

AN EXPERIMENTAL STUDY OF LYMPHATIC VESSEL AUTOTRANSPLANTATION IN THE DOG

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ABSTRACT

To provide greater insight into microsurgical treatment of lymphedema, 7 mongrel dogs underwent autotransplantation of a lymphatic collector into the contralateral lower limb. Two months later, lymphatic vessels were studied using patent blue dye, light microscopy, and histomorphometry.

Patency of the transplanted lymphatics was uniformly observed although there was a notable increase in the width of the intima and a corresponding reduction in the size of the lymphatic lumen. Of note, there was a striking absence of inflammatory infiltrate at the recipient site.

Surgery for lymphedema has greatly advanced since Nielubowicz and Olszewski (1) first performed lymph nodal-venous shunts and later O'Brien (2) direct lymphatic-venous anastomoses to improve lymphatic drainage. Howard (3) and Stevens (4), however, originally suggested direct lymphatic collector repair by means of an end-to-end lymphatic truncal anastomosis as an alternative technique for treating lymphedema. Two decades later, Campisi (5), Baumeister (6), and Mandl (7) have updated this concept by interposing lymph collectors and veins. Transplantation of lymphatic vessels is nowadays considered a plausible but unproven treatment for secondary lymphedema. The object of this transplantation technique is to reconstruct lymphatic continu-

ity and thereby improve lymph drainage in those individuals in whom a local blockage to the circulation of lymph follows lymph node resection, irradiation, or trauma. To shed light on this subject, we examined the evolution and function of lymphatic autotransplantation in dogs.

MATERIALS AND METHODS

In 7 mongrel dogs (BW 20±5kg) under standard general anesthesia, an epifascial lymph collector located in the medial portion of the left thigh of the hind or forelimb was isolated and a dissected fragment 6 to 10cm in length was removed and kept in isotonic saline. In each dog, a lymphatic collector of similar length was excised from the right (contralateral) thigh, after which the lymphatic collector previously removed from the left side was inserted and using microsurgical technique with a Zeiss OPMI-6 microscope, an end-to-end lymphatic-lymphatic autograft anastomosis was performed with 11-0 suture..

The function of the autoimplanted lymphatic grafts was reevaluated two months later and compared to normal lymphatic collectors (controls) of the same region using intradermally injected patent blue dye, light microscopy, and histomorphometry.

Visual inspection with patent blue was performed by injecting the dye into the distal end of the lower limb of the

dog and observing the drainage passage of the dye within the limb and transplanted collector. Standard histologic processing was also performed of the transplanted collector and selected sections were stained with hematoxylin-eosin, Masson-trichrome, and orcein. For histomorphometry, a microscope was used which projects slides over a digital plane. The data was processed in a semiautomatic autoanalyzer Ibas I MOP-videoplan (Kontron), which performed planimetry through an incorporated program of stereology.

After determining the perimeters and areas of each layer of the lymphatic vessel, the maximum external diameter, and percent of media, intima, and lumen size as a function of the total area of the lymphatic collector were compared.

Statistical analysis was based on the Mann-Whitney method (U-test).

RESULTS

Using patent blue dye, patency of the transplanted lymph collector was consistently demonstrated. Lymphatic patency was further confirmed by light microscopy. In contrast to the contralateral lymphatic removed at the same time, disruption of the transplanted lymph vessel wall was not observed.

When control (intact adjacent lymphatic collectors) and transplanted lymphatic vessel was studied with orcein no differences in elastic fibers of the lymphatic wall were found. Both displayed (Fig. 1) a thin, continuous internal elastic lamina separating the intima from the media as well as an area of fragmented elastic fibers in the outer margin of the media. These elastic fibers sometimes formed a segmental external elastic layer marking the dividing line with the adventitia of the lymphatic collector.

The transplanted lymphatic vessels showed, however, differences insofar as the intima was concerned (Fig. 1C and D, Fig. 2) with thickening of the transplanted lymphatics and a corresponding reduction in lumen size. The neointima also at times showed recanalization and the lu-

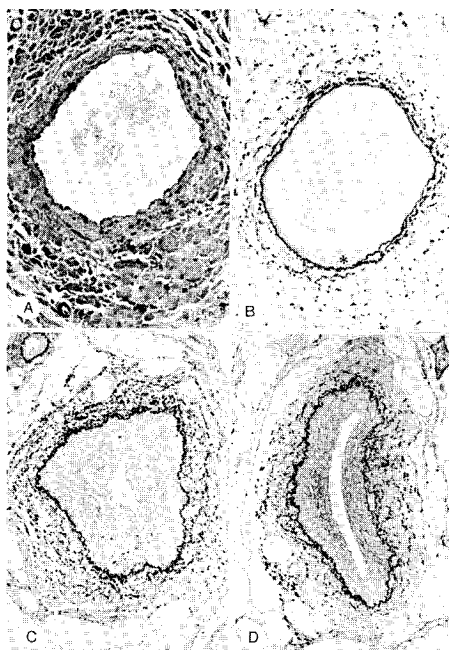


Fig. 1. (A) Normal lymphatic vessel: The wall is formed by collagen fibers and isolated myocytes (Masson trichrome). (B) The intima shows patchy increase (*) with a continuous elastic lamina. The wall also contains fragmented elastic fibers (Orcein of Shikata, MO $\times 40$). (C and D) Transplanted lymphatic vessel: The intima is considerably increased with a corresponding reduction in lumen size. Otherwise, the internal elastic lamina and the wall show no significant changes (Orcein of Shikata, MO $\times 40$).

men remained patent. Histomorphometric studies showed that despite the apparent smaller diameter of the transplanted lymphatics as seen in the operative field, the external diameter of the lymph collector was similar to control lymphatics ($191 \pm 24 \mu$; mean \pm SE vs $172 \pm 53 \mu$; NS). The most significant differences are seen in Fig. 3 where the lumen of the transplanted lymphatics was considerably narrower due to intimal overgrowth. The percentage of the media was, however, decreased in the transplanted lymphatic vessels.

