks and Eestermans, 1983; Drayson and Ford, 1984; Mebius et al., 1991b). Rapid disappearance of macrophages from the lining of the subcapsular sinus after interruption of the afferent lymphatic vessels has also been reported recently (Mebius et al., 1991a).

The deep cortex of a lymph node has been shown to consist of several units, each overlayed by a segment of

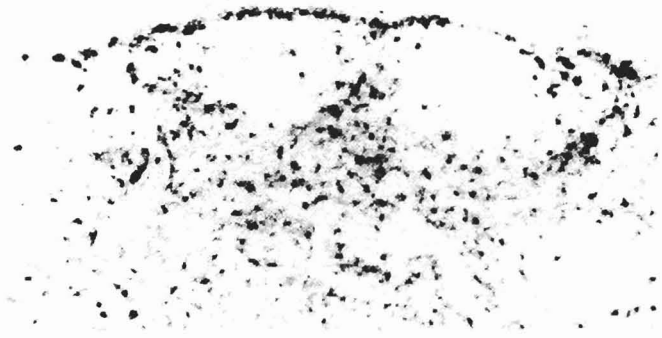


Fig. 17. Cryostat sections of a guinea-pig popliteal node. Portion of a normal node, showing AP-positive macrophages lining the subcapsular sinus. x 120

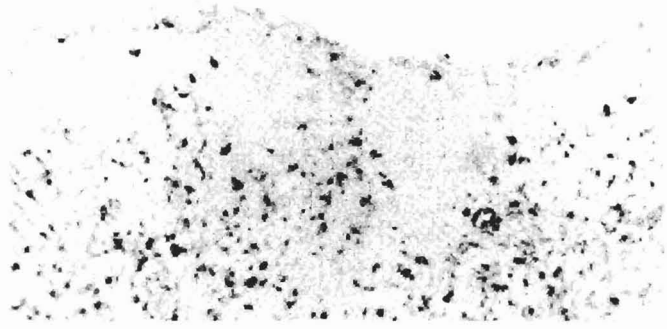


Fig. 18. Cryostat sections of a guinea-pig popliteal node. A treated node obtained 12 weeks after surgery, showing lack of AP-positive macrophages in the lining of the subcapsular sinus. x 120

peripheral cortex and centred under the opening(s) of an afferent lymphatic vessel (Bélisle and Sainte-Marie, 1981a,b; Sainte-Marie et al., 1982). If some of the afferent lymphatic vessels to a node are interrupted, the deep cortical units and overlying peripheral cortex associated with the interrupted lymphatic vessels undergo hypotrophy, owing to the reduced supply of afferent lymph. In an Eb(-)-treated node, all of the 2-5 deep cortical units and all parts of the peripheral cortex underwent hypotrophy, indicating that every afferent lymphatic vessel draining into the Eb(-) node had been carrying lymph from the foot and footpad before the operation. On the other hand, in an Eb(+)-treated node, 2-3 units and their overlying peripheral cortex became hypotrophic, while the remaining 1-2 units and their overlying peripheral cortex were little affected. The latter units and their overlying peripheral cortex may be associated with the lymphatic channel from the tail. From these findings, it is probable that in a rat popliteal node receiving lymphatic drainage from the tail, the nodal architecture is compartmentalized morphologically and functionally into two areas, one being associated with the afferent lymphatic(s) from the tail, and the other with the lymphatics from the foot and footpad.

Surgical interruption of the lymphatic vessels at the lowest edge of the popliteal fossa blocks lymph flow from the foot and footpad into the popliteal node almost completely for weeks or months (Hoshi et al., 1981, 1985). However, an Eb(-)-treated node, receiving no lymphatic channel from the tail, still continued to collect a small quantity of lymph from the surrounding tissue in the popliteal fossa, as has been confirmed in the present experiments. This may explain the persistence of some macrophages in the lining of the subcapsular sinus, various numbers of small lymph follicles in the peripheral cortex, and occasional HEVs in the deep cortex of Eb(-)-treated nodes at 12 and 18 weeks after surgery.

At 18 weeks after the operation, the number of lymph follicles persisting in an Eb(-)-treated node was only 40% of that in the contralateral node, and the persisting lymph follicles may have represented those which were located in nodal regions that continued to be supplied with a small quantity of afferent lymph. During the corresponding period, the weight of the Eb(-)-treated node decreased, and became 20% of the contralateral node. If it is considered that reduction in the weight of a treated node reflects the reduction in the quantity of afferent lymph flowing into it, an Eb(-)-treated node would have received 20% of the normal amount of afferent lymph. If this is the case, the number of lymph follicles persisting in an Eb(-)-treated node at 18 weeks is greater, as compared with an estimated 80% reduction in the quantity of afferent lymph. These observations suggest that some established lymph follicles may tolerate, and can persist despite, a reduced supply of antigen and other factors contained in the afferent lymph.

