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T. P. Szymula von Richter
R. G. H. Baumeister
C. Hammer

Microsurgical reconstruction of the lymphatic and nerve system in small bowel transplantation: the rat model, first results

T. P. Szymula von Richter
Amalienstrasse 89, D-80799 Munich, Germany

R. G. H. Baumeister (✉)
Division of Micro-, Hand and Reconstructive Surgery, Klinikum Grosshadern,
University of Munich,
Marchioninistrasse 15,
D-81377 Munich, Germany

C. Hammer
ICF, University of Munich,
Marchioninistrasse 15,
D-81377 Munich, Germany

Abstract The goal in tissue transplantation is the restoration of all natural (physiological) communication pathways between the host and the graft. To this end, the effects of microsurgical reconstruction of artery, vein, lymphatic vessel, and nerve during grafting were investigated. Allogenic (MHC class II incompatible) and isogenic orthotopic (graft in functional continuity) small bowel recipients with immediate microsurgical lymphatic and nerve anastomosis were observed clinically as well as by immunological and histological examination. To explain the influence of the lymphatic system in allograft survival, short-term therapy was applied with the immunosuppressant cyclosporin A (10 mg/kg i.m.) for only 5 postoperative days. Average allograft survival ended in the control group after 10 days without any therapy, increased up to 20 days after immunosuppressive therapy (in both groups acute rejection and graft-versus-host disease were seen) and increased further to more than 200 days following lymphatic connection of the host and the graft during allografting. In this group no lymphatic edema of the graft was seen. To determine the optimal location of nerve anastomoses between the host and the graft without irritating the host nerve system, isografts in the same model were investigated. No paralysis of graft neighboring tissues was seen when the last ganglion function, and its following nerve plexus, of the host is saved. Nerve reconstruction must be undertaken after this last crossing of regional nerve fibers before entering the organ. The same rule is effective for organ explantation.

Key words Rat small bowel transplantation · Graft rejection · Lymphatic system · Nerve system · Microsurgical anastomoses

Introduction

Today, the importance of the lymphatic system in immunology is understood, although it has been known for 30 years that the lymphatic vessels are pathways of immunological cells [1]. The importance of the nerve system in immunology was proved a few years ago and the peripheral nerves have been known as pathways of information to innervated tissue since the early days of anatomy [2]. Clinically, microsurgeons have reconstructed lymphatic vessels for the last 15 years and nerves for the last 25 years knowing that these pathways regenerate with time [3, 4]. Our initial inspiration for this study comes from the history of human transplantation investigated originally by plastic surgeons [5–10].

Embryologically, the skin and gastrointestinal tract have the same origin, which makes them difficult to transplant allogenically. Both have close associations with the immune system. The goal of understanding this physiological phenomenon is the knowledge that those particular organs are more frequently circulated by immunological cells than others. High levels of po-

tential immunosuppressants are needed to allow the allogenic transplantation of these tissues [11].

In this study we used today's most sophisticated microsurgical reconstruction for transplantation, revealing results of interest to immunologists [12].

Materials and methods

Animals

Young female Brown Norway rats as donors [BN ($RT1^n$) × LEW (RT^l)] and young male Lewis rats as recipients were transplanted allogenically. Young male Lewis rats were used for the isogenic study. The average weight was 200 g.

Explantation

An orthotopic small bowel transplantation model was used, based on the microsurgical experimental methods of Olszewski and Acland [13, 14], the clinical experience of Buncke and Harii [15] for blood vessels, of Millesi [4] for nerves and of Baumeister for lymphatic vessels [3]. Consequently, a less invasive approach and minimal damage to the graft was possible. Whole explantation was done under the microscope. Only 95 % of the donor small bowel was explanted due to the fact that, during transplantation, the remaining 5 % of the explanted host small bowel must remain to preserve the ileo-caecal valve function of the host. After cutting of the portal vein and perfusion with body temperature isotonic solution, the small bowel was explanted with a 2-mm aorta abdominalis stump, to avoid damage of cysterna chyli.

Preparation of the lymphatic system

Careful preparation allowed damage-free lymphatic grafting. At the end of lymphatic preparation, the lymphatic flow of the small bowel led into the cysterna chyli and the following ductus thoracicus only.

Preparation of the nerve system

Thin intestinal nerves lie after the last ganglion and the following nerve plexus and pursue the lymphatic vessels of the mesenteric lymph nodes; thick intestinal nerves are located before the last ganglion.

Allogenic transplantation with lymphatic system reconstruction

The whole operation of allogenic orthotopic small bowel transplantation with ($n = 10$) and without ($n = 10$) microsurgical lymphatic anastomosis was performed under the operating microscope. First, the aorta of the graft was anastomosed with 10-0 sutures end-to-side to the host aorta abdominalis. Cuff techniques that would need an additional kidney explantation, and which may have influenced the host's condition for long-term results, were not employed. Porto-portal anastomoses would damage connections between liver and host cysterna chyli. Therefore, the venous anastomosis was performed between the graft portal vein and the host vene cava inferior with 10-0 sutures end-to-side. Then lymphatic anastomosis was added end-to-side with 12-0 sutures between the graft ductus thoracicus, which remained connected to the graft and the host cysterna chyli during explantation.

