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MICROANATOMY OF THE BLOOD VASCULATURE OF LYMPH NODE FOLLICLES IN THE DOG

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ABSTRACT

We studied the anatomical features of the arterial vasculature of the lymphoid follicles (LF) of tracheobronchial lymph nodes in the dog. The microvascular organization of the lymph node was visualized after systemic perfusion of the canine arterial system with gelatinated colloidal carbon. We found that LF contained a substantial number of blood capillaries forming a vascular network with a density comparable to that of neighboring domains of the node. The density of the vascular supply was greater in the secondary than in the primary LF. The geometric arrangement of LF capillaries was also different in the two types of LF.

These findings are in contrast with the current view that the LF is a vascular-poor domain of the lymph node. We also document the plasticity of the LF blood microvasculature that appears to proliferate in conjunction with lymphoblast turnover and immune reactivity characteristic of secondary LF.

Lymphoid follicles (LF) make up a key functional component of the cortical domain of the lymph node (1,2). They contain lymphoblasts that, in response to an antigenic stimulus, undergo proliferation and differentiation into antibody-producing mature B cells (1,2). The organization of the LF is different in resting (primary) follicles compared with active, immunoreactive (secondary) nodules (2,3). In fact, the secondary follicles are larger than the primary LF as a result of the blastic proliferation of lymphocytes in the LF core, the so-called germinal center. Current views on the arterial blood vessels that supply the LF are derived mainly from observations on small rodents (rats, mice, and guinea pigs) and sheep where these arteries apparently end at the LF periphery (4-8). To pursue this subject further, we investigated the vascular anatomy of the LF in dogs. We used anatomical methods that allowed correlation of broad overviews of the vascular arrangement of the nodules with the identification of the LF type. Contrary to prevailing views, we found the LF domain of the lymph node showed, at least in the dog, a well developed network of blood capillaries.

MATERIALS AND METHODS

Animals

Five male and female adult mongrel dogs were used in this study. The dogs were housed in individual kennels, fed standard commercial dog food, and had unrestrained access to water. The dogs were anesthetized by intravenous injection of sodium pentobarbital (40mg/kg body weight), the

abdominal cavity opened, and the dogs bled by cutting the inferior vena cava. A cannula was introduced in the abdominal aorta and the arterial system was washed with warm saline before intraarterial perfusion with the colloidal carbon marker.

Injection of Colloidal Carbon

Particles of colloidal carbon (Pelikan China ink) were suspended in 30% gelatin and injected into the upper portion of the abdominal aorta in order to visualize the tracheobronchial lymph nodes.

Processing of Lymph Nodes

Three to four tracheobronchial lymph nodes were excised from each dog and fixed in 10% formol. Each lymph node was cut through the hilar domain into three fragments of roughly the same volume. Each was serially sectioned according to three different procedures: (a) 1mm-thick tissue layers that were cleared according to Spalteholz method (9,10); (b) serial frozen sections with widths of 60, 150, and 300µm that were cleared in xylol; (c) 4-6µwide sections that were used for hematoxylin eosin staining after being obtained from paraffin-embedded fragments. Each serial section of the lymph nodes was mounted in a glass slide and studied by light microscopy.

RESULTS

Blood vessels were readily identified in hematoxylin-eosin stained sections of lymph nodes by the dark pigment left in the lumen of these vessels by the presence of gelatinated colloidal carbon particles as illustrated in *Fig. 1*. Although in these relatively thin sections the general distribution and architecture of the arterial vasculature was not easily recognized, the hematoxylin-eosin sections, nonetheless, allowed accurate identification of primary and secondary LF. In favorable views of these preparations, arterial capillaries were seen penetrating deeply into the LF domains *(Fig. 2)*.

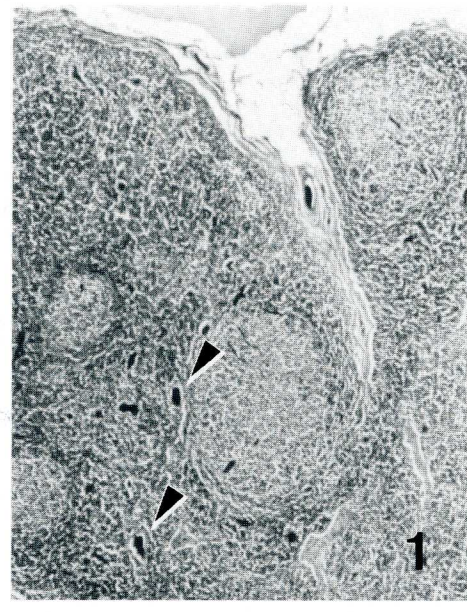

Fig. 1. Light micrograph of a section of the cortex of a dog tracheobronchial lymph node showing the black pigment of colloidal carbon (arrowheads) filling the lumen of the arterial vessels. Hematoxylin-eosin stained section of a paraffin-embedded tissue fragment (x50).