The present observations of the rat popliteal node after blockage of the afferent lymphatics are in keeping with our previous conclusion that after interruption of the afferent lymphatics, the nodal structures undergo a decrease in size and number, until the lymphatic organization of the node reaches equilibrium with the reduced supply of afferent lymph (Hoshi et al., 1985).

In the present study, young adult guinea pigs were also subjected to surgical interruption of the afferent lymphatics to the popliteal node, using the same method as that employed in rats. Interestingly, in the guinea pig, the treatment completely blocked the afferent lymph flowing into the treated node, not only from the foot and footpad, but also from the surrounding tissue of the popliteal fossa. The regressive changes in the treated nodes of the guinea pig during the first 8 weeks after the operation were slightly more evident than, but not very different from, those seen in Eb(-)-treated nodes of the rat. In the following weeks, the treated nodes of the guinea pig continued to undergo atrophy, and ultimately became rudimentary or even disappeared by 16 weeks after surgery.

It has been reported that interruption of the afferent lymphatics of the rat popliteal node results in the disappearance of HEV and immigrating lymphocytes within 3 weeks (Hendriks et al., 1980; Hendriks and Eestermans, 1983) or 6-12 weeks (Drayson and Ford, 1984). In this study, the HEV appeared to decrease during the first 4 weeks after surgery, but some of them persisted for at least 12 weeks, at which time the treated node showed a minimal level of lymphoid organization. Complete disappearance of HEV was noted at 16 weeks after surgery, when the nodal structures had become rudimentary and devoid of lymphocytes. The variation in the period required for the popliteal node to lose all of the HEV after interruption of the afferent lymphatic vessels may depend on the method employed to interrupt the vessels.

Each popliteal node in untreated guinea pigs generally contained more than 100 lymph follicles, about 30% being primary follicles without germinal centres, and the others secondary follicles containing germinal centres. It is known that germinal centre activity within a lymph follicle is maintained by stimulation with antigen retained by follicular dendritic cells, and that each germinal centre becomes inactive as the retained antigen becomes degraded (Nossal et al., 1964; Nossal and Ada, 1971; Klaus et al., 1980; Mandel et al., 1980; Nieuwenhouis and Opstelten, 1984; Hoshi et al., 1989). During 4 weeks after surgery, nearly half the number of lymph follicles disappeared from the treated node. Since during this period the HEVs were present, even though at a decreased level, all of the existing lymph follicles may have had a chance to be supplied with reduced numbers of immigrating lymphocytes. Nevertheless, some follicles and germinal centres disappeared, while the others persisted. This observation indicates that some primary and secondary follicles require, for their persistence, a constant supply of antigen and other stimulating factors via the afferent lymph. Lymph

follicles with and without germinal centres that persisted for weeks may have been those which retained antigen of such a type that was resistant to degradation. Progressive reduction in the supply of immigrating lymphocytes due to progressive disappearance of HEVs may thus become an important factor determining the disappearance of persisting lymph follicles at later stages.

The present study showed that after surgery, the nodal structures of the guinea pig popliteal node ultimately became depleted of lymphocytes and macrophages, and finally underwent involution, being replaced by fat. This finding is of interest, since it indicates that stromal cells of the lymph node cannot preserve their intrinsic character under conditions where free cells normally occupying the mesh of stromal cells are absent. However, the present observations differ from the previous ones for the rat, where the lymph node deprived of afferent lymphatics ultimately became depleted of macrophages, interdigitating cells and lymphocytes, leaving only stromal cells (Hendriks et al., 1980; Drayson and Ford, 1984). The difference may be attributable to interspecific variation in the response of the lymph node stromal cells to the depleted state.

Acknowledgements. We thank Mr. Shigeharu Okabe and Mr. Akio Ito for photography and Mrs. Sonoko Araki for technical assistance. This research was supported by a grant from the Japanese Ministry of Education, Culture and Science.

References

Balfour B.M., Drexhage H.A., Kamperdijk E.W.A. and Hoefsmit E.Ch.M. (1981). Antigen-presenting cells, including Langerhans cells, veiled cells and interdigitating cells. Ciba Found. Symp. 84. Pitman Medical. London. pp 281-300.

Bélisle C. and Sainte-Marie G. (1981a). Tridimensional study of the deep cortex of the rat lymph node. I. Topography of the deep cortex. Anat. Rec. 199, 45-59.

Bélisle C. and Sainte-Marie G. (1981b). Tridimensional study of the deep cortex of the rat lymph node. II. Relation of deep cortex units to afferent lymphatic vessels. Anat. Rec. 199, 61-73.

Burstone M.S. (1958). Histochemical comparison of naphthol AS-phosphates for the demonstration of phosphatase. J. Natl. Cancer Inst. 20, 601-610.