DISCUSSION

Although numerous clinical and experimental papers deal with the diagno-

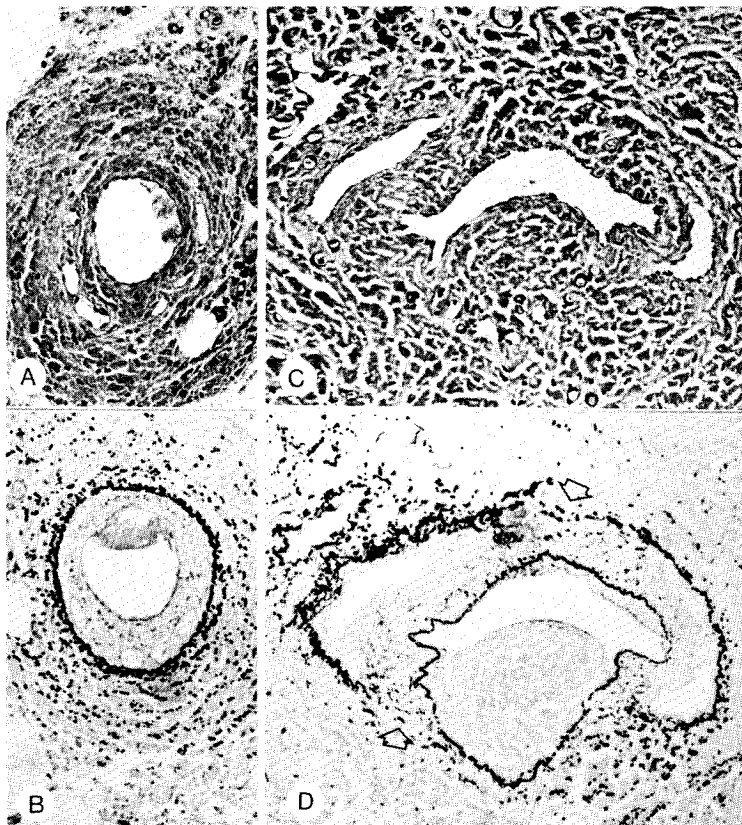


Fig. 2. (A and B) Transplanted lymphatic vessel: A thickened intima shows small vascular channels. The other vascular layers are normal (A: Masson trichrome; B: Orcein of Shikata, MO x40). (C and D) Transplanted lymphatic vessel: Signs of recanalization are more evident with disruption of the internal elastic lamina (arrows--D) In the center of the image (D) there is neovascularization with formation of a new internal elastic lamina (C: Masson trichrome; D: Orcein of Shikata, MO x40).

sis (8) and treatment (9) of lymphedema, we examined healthy dogs to avoid distortions in lumen size from an artifactual increase in intraluminal lymphatic pressure associated with lymphedema. Working with a low pressure lymphatic system made functional continuity of the autografts more difficult but, on the other hand, assured vessel function under more favorable conditions. As Krilov (10) demonstrated, for example, lymphatic-venous anastomoses were patent only as long as intraluminal lymphatic pressure remained high. Nonetheless, in our experiments using a normal (low) pressure system, transplanted lymphatic collectors were

shown to remain patent for as long as two months following autografting using direct visualization (patent blue) and light microscopy.

Examination of lymphatic viability and structure using histomorphometry was also useful as objectively demonstrating a reduction in luminal area of autografted lymphatics while at the same time quantifying thickness of the different layers in the transplanted lymphatic collectors.

At this stage, transplantation of lymphatic vessels carries a definite risk of lumen occlusion as suggested by early (two months) intimal proliferation. How-

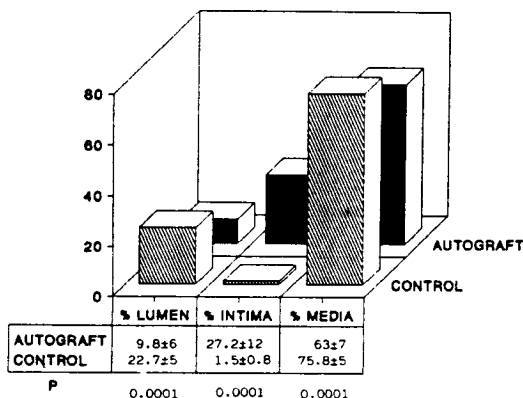


Fig. 3. Percent (mean \pm SE) of media, intima, and lumen of the grafted lymphatic collector as compared to control. Note increased intimal thickness, slight reduction in media, and decreased lumen size in the transplanted lymphatic. Significant differences are $p < 0.0001$.

ever, in two instances recanalization was also observed with continued vessel patency suggesting potential reversibility of the proliferating process.

The reason for intimal proliferation is unclear although operative trauma itself may be a contributing factor. Another possibility is a disturbance in lymphatic contractility and flow as, for example, from lymphangion denervation as a result of free graft transplantation. It is also worth noting that the recipient site of lymphatic implantation was strikingly devoid of an inflammatory infiltrate.

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