Original articles

Spontaneous reanastomosis between lymphatic vessels following syngeneic transplantation of the small intestine in the rat

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Abstract

Abstract Spontaneous lymphvascular reanastomosis (SLR) following small bowel transplantation in rats is of clinical relevance for the resorption of long chain fatty acids. Detailed morphological and molecular data concerning the process of lymphvascular reanastomosis are not available in the literature. In this study SLR was investigated using microradiology and scanning electron microscopy. Between the 8^{th} and 21^{st} postoperative days following transplantation SLR does not occur between the intestinal trunk of the transplant and the thoracic duct of the recipient. Instead, an indirect connection was observed between the inserted advential lymphatic vessels of the mesenteric artery and lymphatic vessels of the aorta or ductus deferens, which are connected with the thoracic duct.

Surgical reanastomosis of interrupted lymphatic vessels in organ transplantations is not performed in man, hence temporary postoperative edema of the graft may be anticipated. When performing transplantations of the small intestine, the efficacy of spontaneous or surgical lymphvascular reanastomosis must be critically assessed because of the absorption of fat through the lymph vessels. Spontaneous lymphvascular reanastomosis (SLR) of the graft is of significance after transplantation of the small intestine for short bowel syndrome, as is intestinal lymphangiectasis [6]. In the present study "SLR" is defined as the spontaneous (non-surgical) reanastomosis of two lymphatic vessels between transplant and recipient.

Following transplantation of the small intestine in the rat, it is possible to perform surgical reanastomosis [10] of the lymphatic drainage ducts of the transplant with the thoracic duct [11]. There are controversial animal experimental data concerning the time span of the corresponding

spontaneous lymphvascular reanastomosis 3 days for the rat [18] and 4 weeks for the pig [17].

Conventional radiological and microscopic investigative techniques have been used unsuccessfully in providing a morphological characterization of the progressive lymphvascular reanastomosis between transplant and recipient.

In the present study microradiological and scanning electron microscopic investigations were conducted to investigate possible problems with lymphatic drainage after organ transplantation. Using this combined investigative procedure, devised by Poulsen Nautrup and Berens v. Rautenfeld [14], it was possible to demonstrate both the radiological and microstructural aspects of the initial lymph vessel reanastomosis.

Material and methods

Syngeneic heterotopic transplantation of the small intestine was performed on 22 male Lewis rats using the technique of Preissner et al [15]. The weight of the donors was on average 311 g and that of the recipients 325 g.

Transplantation technique

The small intestine was removed from the donor from the lig. of Treitz to 2 cm proximal to the ileocaecal valve with the mesentery, the cranial mesenteric v., the portal v., the cranial mesenteric a. with short aortic segments and the jejunal lymph nodes with the intestinal trunk. The transplants were stored in 0.9% sodium chloride solution until the time of transplantation. The average ischemic timewas 61 ± 10min.

The aorta and caudal vena cava of the recipient were surgically prepared approximately 1 cm distal to the renal v. and clamped proximally and distally. Using an operating microscope end-to-side anastomoses were performed between the portal v. of the transplant and the caudal vena cava of the recipient, as well as between the cranial mesenteric a. via the aortic segment of the transplant and the aorta (Fig. 1) of the recipient. The transplanted and separated intestinal trunk was left exposed and was not reanastomosed. The proximal jejunal end of the transplant was closed as a blind pouch, while the ileal end was anastomosed end-to-side at the exposed antimesenteric ileum of the recipient close to the ileocaecal valve.