For more complete views of the arterial vessels, the xylol-cleared thick sections showed that the LF displayed blood capillaries in densities that were comparable to the capillary density of surrounding domains of the dog lymph node *(Fig. 3)*. The density and topographic organization of the arterial vasculature was different in primary *(Fig. 3**A**)* and secondary *(Fig. 3**B**)* LF. Thus, arterial capillaries were sparser in primary LF and appeared to originate as straight lines from a spherical network located at the periphery of the nodules *(Fig. 3**A**)*. Inside secondary LF, the arterial capillaries were more numerous and appeared to originate from a centripetal branching of larger arterial vessels that penetrated the core of the follicles *(Fig. 3**B**)*.

DISCUSSION

Recirculation of lymphocytes through lymph nodes is a key feature of the

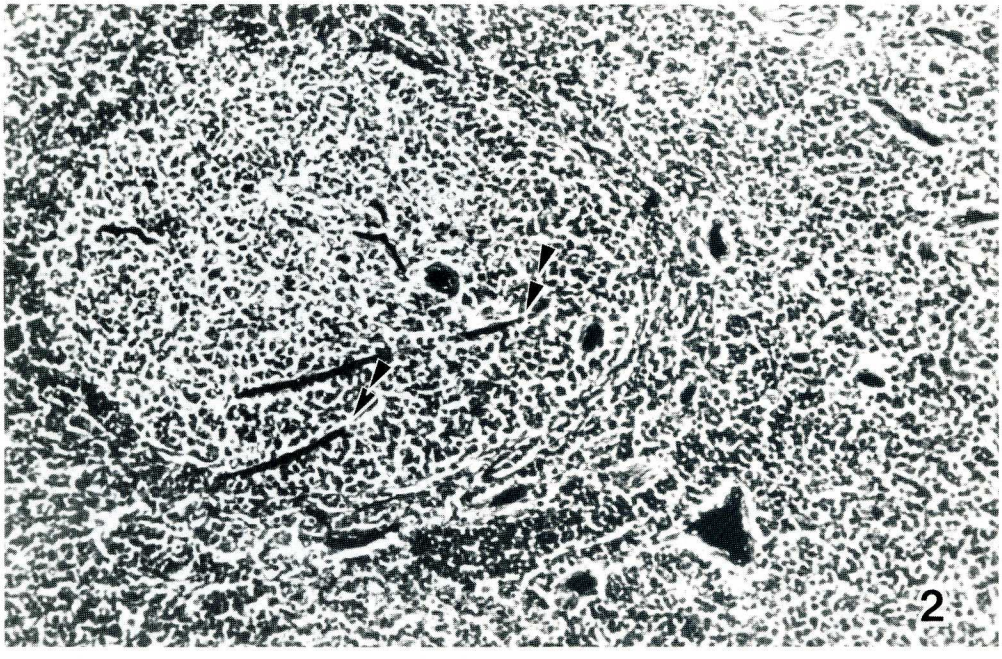

Fig. 2. Light micrograph of hematoxylin-eosin stained section of paraffin-embedded tracheobronchial lymph node of the dog showing the penetration of a lymph follicle by arterial capillaries as visualized by colloidal carbon filling of the lumen of the blood vessels (arrowheads) (x180).

physiology of the immune system as was demonstrated in a classical series of experiments by Gowans and co-workers (11). Since then, the importance of this physiological feature has led to a number of studies particularly aimed at a detailed delineation of the anatomical basis of circulation in the lymph node (12-21). These investigations have revealed that lymph nodes are highly compartmentalized organs made up of multiple histological domains where blood vessels follow particular patterns of development and arrangement. As with high endothelial venules (22), this blood vascular pattern directly relates to the pathways of lymphocyte migration in the lymph node.

