

## INHIBITION OF MITOSIS IN THE GUINEA PIG THYMUS AFTER LIGATION OF DRAINING EFFERENT LYMPHATICS

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### ABSTRACT

*Ligature of the cervical lymph duct in guinea pigs resulted in marked dilatation of the lymphatics draining the thymus and marked reduction of mitosis in the thymus. A decrease of about 50% in the number of mitoses was found in the cortex within one day after ligation and in the medulla within five days after ligation. The thickness of the cortex also decreased rapidly and markedly in direct proportion to the decrease in cortical mitosis. Mitosis in the thymus recovered within 10 days after ligation, presumably after regeneration of lymphatics or collateralization. Thickening of the walls and valves of the efferent lymphatics of the thymus after lymph congestion was also demonstrated.*

The thymus has an essential immunological function as a central lymphoid organ by producing lymphocytes, that are primarily discharged into the general circulation by the lymphatic route (9,1). The present study investigated the important role of thymus gland lymphatics in lymphopoiesis.

### MATERIALS AND METHODS

Male guinea pigs of the outbred Hartley strain, 4-5 weeks of age and weighing 240-260g were used in this study. In the guinea pig, the thymus is located in the upper ventral part of the neck, and its efferent lymphatic vessels enter the cervical lymph duct that descends

towards the cervical lymph nodes. Through a midline incision in the neck under ether anesthesia, the cervical lymph nodes on both sides were exposed by blunt dissection, ligated tightly as a whole, and removed, resulting in ligation of the cervical lymph ducts. The cervical lymph ducts became dilated superiorly immediately after this maneuver. The guinea pigs were killed by ether inhalation 1 to 15 days after ligation and the thymic lobes were excised with the surrounding connective tissue. Six hours before sacrifice, the guinea pigs were injected subcutaneously with colchicine at a dose of 0.1 mg/100 g body weight. The mid one-third of each lobe was fixed in Carnoy's solution, embedded in paraffin wax, sectioned at a thickness of 6  $\mu$ m and then stained with hematoxylin and eosin. The thickness of the cortex was measured using a micrometer at locations that were vertically sectioned. The medulla was not measured because of its indefinite form. The ratio of the number of cells in mitotic metaphase to the total number of cells (1130-2070) in each cortex and medulla was calculated. The statistical significance of differences in mean values was assessed using Student's t-test: differences at  $P < 0.05$  were considered significant. The thymus was examined 5 days after exposure of the cervical lymph nodes in the same manner except without ligation and extirpation (sham-operated controls). The thymus of non-treated guinea pigs was also examined as normal controls.

**TABLE 1**  
**Effect of Ligation of the Cervical Lymph Duct on Guinea Pig Thymus Lymphopoiesis**

Days after ligation	Thickness of cortex ( $\mu\text{m}$ )	Percentage of mitotic cells <sup>1</sup>	
		Cortex	Medulla
Normal	25.2 $\pm$ 0.7 <sup>2</sup>	48.6 $\pm$ 1.9	7.3 $\pm$ 0.5
Sham-operated <sup>3</sup>	26.8 $\pm$ 1.2	50.1 $\pm$ 2.8	7.8 $\pm$ 0.7
1	22.8 $\pm$ 0.5*	29.0 $\pm$ 2.4*	5.7 $\pm$ 1.0***
3	17.1 $\pm$ 0.5*	22.4 $\pm$ 1.6*	5.9 $\pm$ 0.8***
5	17.8 $\pm$ 0.6*	18.5 $\pm$ 2.9*	3.6 $\pm$ 0.6*
7	22.2 $\pm$ 0.6*	23.4 $\pm$ 2.9*	4.5 $\pm$ 0.5**
10	23.3 $\pm$ 0.5**	50.5 $\pm$ 1.7	6.2 $\pm$ 1.2
15	23.1 $\pm$ 0.5**	48.6 $\pm$ 2.7	7.1 $\pm$ 0.4

<sup>1</sup>The number of mitotic cells was calculated 6h after colchicine administration.  
<sup>2</sup>Mean  $\pm$  S.E. of 3-5 guinea pigs (6-8 lobes).  
<sup>3</sup>5 days after exposure of the cervical lymph nodes without ligation and extirpation.  
\*P<0.001, \*\*P<0.001, \*\*\*P<0.05.