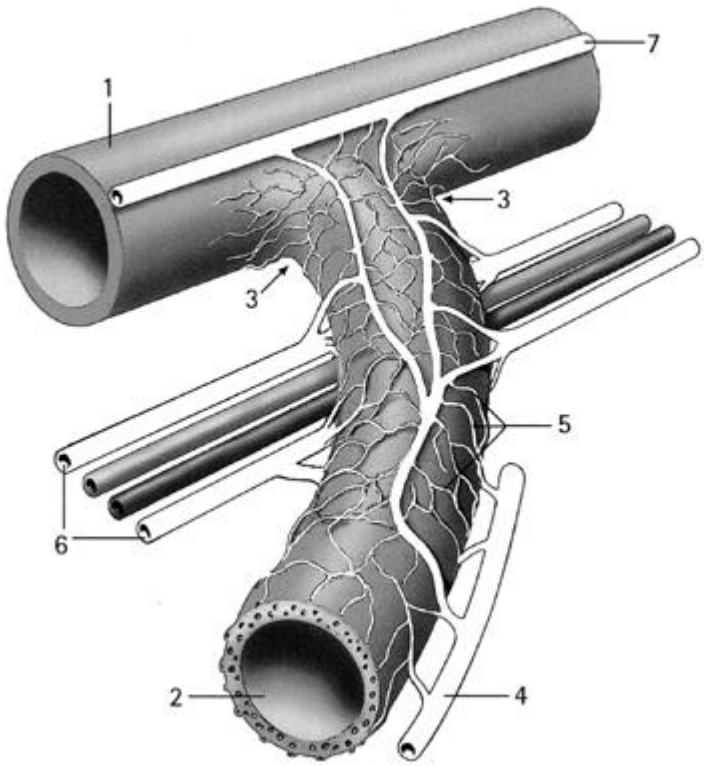

Fig. 1 Schematic representation of the spontaneous lymphvascular reanastomosis following transplantation of the small intestine. The testicular lymphatic vessels flow into the thoracic duct cranial to the mesenteric a.. *1*, aorta *2*, cranial mesenteric a. *3*, area of the arterial end-to-side anastomosis *4*, intestinal trunk *5*, initial adventitial lymphatic vessels *6*, left testicular lymphatic vessels associated to the vein and artery *7*, thoracic duct

Microlymphography

The recipients of the transplant (n = 22) were subdivided into 6 groups. The lymphographic and scanning electron microscopic investigations were performed on the 2^{nd} (n = 3), 4^{th} (n = 4), 6^{th} (n = 4), 8^{th} (n = 4), 10^{th} (n = 4) and 21^{st} (n = 3) postoperative days, respectively. Microlymphography was carried out using a protective X-ray system FXS 100.22 (Feinfocus X-ray System Co., Wunstorf).

The rats were first anaesthetized with Ketanest® and Rompun® and laparotomized in the supine position. The small intestine transplant was laid free of pressure and placed to the left side of the animal. Each rat received X-ray contrast medium Iotasul® (developing preparation) or Isovits® 300 (Schering Co., Berlin) using an injector pump (Precidor 5003, Heinemann Co., Schwäbisch Gmünd) for a period of 90mn with an administration rate of 0.02175 ml/mn into one of the jejunal lymph nodes of the transplant. Under identical perfusion conditions X-ray contrast medium was placed into the interstitium of the left testicle in 8 of the 22 rats to fill the testicular lymphatic vessels. For this procedure the rat was placed on a Plexiglas plate together with the injector within the closed X-ray system.

Microlymphographic documentation was undertaken using still radiographs with a high-resolution film (AGFA Structurix D7 size 18 x 24 cm), as well as continuous fluoroscopic videos beginning 5mn and continuing to 90mn into the perfusion of each animal.

To increase the quality of the video film, subtraction angiography or pseudo-3D-presentation was introduced using deviation-image subtraction with an image evaluation system Hamamatsu DVS 3000.

Scanning electron microscopy (SEM) procedure

Intranodal perfusion of Iotasul® and Isovist® was also conducted for rinsing and distending the operative site in preparation for perfusion fixation of the lymph drainage tract between transplant and recipient. Immediately following lymphvascular rinsing (after 90 minutes), intranodal perfusion fixation was performed with 2.5% glutaraldehyde using the same administration rate (0.02175 ml/mn) for up to 180mn.

From each of the 26 animals, 5 SEM specimens were taken from the following areas perfused jejunal lymph nodes, intestinal trunk, mesentery, cranial mesenteric a. and aorta. Preparation and assessment of the SEM specimens were performed according to the method of Berens v. Rautenfeld et al. [5] using the Philips SEM 505.

Results

Spontaneous lymphvascular reanastomosis (SLR) took place in two chronological phases, as shown by microfocus lymphography and scanning electron microscopy (SEM).

Phase I Provisional lymphvascular reanastomosis (2nd to 6th postoperative days)

On the 2nd postoperative day, after application of Isovist® into one of the jejunal lymph nodes, abrupt termination of contrast medium in the region of the intestinal trunk of the transplant resulted in all investigated animals (Fig. 2). Only one of three animals showed a contrast medium flow through the extranodular veins of the jejunal lymph nodes into the caudal vena cava of the recipient on the 2nd postoperative day. SEM showed evidence of intranodular, as well as dilated lymph sinuses and veins, however no lymphovenous anastomoses were detected. Eighteen of the 22 animals showed extranodal venous contrast medium flow in each group from the 6th to the 21st postoperative day. No evidence of other lymph vessels within and outside the transplant was found.