Immunological therapy

Allogenic orthotopic small bowel recipients with and without microsurgical lymphatic anastomosis were treated with cyclosporine A (CsA; 10 mg/kg i.m. diluted in 0.3 ml Intralipid) for 5 postoperative days only.

Isogenic transplantation with nerve system reconstruction

Isogenic orthotopic small bowel transplantation with microsurgical nerve anastomoses before ($n = 5$) and after the last ganglion ($n = 5$) were also performed under the operating microscope. In this study, the reconstruction of the intestinal nerves was done end-to-end with 12-0 sutures between the prepared intestinal nerves of the graft and the host. To study the importance of the location for nerve repair, the nerve anastomoses were designed in five animals after and in five animals before the donor's and the host's last ganglions.

Transplantation long-term follow-up

The outcome of grafts was studied up to 1 year by clinical observation, including weight measurements as well as histological studies. In the allogenic group, cytoimmunological monitoring was added.

Results

First, the lymphatic anastomosis prolongs allograft survival after only 5 days immunosuppression with 10 mg/kg i.m. cyclosporin (Fig. 1). The average small bowel survival was more than 200 days when lymphatic anastomosis was added during transplantation, compared with 20 days of average allograft survival in animals without this microsurgical technique. When immunosuppressant was not used, all participents bearing MHC class II incompatibility of the small bowel died, on average, at day 10 after grafting due to rejection, showing weight loss from day one. Animals receiving allogenic organ transplantation with lymphatic repair showed no signs of acute rejection after 5 days immunosuppressive therapy compared to those undergoing allografting without lymphatic anastomosis.

Second, in the isogenic model, transections of recipient intestinal nerves caused no paralysis of the remaining recipient gastrointestinal tract if placed after the last ganglion. The optimal position for the intestinal nerve anastomoses between the graft and the host, therefore, was found to be after the last ganglion.

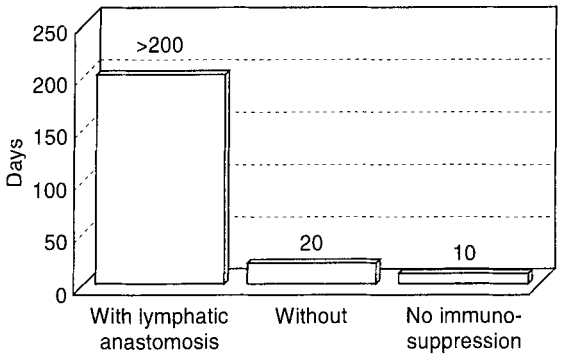

Fig. 1 Average allograft survival after only 5 days of immunosuppressive therapy in orthotopic small bowel transplantation in the rat

Discussion

In discussing the lymphatic influence in allografting, it is of great importance to understand that allogenic sensitization occurs primarily in draining lymph nodes and, in keeping with the principles of fundamental immunology, that naive T-cells are kept within the lymphatic tissue before any kind of rejection starts and that only activated or memory cells are allowed to circulate into peripheral tissue [16]. Since most sensation occurs in the peripheral tissue of the host, the early cell-mediated response via the lymphatic vessels of the graft into the host lymphatic system with the repair of lymphatic vessels makes sense. Most donor cell traffic leaves the allograft directly through the veins and lymphatic vessels in our model. In former models, without lymphatic vessel repair, this traffic occurred at the outset through the vein and tissue connections between the edemic graft and the host. The inflammation which followed may have been due to lymphatic tissue edema that occurs after invasion of immunological cells stimulated by the MHC incompatibility. Lymphoid and dendritic cell replacement may, therefore start, earlier in our lymphatic model, because of the earlier two-way immunological cell migration between the graft and the host [17]. Moreover, the fat-soluble immunosuppressive drug, CsA, may be able to recirculate into the recipient lymphatic system after being eliminated into the gastrointestinal tract, because lymphatic vessels transport long-chain fatty acids that contain CsA transport molecules. Hence, the highest body concentration of CsA is located in the thoracic duct. This may explain the 1-year survival of one participant in the MHC class II incompatible model who showed no histological signs of rejection after only 5 days of CsA therapy.

The future problem in transplantation of some organs may be the nerve regulation of transplanted tissue successful long-term survival, with the goal of maximal original function of the graft [18]. Since it is known that postganglionic parasympathetic nerves survive in the allograft, evidenced by histological studies of postganglionic autonomic nerve fibers, surgical reinnervation after transplantation is the next step to be investigated [17]. Grafting with microsurgical nerve anastomoses allows host nerve axons to grow into the graft using the original nerve pathways. To study the behavior of nerve anastomoses during grafting, and to demonstrate nerve regeneration in organ transplantation, isogenic investigations were first planned. Experimental grafting in plastic surgery suggests that nerve axons find the position of their original nerve synapses after correct nerve anastomoses. Less nerve regeneration time is needed in comparison to incorrect nerve restoration. The more exact the anastomosis is, the better functional results can be achieved.

We therefore think that realisation of primary lymphatic anastomoses during allogenic transplantation could lower immunosuppressive drug consumption and their side-effects as well as prolong graft survival. Concerning the long-term function of grafts, correct nerve reconstruction may also be of importance for integration into the host nerve system. The microsurgical reconstruction of the natural communication pathways of the organ, the arterial, vein, lymphatic, and nerve system, is now approaching reality and should be taken into consideration in transplantation surgery in the future.

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