Davis B.J. and Ornstein L. (1959). High resolution enzyme localization with a new diazo reagent «hexazonium pararosaniline». J. Histochem. Cytochem. 7, 297-298.

Delemarre F.G.A., Kors N., Kraal G. and van Rooijen N. (1990). Repopulation of macrophages in popliteal lymph nodes of mice after liposome-mediated depletion. J. Leukocyte Biol. 47, 251-257.

Drayson M.T. and Ford W.I. (1984). Afferent lymph and lymph borne cells: their influence on lymph node function. Immunobiology 168, 362-379.

Gowans J.L. and Knight E.J. (1964). The route of re-circulation of lymphocytes in the rat. Proc. R. Soc. Lond. (Biol) 159, 257-282.

Hendriks H.R. and Eestermans I.L. (1983). Disappearance and reappearance of high endothelial venules and immigrating lymphocytes in lymph nodes deprived of afferent lymphatic vessels: a possible regulatory role of macrophages in lymphocyte migration. Eur. J. Immunol. 13, 663-669.

Hendriks H.R., Eestermans I.L. and Hoefsmit E.C.M. (1980). Depletion of macrophages and disappearance of postcapillary high endothelial venules in lymph nodes deprived of afferent lymphatic vessels. Cell Tissue Res. 211, 375-389.

Hendriks H.R., Duijvestijn A.M. and Kraal G. (1987). Rapid decrease in lymphocyte adherence to high endothelial venules in lymph nodes deprived of afferent lymphatic vessels. Eur. J. Immunol. 17, 1691-1695.

Hoshi H., Horie K., Nagata H. and Sato M. (1989). A histological and experimental study of the fate of an increased number of lymph follicles produced in the mouse popliteal lymph node by exogenous antigen stimulation. Arch. Histol. Cytol. 52, 485-491.

Hoshi H., Kamiya K., Aijima H., Yoshida K. and Endo E. (1985). Histological observations on rat popliteal lymph nodes after blockage of their afferent lymphatics. Arch. Histol. Jpn. 48, 135-148.

Hoshi H., Kamiya K. and Endo E. (1981). Cortical structure of the lymph node. I. Effect of blockage of the afferent lymph flow to mouse popliteal nodes for protracted periods. J. Anat. 133, 593-606.

Kamperdijk E.W.A., Raaymakers E.M., de Leeuw J.H.S. and Hoefsmit E.Ch.M. (1978). Lymph node macrophages and reticulum cells in the immune response. I. The primary response to paratyphoid vaccine. Cell Tissue Res. 192, 1-23.

Kelly R.H., Balfour B.M., Armstrong J.A. and Griffiths S. (1978). Functional anatomy of lymph nodes. II. Peripheral lymph-borne mononuclear cells. Anat. Rec. 190, 5-22.

Klaus G.G.B., Humphrey J.H., Kunkl A. and Dongworth D.W. (1980). The follicular dendritic cell: its role in antigen presentation in the generation of immunological memory. Immunol. Rev. 53, 3-28.

Mandel T.E., Phipps R.P., Abbot A. and Tew J.G. (1980). The follicular dendritic cell: long term antigen retention during immunity. Immunol. Rev. 53, 29-59.

Mebius R.E., Bauer J., Twisk A.J.T., Breve J. and Kraal G. (1991a). The functional activity of high endothelial venules: a role for the subcapsular sinus macrophages in the lymph node. Immunobiology 182, 277-291.

Mebius R.E., Streeter P.R., Breve J., Duijvestijn J. and Kraal G. (1991b). The influence of afferent lymphatic vessel interruption on vascular addressin expression. J. Cell Biol. 115, 85-95.

Miotti R. (1965). Die Lymphknoten und Lymphgefasse der weissen Ratte *(Rattus norvegicus berkenfout, Epimis Norvegicus)*. Acta Anat. 62, 489-527.

Nieuwenhuis P. and Opstelten D. (1984). Functional anatomy of germinal centers. Am. J. Anat. 170, 421-435.

Nossal G.J.V. and Ada G.L. (1971). Antigens, lymphoid cells, and the immune response. Academic Press. New York-London.

Nossal G.J.V., Ada G.L. and Austin C.M. (1964). Antigen in immunity. IV. Cellular localization of ^{125}I- and ^{131}I-labelled flagella in lymph nodes. Aust. J. Exp. Biol. Med. Sci. 42, 311-330.

Sainte-Marie G., Peng F.S. and Bélisle C. (1982). Overall architecture and pattern of lymph flow in the rat lymph node. Am. J. Anat. 164, 275-309.

Tilney N.L. (1971). Patterns of lymphatic drainage in the adult laboratory rat. J. Anat. 109, 369-383.

Accepted February 25, 1993