Progress in experimental intestinal transplantation in small animal models

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Abstract

The unique immune response after small bowel transplantation (SBT) has been the subject of extensive research using small animal models in rats and mice. These animals are inexpensive, for most societies ethically acceptable and the existence of inbred strains allows for reproducibility and defined immunobiological conditions. The basic immunological reactions, such as graft-versus-host-reactions (GVHR), host-versus-graft-reactions (HVGR), a combination of both reactions, chronic rejection and tolerance have been described. Almost all immunosuppressive agents of proven or potential clinical relevance have been tested for their efficacy in small bowel transplantation. All techniques which are applied to intestinal transplantation in humans including multiorgan transplantation, can also be performed in rats. Intestinal transplantation in mice is methodically restricted to heterotopic transplantation. The mouse however, offers several advantages compared to the rat model. A large number of congenic and knockout strains is available as well as many analytical tools. In the future, intriguing new insights into the unique immunological mechanisms of allograft rejection will be discovered using murine models. (Acta gastroenterol. belg., **1999**, 62, **216-220**).

Key words : small bowel transplantation, rats, mice.

1. Introduction

In the past two decades, small bowel transplantation (SBT) has become applicable reality for patients with end-stage intestinal failure. Despite the progress in clinical management, the outcome after SBT is still not comparable to that after other solid organ transplants. The major problems are graft rejection and infections after transplantation due to high immunosuppression. Many questions around the immunological phenomena after SBT are unanswered and have to be clearified by experimental research.

In contrast to large animals, rat models as well as recently reported mouse models provide several advantages. Most important is the availability of well defined inbred strains with known MHC-antigens. Theoretically, two types of immunological reactions are possible after small bowel transplantation : HVGR, the reaction of the immune system of the recipient against the donor, and GVHR, the reaction of the immunocompetent cells of the graft against the recipient. In species, in which inbred strains are available, these reactions can be studied either in combination or separately.

In the early 1990's Squiers *et al.* (1) and Zhong *et al.* (2) simultaneously developed the mouse small bowel transplant model. For experiments in mice a wide variety of analytical tools as well as many genetically well-defined strains, including those congenic at MHC class I and class II antigens and several transgenic and knockout animals, are available.

2. Small bowel transplantation in rats

2.1. *Technical considerations*

There are two techniques for small bowel transplantation in rats : (1) heterotopic and (2) orthotopic transplantation. In this technique the recipient's own small bowel is removed and the recipient's survival is depending on the function of the graft. High quality of microsurgical techniques is necessary to guarantee a constant success rate of the procedure. In the donor, the small bowel and the colon have to be separated, then the vessels of the graft are dissected, resulting in an aortic segment with a superior mesenteric artery and the portal vein. Finally, the graft is perfused and removed. In the recipient the aortic segment of the graft is anastomosed end-to-side to the recipient's aorta and the portal vein of the graft is either anastomosed to the infrarenal vena cava (systemic drainage) or to the portal vein of the recipient (portal drainage). Although technically more demanding, porto-portal drainage establishes an anatomically and physiologically normal state. The main advantages of portal venous drainage potentially include (1) filtering of translocated organisms and toxins from the small bowel graft by the native liver, and (2) protection from rejection (3-5). Beneficial effects of this route of venous drainage on graft survival have been reported, but in most studies the route of venous drainage does not seem to be significant (6-7).

Concerning the lymphatic drainage in SBT, a novel microsurgical model that reconstitutes the lymphatic drainage immediately after transplantation was reported by von Richter *et al.* 1996 (8). Kellersmann and coworkers modified this model, achieving a success rate of 87% in reconstituting the draining lymphatic vessels (9). They hypothesised, that complications following SBT such as fat malabsorption, protein-losing enteropathy, and bacterial translocation may be minimised by lymphatic reconstruction. Further investigation will clarify the effect of lymphatic drainage on immunological responses.

2.1.1. Heterotopic techniques

The heterotopic techniques vary in the management of the ends of the graft. These ends can either be

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exteriorised as stomas or one end can be anastomosed to the recipient's gastrointestinal tract with or without a stoma of the other end (10). One disadvantage of the otherwise relatively simple and reliable technique of heterotopic small bowel transplantation is the fact that rejection is not exactly defined in these animals. Usually, they do not die in the course of rejection, so that rejection has to be diagnosed by repeated open biopsies. These problems can be overcome by a technique developed in our laboratory, which we called videomicroscopic imaging. The stoma is observed using a 30 times magnification and the pictures are stored on a computer. We could demonstrate that the mucosal surface alterations of the graft correlate with the histological stages of small bowel rejection. During the course of rejection, one can see broadening of the villi which are covered by cell detritus and widening of the crypts (11). In preliminary observation made by Tzakis and co-workers in Miami it appeared that a similar correlation between mucosal surface alterations and rejection of grafts can be shown in humans as well (12).