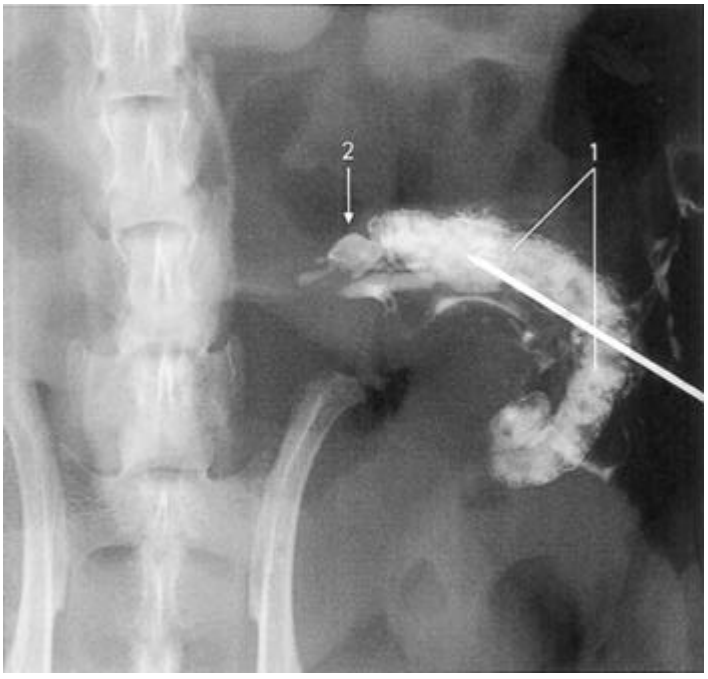

Fig. 2 Microfocus lymphography after application of Isovist® into one of the jejunal lymph nodes, 2nd postoperative day (60kV 0,2mA 35sec direct radiological magnification = 4x). *1*, injected jejunal lymph node *2* intestinal trunk with contrast medium

On the 4th postoperative day, in addition to termination of the contrast medium in the intestinal trunk, a network of initial lymphatic vessels was found close to the cranial mesenteric arterial wall within the transplant (Fig. 1). Contrast medium was seen as far as the anastomosis of the cranial mesenteric a. of the transplant with the aorta of the recipient, however no contrast was seen to cross into the recipient. Using SEM the initial adventitial lymphatic vessels demonstrated typical structural characteristics of the lymphvascular endothelial layer interendothelial openings, endothelial bridges, connective tissue trabeculae and subendothelial filaments. Within the intestinal trunk distended pore-like endothelial openings were seen in the form of outflow valves. It was not possible to detect any lymphvascular or interstitial channels containing contrast medium between the intestinal trunk and the adventitial network of the initial lymphatic vessels (lymphatic capillaries and precollectors).

On the 6th postoperative day SLR was observed in one animal between the adventitial initial lymphatic vessels in the wall of the cranial mesenteric a. of the transplant and the adventitial initial lymphatic vessels of the aorta of the recipient. This was confirmed by SEM (Fig. 3).

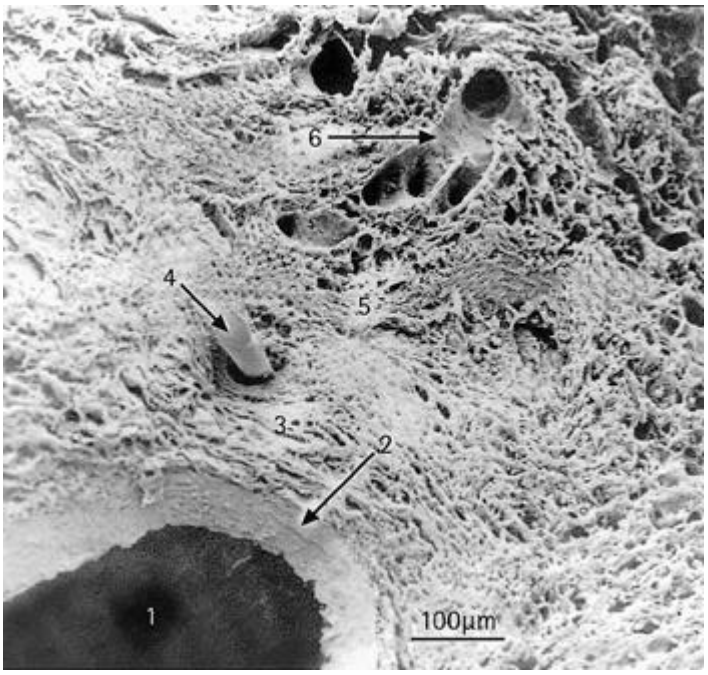

Fig. 3 SEM wall of the cranial mesenteric a. near to the aorta. *1*, intravasal lumen *2*, intima *3*, media *4*, suture material (Ethicon®) *5*, adventitia *6*, initial lymphatic vessels (capillaries and precollectors)

Phase 2 Final lymphvascular reanastomosis (from the 8th postoperative day)

All four animals studied on the 8th, and all animals studied up to the 21st postoperative days demonstrated SLR in the adventitia of the mesenteric a. and the aorta and a new lymphvascular reanastomosis (Figs. 4, 5). This new connection was formed between one or two markedly extended lymphatic vessels in the adventitia of the mesenteric a. of the graft and the ascending testicular lymphatic vessels of the recipient. Renal and lumbar paraaortic lymph nodes can drain into this lymphatic channel.