## RESULTS

### *Changes in Mitosis in the Thymus*

No differences were found between normal and sham-operated controls either in mitosis in the thymus or in thickness of the thymic cortex (*Table 1*).

Mitosis in the thymus decreased rapidly and markedly after ligation of the cervical lymph duct (*Fig. 1*). A decrease of about 50% in the number of mitoses was found in the cortex within one day after ligation and in the medulla within five days after ligation. The number of mitoses in the thymus recovered within 10 days after ligation. A significant decrease in cortical thickness also occurred rapidly in direct proportion to the decrease of mitosis in the cortex.

### *Changes in the Lymphatics Draining the Thymus*

The efferent lymphatics of the thymus showed marked dilatation within one day after ligation of the cervical lymph duct. Dilatation of the efferent lymphatics progressed further during 3 to 7 days after ligation (*Fig. 2*), and the capsular lymphatics also became dilated (*Fig. 1b*). The wall of the dilated lymphatics became partially thickened 3-5 days after ligation (*Fig. 2*), and almost the total length was involved by 7-10 days (*Fig. 3*). Pronounced thickening of the valves of the efferent lymphatics was also observed (*Figs. 2 and 3*). In contrast, no changes were observed in the accompanying artery and vein. The lymphatics which formed a network in the pronounced interlobular accumulation of lymphocytes dilated greatly (*Fig. 4*). Moreover, tiny tubular structures containing various numbers of lymphocytes were often seen in the medulla (*Fig. 4*), where the endothelial cell lining was barely detectable. Fifteen days after ligation, the efferent lymphatics were

