

The Distribution and Ultrastructure of Renal Lymphatic Vessels Proliferating in Response to Kidney Injury in the Dog

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Summary

A study of lymphatic proliferation around the sites of chronic injury has clarified the process by which new lymphatic vessels are formed. Sterile wooden splinters were introduced through the renal capsule into the cortex and the medulla. Retrograde carbon injection of lymphatic trunks four weeks after splinter implantation revealed a profusion of new lymphatic vessels in the renal cortex with an extension of these vessels into the outer medulla of the dog. Ultrastructurally, these new lymphatic vessels were characterized by a large leading cell and absence of basement membrane and anchoring filaments. Carbon particles used as a lymphatic marker were found in both the lymphatic lumen and penetrating a small canal formed by the leading cell. Thus, the present study supports the hypothesis that new lymphatics arise from endothelial sprouts of pre-existing lymphatic vessels.

Introduction

In a previous publication it was shown that the lymphatics of the renal cortex were distributed primarily along the arteries and veins of the interlobular spaces (1). An extension of these studies elucidated the ultrastructure of lymphatic vessels in relation to other intrarenal structures (2). During the course of these studies focal surface lesions were observed in some kidneys. India ink injection of lymphatic trunks in these areas revealed a dense lymphatic plexus in the parenchyma surrounding the site of injury. The present study was designed to further investigate this phenomenon in kidneys with experimentally induced lesions and to shed light on the process by which new lymphatic vessels are formed.

Methods

Dogs were anesthetized with sodium pentobarbital (30 mg/kg) and the left kidney exposed through a flank incision. Four sterile splinters (3 x 20 mm) were implanted into the anterior pole of each kidney. The incision was closed and the animal allowed to recover. Each animal received 600,000 units of Benzathine Penicillin G intramuscularly over a three day period following surgery.

These animals were reanesthetized four weeks following the implantation procedure. The left kidney was again exposed and examined for prominent capsular lymphatic vessels in the areas of the implanted splinters. When such vessels were observed, they were injected with India ink in a retrograde manner as previously described (1). After sacrificing the animal, 10 ml of 6.25% glutaraldehyde in phosphate buffered saline was introduced into the kidney via the renal artery and the kidney was removed. Blocks of renal tissue containing the splinters were fixed in additional glutaraldehyde and post fixed in 1% phosphate buffered osmium tetroxide for two hours. The blocks were dehydrated in graded ethanol and imbedded in araldite. The polymerized blocks were sectioned,

mounted on copper grids and stained with uranyl acetate and lead citrate (3). Micrographs were taken at various magnifications on a Hitachi HU-11B electron microscope. Additional specimens for light microscopy were fixed in buffered formalin and macro-sectioned with a Stadie microtome. Sections were dehydrated in graded alcohol, cleared in xylene and mounted.

Results

A typical pattern of injected lymphatic vessels around a splinter site is shown in Fig. 1. This low magnification photograph shows the dense cortical plexus and an extension into the outer medullary area.

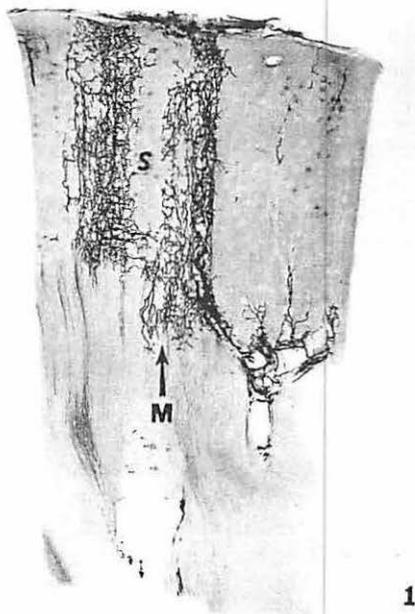


Fig. 1. A Stadie microtome section showing the dense plexus of newly formed lymphatic vessels around a splinter site (S). Extension of the lymphatic plexus into the outer medulla is seen (M). X 5,5



Fig. 2. This micrograph depicts the scarred zone around the splinter site. The upper edge shows the epithelial layer (E) immediately adjacent to the splinter. The dense, collagenous scar tissue (S) is bisected by a lymph capillary (L) containing injected carbon. X 7,750