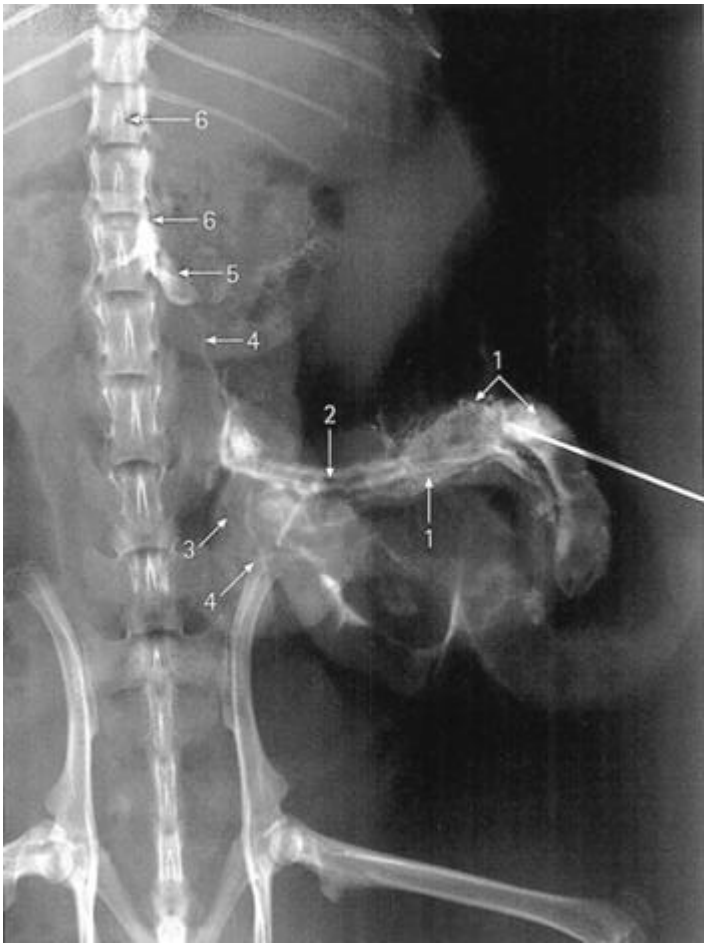

Fig. 4 Microfocuslymphography after application of Isovist® into one of the jejunal lymph nodes and into the left testis on the 8th postoperative day (70kV, 0,1mA, 80sec, direct radiographic magnification = 4x). *1*, injected jejunal lymph node *2*, cranial mesenteric a. with initial adventitial lymphatic vessels (capillaries and precollectors) *3*, initial adventitial lymphatic vessels in the area of the arterial anastomosis between the cranial mesenteric a. and the aorta *4*, left testicular lymphatic vessel *5*, left renal lymph node *6*, thoracic duct