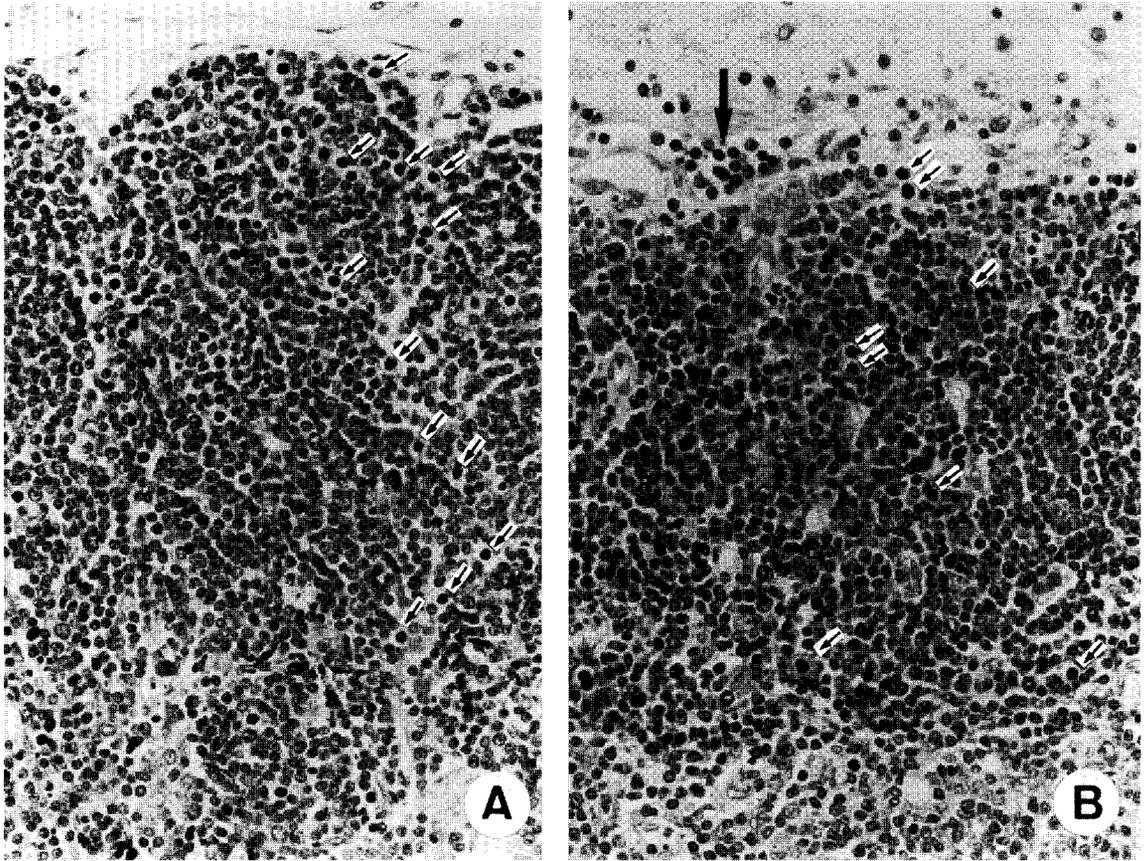


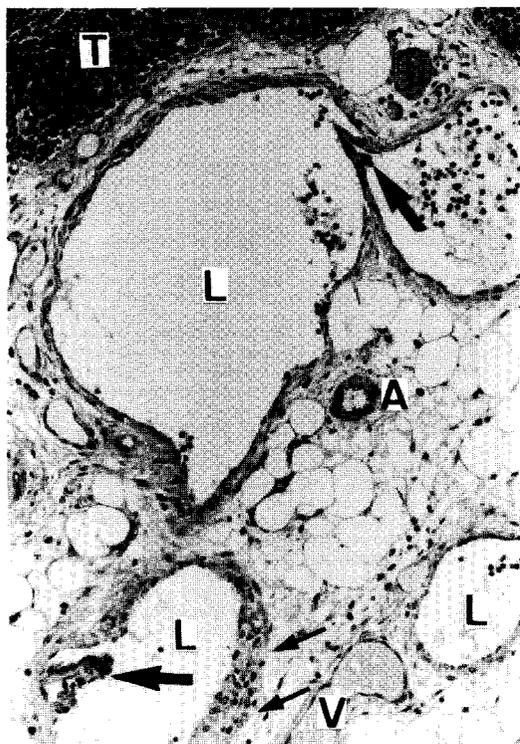
Fig. 1. Photomicrographs of (A) normal guinea pig thymus (control) and (B) thymus 7 days after ligation of the draining lymphatics. Note that mitotic figures (small arrows) in the thymus are comparatively scarce. Colchicine was injected 6 h before sacrifice. A dilated capsular lymphatic vessel is also displayed (large arrow). H. & E. x280.

still dilated but were not as conspicuous in two of the three guinea pigs. The thickening of the walls and valves of the efferent lymphatics thereafter mostly disappeared.

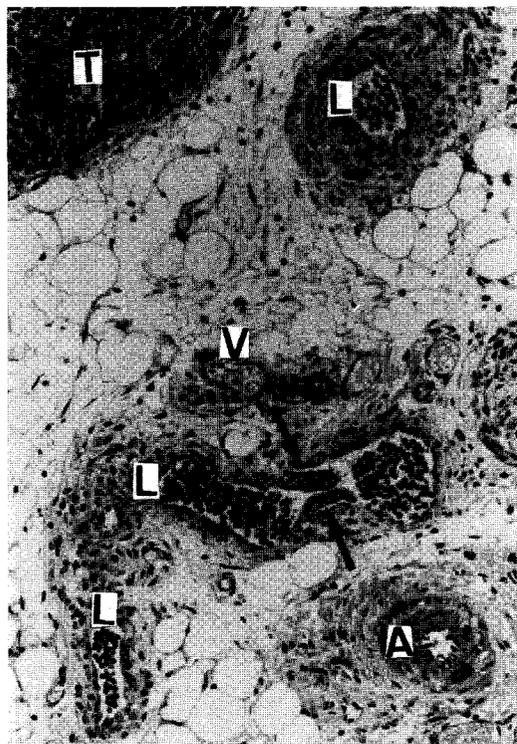
#### DISCUSSION

The lymphatics or an analogous draining system are essential for the function of each organ. For example, testicular lymphatics have a close relationship to spermatogenesis and testosterone secretion (11). In the thymus the lymphatics contribute to the transport of thymic lymphocytes into the general circulation (9,10). The present study demonstrated another important role of the thymic