2.1.2. Orthotopic techniques

Since the first report of an orthotopic rat SBT model (OSBT) by Kort *et al.* in 1973 (13) many studies have been published using this technique. In the orthotopic situation, the recipient's small bowel is resected and the graft is placed in continuity between the remaining duodenum and terminal ileum of the recipient. In skilled hands the technical success rate for the orthotopic technique is not significantly different from the heterotopic procedure (14). In comparison with the heterotopic technique, the main advantage of OSBT is the fact that the graft is exposed to a normal intraluminal environment, which may improve gut barrier function and delay the onset of rejection after OSBT. However, recipient's well-being depends on the integrity of the graft and rejection of the small bowel graft in the orthotopic position usually causes the recipient's death, while rejection in the heterotopic graft is often well tolerated. Additionally there are immunological differences regarding acute and chronic rejection as well as GVHR between both models (15, 16).

2.2. *Basic immunological reactions after SBT*

Using a heterotopic transplantation model, Monchik and Russell systematically analysed the immunological reactions characteristic for small bowel transplantation 1971 (17). (1) Syngeneic grafts survived long-term. (2) When F1 hybrid grafts were transplanted into parental strain animals (BN/LEW-LEW), rejection occurs within 9 days. (3) Transplantation in the reversed semiallogeneic combination (LEW-BN/LEW) resulted in isolated GVHR. Recipients died after 17 days as a result of GVH disease. When these grafts were irradiated, the recipients survived long-term without any signs of GVH disease. (4) In fully allogeneic donor-recipient strain combinations without immunosuppression all small bowel grafts were rejected. The time course of rejection was dependant on the strain combination used. Recent data by Fändrich *et al.* suggest that the severity of the GVHR seems to be regulated by NK-cells of the recipient (18).

2.2.1. Effects of immunosuppressive agents

The effects of Cyclosporin A and FK 506 have been studied extensively after SBT using different semiallogeneic or fully allogeneic rat models. For FK 506 it has been well demonstrated by Lee *et al.*, that long-term graft survival in the orthotopic situation is achieved by short courses of 2 mg/kg FK 506 on days 0-4 post-transplantation in the BN-LEW model (19). Similar results were reported by Langrehr *et al.* (20) with an indefinite graft survival for more than 400 days in this combination. Using a comparable protocol of 2 mg/kg FK 506 for 5 days in the same allogeneic BN-LEW model we found a prolonged allograft survival of about 100 days with histologically confirmed chronic rejection in the graft. Only a prolonged administration of this high dose protocol for 9 days resulted in an indefinite graft survival (Fig. 1). Histological studies of these long-term surviving grafts (> 200 days) demonstrated intact mucosal morphology with only occasional cryptitis. In these long-term surviving recipients a state of "split tolerance" was observed. In vivo these animals accepted donor-specific heart allografts whereas third party grafts were rejected. At the same time $CD4^+$ T cell reactivity against donor-specific antigens was not suppressed.

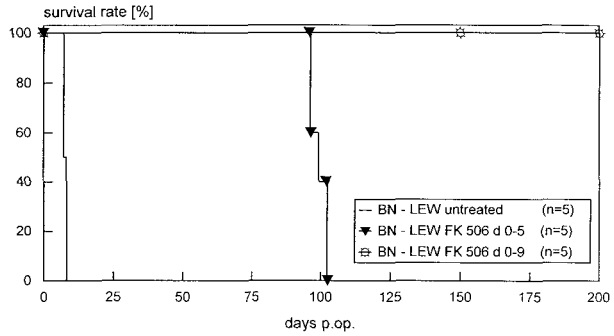

Fig. 1. — Survival rate of recipients after allogeneic orthotopic small bowel transplantation in the strain combination BN-LEW. FK 506 was given at a dose of 2 mg/kg/day either from day 0 to day 5 or days 9 postoperatively.

In conclusion, these data clearly demonstrate the effectiveness of FK 506 in suppressing small bowel allograft rejection. Only high dosages of FK 506 result in long-term graft acceptance after SBT. Lower dosages that induce long-term graft survival in most other vascularized organs lead to chronic or delayed acute rejection in small bowel grafts. Comparable results have also been demonstrated for Cyclosporin A (21). Immunosuppressive effects but not induction of tolerance have been described for other agents like rapamycin (22), brequinar (23) and FTY 720 (24).