The lymphoid follicle (LF) makes up the most dynamic domain of the lymph node since it is capable of undergoing rapid and profound enlargement due to lymphoblast proliferation in response to antigenic stimuli (1,3). In spite of its pivotal role and plasticity, previous studies have suggested that the blood vasculature of LF is poorly developed (8,12-14). In fact, LF are generally considered to contain just a few blood capillaries located at the periphery of the nodules (5-7). This observation has been interpreted to indicate the relative independence of reactive LF to circulating substances in the bloodstream (8). In the present study, however, we describe anatomical observations in dog lymph nodes that disagree with this prevailing view which has heretofore been based largely on experiments in small rodents. Indeed, not only is there not a paucity of blood vessels in LF in the dog, but the LF appear as rich in arterial vessels as the adjacent surrounding cortical area of the lymph node. Moreover, the density of the LF blood capillaries is also increased in secondary LF as compared with primary nodules. This enhancement in blood capillaries in secondary LF conforms with the previous reports of Herman and co-workers (23,24). Furthermore, the architectural arrangements of the dog blood

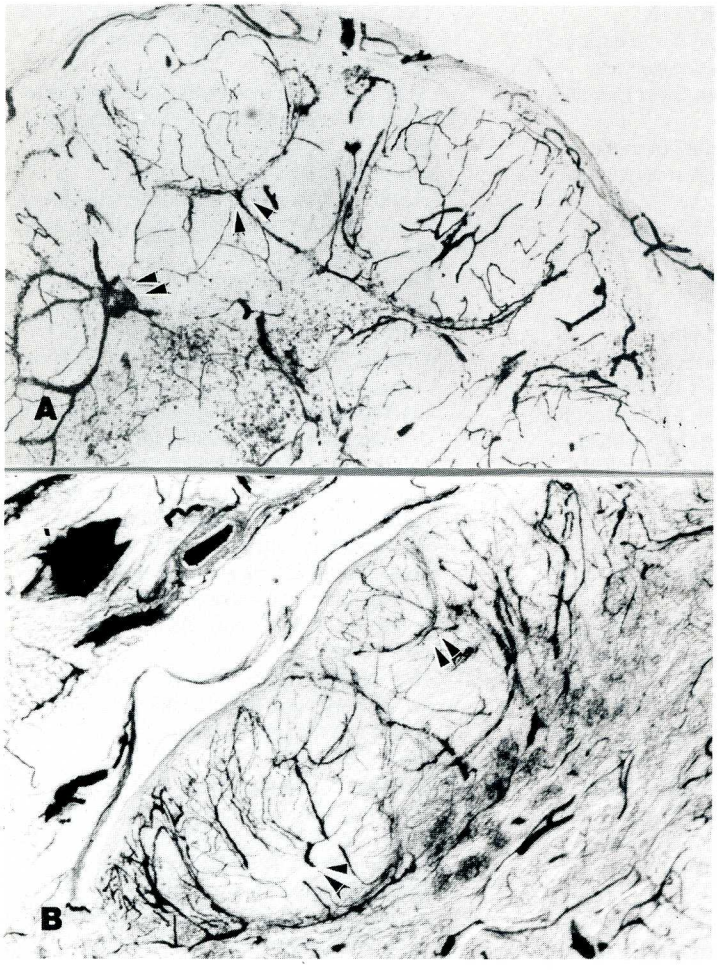

*Fig. 3. Light micrographs showing the tracing of arterial vessels associated with primary (**A**) and secondary (**B**) follicles of tracheo-bronchial lymph nodes of the dog. In primary follicles (**A**), the network of capillaries is composed primarily of straight lines emanating from the periphery of the follicle; the centripetal branching of the arterioles into the follicles is indicated by arrowheads. The centrifugal branching of arterioles in the secondary follicles is seen in **B** (arrowheads) where a denser network of capillaries is seen inside the follicles (**B**). (**A**) x110 and (**B**) x125.*

vessels inside the LF is distinct in the two types of LF. Thus, primary LF show a centripetal microvascular arrangement that is in contrast to the centrifugal branching observed in secondary LF. These patterns are at variance with those recently proposed by Bélisle and Sainte-Marie (8). In conclusion, the blood microvasculature of LF is well developed, at least in the dog, and shows even a greater density in secondary than primary lymphoid follicles.

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