lymphatics in influencing lymphopoiesis. The production of immunocompetent T lymphocytes in the thymus was markedly inhibited by lymph congestion. The nature of the signal necessary for maintaining lymphocyte mitosis in the thymus is still not clear. In contrast to lymph nodes, the thymus is a central lymphoid organ lacking afferent lymphatics through which antigens stimulating lymphopoiesis are transported from the periphery. In the thymus, it seems likely that thymic epithelial (7) or nurse cells (3) play a dominant role in T-cell differentiation through secretion of certain thymic hormones. The microenvironmental disorders of thymic cells including epithelial or nurse cells



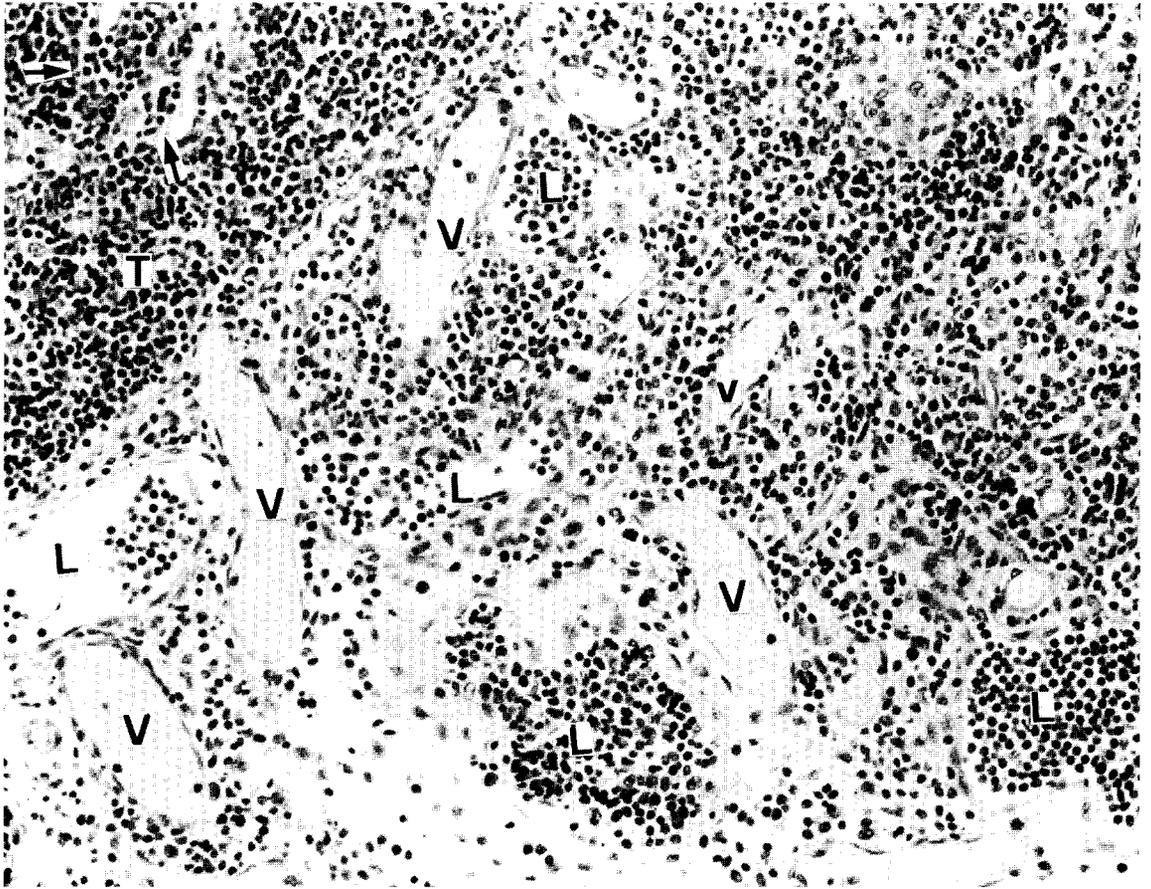
*Fig. 2. Photomicrograph of guinea pig thymus 3 days after ligation of draining lymphatics. The efferent lymphatic vessels (L) of the thymus (T) are greatly dilated. The walls of these lymphatics show partial but conspicuous thickening (small arrows). Although the thickening of a lymphatic valve (large arrow) in the upper field is slight, another valve (large arrow) in the lower field is markedly thickened. The accompanying vein (V) is filled with erythrocytes. (A), artery. H. & E. x120.*