While ten dogs were utilized in this study, only the three best specimens were selected for electron microscopy. Ultrastructurally, the tissue adjacent to the splinter resembled scar tissue. There was an epithelialization of the tissue-splinter interface and a dense collagenous zone between this epithelial layer and the normal renal parenchyma. Within the scarred area there were many carbon-filled lymphatics (Fig. 2); the largest of these seemed to run parallel to the course of the splinter (Fig. 3). From these large lymphatics, lateral branching smaller lymphatic vessels crossed the scar tissue and penetrated into the interstitium surrounding the undamaged renal parenchyma (Fig. 4). In the same areas there were large numbers of blood vascular components, which, on occasion, ran parallel to the lymphatics.

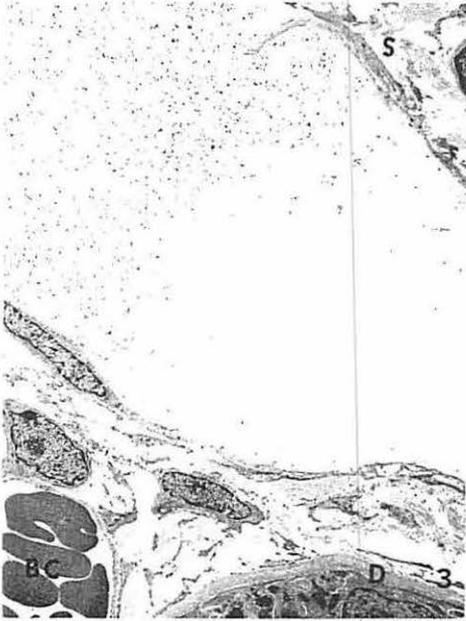


Fig. 3. This picture illustrates the size of the lymph veins which parallel the length of the splinter and appear to give off lateral branches into nearby parenchyma. Distal convoluted tubules (D), blood capillary (BC), scar tissue (S) are shown. X 4,500



Fig. 4. Scar zone adjacent to proximal tubules (P). Note the carbon containing lymph capillary (L) in the collagenous zone which appears to be avoiding a pre-existing blood vessel (B). The leading cell (C) of this lymph capillary has numerous vacuoles which contain carbon particles (→), indicating that a patent channel exists in the cytoplasm. X 7,750

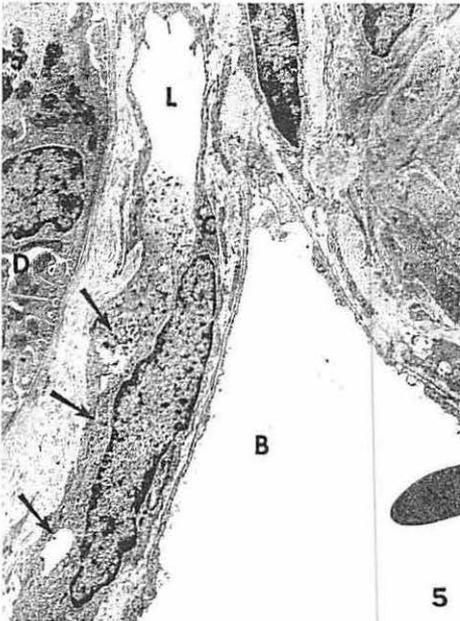


Fig. 5. Parenchymal area immediately adjacent to the scar zone. A lymph capillary is shown containing carbon in the lumen (L) is seen to be continuous with the small channel which is forming in the cytoplasm. Note the carbon particles (→) in the newly formed channel. X 7,750

The newly formed lymph vessels were characterized by an absence of both basement membrane and anchoring filaments (Fig. 2). In addition, a large leading cell was located at the tip of the newly formed lymphatic sprout. These leading cells appear to have advanced in the direction of least resistance but were always in direct contact with lymphatic endothelium.

Carbon particles injected into the lymph trunk were observed in both lymphatic capillary lumina and groove-like spaces adjacent to the cytoplasm of the leading cells (Fig. 5). This cytoplasmic groove appeared to be continuous with the lumen of the lymph capillary.