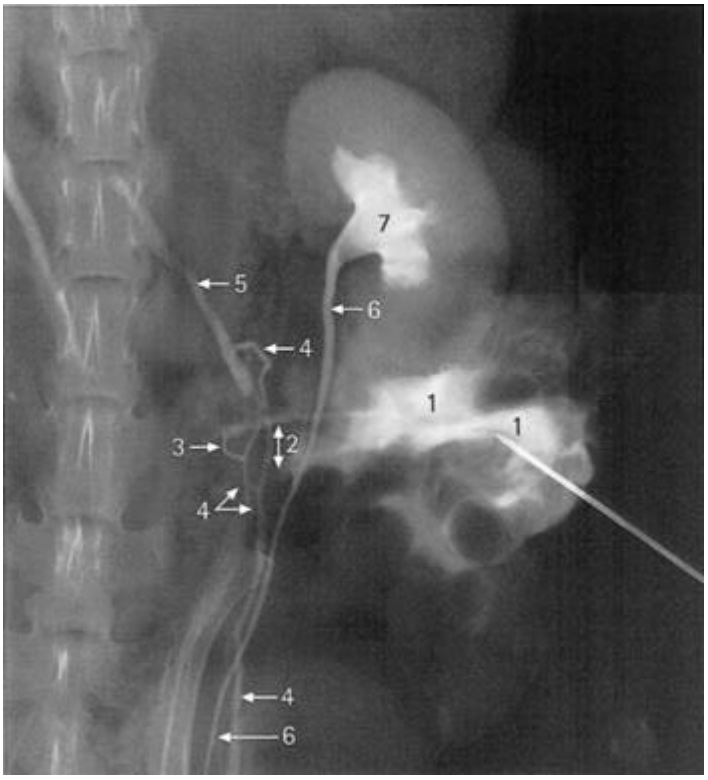

Fig. 5 Microfocuslymphography after application of Isovist® into one of the jejunal lymph nodes and into the left testis on the 21st postoperative day (60kV, 0,2mA, 35sec, direct radiographic magnification = 4x). *1*, injected jejunal lymph node *2*, cranial mesenteric a. with initial adventitial lymphatic vessels (capillaries and precollectors) *3*, lymphatic vessel (collector) *4*, testicular lymphatic vessels *5*, thoracic duct *6*, ureter *7*, kidney

Discussion

The study has demonstrated that the initial lymphatic vessels form a reparative outflow passage between the transplant and donor. Radiological evidence of the initial lymphatic vessels can only be determined microlymphographically [14], which with electron microscopic investigations provide supplementary evidence of the typical structural elements of the initial lymphatic vessels [4]. Bellmann and Oden [3] and Walzer [22] were unable to characterize ‘lymphatic vessel regeneration' as initial lymphatic vessels after separation of lymphatic vessels in rabbit ears.

The first evidence of contrast medium outflow from the lymph system into the blood system in the current series was seen in the contrasted jejunal lymph nodes of the graft. This occurred in one animal on the 2nd postoperative day and subsequently in 18 of the animals. This lymphovenous outflow could indicate a temporary overflow valve function prior to regeneration of lymphovenous anastomoses. Intranodal lymphovenous anastomoses can appear even after experimental sealing of the thoracic duct [8]. In contrast to humans, extranodal lymphovenous anastomoses can be regularly observed in animals [2]. However, no physiological lymphovenous anastomoses have been found within the lymph nodes of the rat [7], indicating that this outflow phenomenon could be an artificial lymphovenous anastomosis.

As expected SLR did not take place directly between the intestinal trunk of the transplant and the thoracic duct of the recipient. Instead, from the 8th postoperative day initial lymphatic vessels provided a lymphvascular outflow passage from the wall of the cranial mesenteric a. of the transplant to the adventitial lymphatics of the aorta of the recipient. The arterial anastomosis between the mesenteric a. of the graft and the aorta of the recipient approximates the adventitia of both vessels, enabling the first by-way anastomosis of the adventitial initial lymphatic vessels of both arteries

(Fig. 3).

The final phase of SLR was demonstrated in all animals after the 8th postoperative day (Figs. 4, 5), being characterized by significantly dilated initial lymphatic vessels in the mesenteric arterial wall, draining via the testicular lymph vessels to the thoracic duct [21]. Schmid et al. [18] have reported a primary lymphvascular anastomosis following transplantation of the small intestine in the rat on the 3rd postoperative day, however without any anatomic characterization of the outflow passage.

Two competing models for the new formation of lymphatic vessels are considered sprouting of lymphatic vessels from the venous system (centrifugal or venous theory of Ranvier [16]) and the formation of isolated interstitial, and subsequently endothelialized, vessels from the mesenchyme (central or mesenchymal theory of Gulland [9]). However, microradiography does not allow discrimination between these two models, since it is not possible to detect isolated lymphatic vessels. In subsequent studies reconstructions of serial sections, in combination with molecular markers may be helpful. To determine the underlying molecular processes for the observed new formation of the initial lymph vessels between the intestinal trunk of the graft and the adventitia of its cranial mesenteric a. in this study, molecular biological methods need to be applied. It has recently been shown that vascular endothelial growth factors -C and -D (VEGF-C, -D), respectively and the VEGF-receptor-3 are key molecules for lymphangiogenesis [13, 23]. Their expression correlates with lymphatic metastasis in mouse tumor models [12, 19, 20]. In addition, lymphatic vessel endothelial hyaluronan receptor -1 (LYVE-1), a homologue of the glycoprotein CD44, is expressed almost exclusively in lymphatic vessels, but is absent in blood vessels [1]. These molecules will be used in subsequent investigations of the intestine transplantation model as molecular markers and for exogenous applications.

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