*Fig. 3. Photomicrograph of guinea pig thymus 10 days after ligation of draining lymphatics. The walls of the efferent lymphatic vessels (L) of the thymus (T) are thickened along the total length. The thickest wall of the lymphatics has a thickness similar to the wall of the accompanying artery (A), which is clearly distinguishable from the lymphatics by its compact media. Unlike the accompanying artery, the lymphatics contain a large number of lymphocytes in their wide lumina. The accompanying vein (V) is filled with erythrocytes. A markedly thickened lymphatic valve is indicated by arrows. H. & E. x120.*

due to stasis of tissue fluids are thought to be a major cause of inhibition of lymphocyte proliferation (8). It has been reported by Osogoe (14) in lymph nodes of rabbits that lymphocytes within the dilated lymphatic sinuses become scanty when the efferent lymphatics are blocked. Moreover, the cortex is reduced both in cellularity and amount and the medullary cords are thickened through fibrosis. All these changes in lymph nodes are also ascribed to high lymph pressure produced by the obstruction of lymph flow (13,21).

In the present study, the cervical lymph duct was ligated in order to obstruct the flow of lymph from the thymus. Therefore, the function of various organs other than the thymus may have been concomitantly affected by lymph congestion. In particular, dysfunction of the thyroid gland may have altered lymphopoiesis in the thymus (1,18). Since cerebrospinal fluid drains into the cervical lymph duct, and its obstruction induces lymphostatic encephalopathy (4), disruption of the hypothalamic-pituitary axis may also have contributed to inhibition of



*Fig. 4. Photograph of guinea pig thymus 7 days after ligation. The lymphatic vessels (L) forming a network in the pronounced interlobular accumulation of lymphocytes show great dilatation and contain a large number of lymphocytes only in their lumina. The veins (V) are filled with erythrocytes. The tiny vascular structures containing lymphocytes in the medulla of the thymus (T) are indicated by arrows. H. & E. x220.*

lymphopoiesis in the thymus perhaps related to growth hormone and prolactin output (2,15). Lymphocyte proliferation in the thymus, however, recovered rapidly within 10 days after lymphatic ligation, presumably through regeneration of the lymphatics or collateralization. Lymphatic pathways are generally reconstituted within two to three weeks after their severance or ligation including the larger lymphatic trunks (16,21).

A characteristic change seen in the obstructed lymphatics was thickening of their walls and valves. Gross expansion of the lymph vessels and notably increased intraluminal lymph pressure along with

“obliterative lymphangitis” (21) may have induced this thickening. Rapid thickening due to active fiber formation in the walls of the deep cervical lymph duct after ligation has previously been reported in rabbits (12). Tiny tubular structures containing lymphocytes often became visible in the medulla only after lymph congestion. Opinions on the existence of lymphatic vessels within the thymic parenchyma are still controversial. Some maintain that the lymphatics begin in the medullary parenchyma (5,6,19) whereas others claim that they begin in the perivascular spaces (17) formed by protrusion of the interlobular connective tissue into the

medulla or the corticomedullary junction (9,10,20). In this study, it was unclear whether the tiny tubular structures visualized are true initial lymphatic vessels or prelymphatic tissue spaces. Nonetheless, the ligation model seems useful to define the presence of initial lymphatics in the thymic parenchyma using electron microscopy or histochemistry.

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