Discussion

Pullinger and Florey (4) studied lymphatic proliferation following the subcutaneous injection of either turpentine or amorphous silica in rat ears. In these experiments, a rich plexus of newly formed lymphatic vessels were observed around the injection site. A new growth of lymphatics was noted 12 days after the injection and seemed to increase up to one month. After three months the number of lymphatics decreased around the turpentine site but persisted up to 6 months around the silica site.

In the present study the growth of new lymphatics around a wooden splinter was studied following implantation for one month. The results demonstrate a dense plexus of new lymphatic vessels around the site of chronic injury similar to those described by *Pullinger and Florey*. In chronically injured kidneys, this plexus was formed by proliferation of cortical lymphatics at the site of injury and a downward extension of these vessels into the medulla. This contrasts with previous studies on normal kidneys, in which the lymph vasculature was distributed mainly along major blood vessels of the cortex, but was absent from the medulla (1, 5).

Electron microscopic studies on renal lymphatics of the normal kidney have been conducted by several investigators (2, 5, 6, 7, 8). The ultrastructural characteristics of newly formed lymphatics around the splinter site in the present study were similar to those seen in normal kidneys with two exceptions, i.e., the complete absence of basement membrane and anchoring filaments. This does not exclude the possibility that a basement membrane and/or anchoring filaments might be formed at some later stage.

Many anatomical studies have shown that new blood capillary formation proceeds by endothelial sprouts branching laterally from larger vessels (9).

These sprouts are described as having leading cells which advance in front of the lumen and either form the lumen by direct extension of the lumen into its cytoplasm or by coalescence with mesenchymal cells in the surrounding tissue. The present study supports the first of these processes in the formation of new lymphatic capillaries, since groups of free cells have not been observed in these areas. Further, the large leading cell appears to be taking part in the formation of the lumen. In addition, the carbon that was injected into capsular lymphatics has filled the new lymphatic plexus and may be found in the small canal adjacent to the cytoplasm of this cell. Since there was no evidence of extravasation of carbon it appears that a direct communication exists between capsular lymphatics and the growing tip of the lymphatic sprout. However, it is difficult to prove conclusively if the lumen is formed by direct extension into the mass of the cytoplasm of the leading cell or if the cell forms the lumen by wrapping about

itself. This study suggests that this process is accomplished by a single cell, although serial sections of this area do not preclude other interpretations.

A c k n o w l e d g e m e n t s

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References

- 1 *Bell, R.D., M.J. Keyl, F.R. Shrader, E.W. Jones, L.P. Henry*: Renal Lymphatics: the internal distribution. *Nephron* 5 (1968) 454-463
- 2 *Nordquist, R.E., R.D. Bell, R.J. Sinclair, M.J. Keyl*: The distribution and ultrastructural morphology of lymphatic vessels in the canine renal cortex. *Lymphology* 6 (1973) 13-19
- 3 *Reynolds, E.S.*: The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J.Cell Biol.* 17 (1963) 208-212
- 4 *Pullinger, B.D. and H.W. Florey*: Proliferation of lymphatics in inflammation. *J.Path. Bact.* 45 (1937) 157-170
- 5 *Kriz, W. und H.J. Dietrich*: Das Lymphgefäßsystem der Niere bei einigen Säugetieren. *Z.Anat.Entw.Gesch.* 131 (1970) 111-147
- 6 *Rhodin, J.*: Fine structure of the peritubular capillaries of the human kidney. In: *Progress in Pyelonephritis*. Ed. E.H. Kass. F.A. Davis, Philadelphia 1965
- 7 *Huth, F.*: Beiträge zur Orthologie und Pathologie der Lymphgefäße der Nieren. *Beitr. Path.Anat.* 136 (1968) 341-412
- 8 *Ohkuma, M.*: Fine structure and function of cutaneous and renal lymphatic capillary. *Bull.Tokyo Med.Dent.Univ.* 17 (1970) 103-111
- 9 *Clark, E.R., W.J. Hirschler, H.T. Kirby-Smith, R.O. Rex, J.H. Smith*: General observations of the ingrowth of new blood vessels into standardized chambers in the rabbit's ear and the subsequent changes in the newly grown vessels over a period of months. *Anat.Rec.* 50 (1931) 129-167

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