2.2.2. Immunosuppression using various modalities

In order to reduce the side effects of immunosuppression based on high doses of FK 506 or Cyclosporin A other immunosuppressive protocols have been developed. The efficacy of different murine monoclonal antibodies (MAbs) against surface molecules of T cells, the T cell receptor or adhesion molecules was tested. Protocols involving the injection of anti-CD4 and anti-CD8 MAbs have been shown to induce tolerance for liver and kidney allografts in rats (25). When similar regimens were tested in experimental SBT, only a limited prolongation of graft survival was observed (26). Another strategy is the blockage of the IL-2 pathway in the early postoperative phase after transplantation with MAbs against the IL-2 receptor. Injection of the anti-IL-2 receptor MAb NDS 61 in combination with low dose FK 506 therapy did also prolong small bowel graft survival (27).

Other attempts to achieve graft acceptance are based on the induction of chimerism in the recipient. Orloff *et al.* repopulated sublethally irradiated recipients with T cell depleted bone marrow of both donor and recipient origin thus inducing mixed allogeneic chimerism. In these animals small bowel allografts survived long-term (28). Permanent graft survival was also observed after sublethal total body irradiation and intrathymic inoculation of resting donor T cells (29). An immunosuppressive protocol without the need to irradiate the recipient was recently presented by Murase *et al.* (30). In this experiment BN rats received LEW small bowel allografts. The grafts were irradiated to prevent GVHR. The recipients received donor bone marrow at the time of transplantation and FK 506 postoperatively. Neither rejection nor GVHR were observed and the recipients survived long-term.

2.2.3. SBT combined with other organs

A liver allograft can prevent rejection of other grafts transplanted either together with or secondary to the liver in rodent models (31). This is of special relevance for clinical intestinal transplantation. In certain situations e.g. liver cirrhosis after long-term parenteral nutrition, patients require both, a small bowel and a liver graft. In experiments using multivisceral transplantation in the rat (liver, pancreas and stomach together with large and small bowel) Murase *et al.* did not observe a tolerogenic effect of the liver. Survival of animals with multivisceral grafts was not different from those with isolated small bowel grafts (32). In 1991 Zhong and co-workers published experiments using a non-arterialized liver allograft and a heterotopically placed small bowel graft with systemic venous drainage. GVHR was observed in recipients of this combined liver/small bowel allograft, which, usually reject an isolated small bowel graft (33). This was the first evidence that a liver graft altered the immune reactions after SBT. In 1992, Sanacki and co-workers described a sequential model, in which liver and small bowel was accepted long-term for the first time : A small bowel graft was transplanted secondarily (17 days) to a liver allograft. Both grafts were taken from the same inbred strain and a donor recipient combination was used, in which an isolated liver allograft is spontaneously accepted. This experimental model demonstrated for the first time the tolerogenic effect of the liver towards a small bowel graft. However, this model is clinically not applicable.

Therefore, at our institution, the colleagues Gassel and Meyer developed two models of combined liver/small bowel transplantation with three major advantages (34) : (1) the liver allograft is arterialized, (2) the small bowel allograft has a portal venous drainage and (3) both grafts, simultaneously transplanted, are from the same donor. In the first model the small bowel allograft is placed in a heterotopic position, in the second model orthotopically. The following results could be observed in the BN-LEW strain combination, in which isolated liver allografts are tolerated in 80% of the cases, whereas isolated small bowel allografts are rejected : (1) a combined liver/heterotopic small bowel allograft is spontaneously tolerated in 70% of the cases (35). (2) As mentioned above, recipients of orthotopic isolated small bowel allografts in the BN-LEW combination survive approximately 100 days when the animals are treated with 2 mg FK 506 for 5 days. At that time, the graft is chronically rejected (36). When the small bowel is transplanted orthotopically together with a liver the animals only need 0.5 mg FK 506 for 5 days to survive indefinitely. In addition, they do not have any signs of chronic rejection. This clearly demonstrates the protective effect of a liver allograft towards a concomitant small bowel.

3. Small bowel transplantation in mice

A relatively new and fascinating field of experimental SBT is the transplantation in the mouse. Techniques for this model were developed by Squiers and Zhong (1, 2). They described a heterotopic transplantation model with porto-systemic venous drainage. However, this model has not been widely used due to its technical complexity.

Basic immunological phenomena after small bowel transplantation in various mouse strains in comparison to other organs have been analysed by Zhang *et al.* In their experiments, rapid rejection was observed in all strain combinations examined. This is in contrast to other organ grafts such as liver and kidney which are sometimes spontaneously accepted (37). A detailed analysis of the cytokines and adhesion molecules expressed during rejection of small bowel grafts was published by Quan *et al.* in 1994 (38). The role of MHC antigens in intestinal graft rejection was examined using MHC-class I deficient and MHC class II-deficient donors. Both types of grafts showed prolonged survival as compared to normal grafts (39). The role of T cells subsets in intestinal allograft rejection was analysed using T cell receptor ß or δ chain knockout mice and

depleting antibodies against $CD4^+$ or $CD8^+$ T cells. It could be demonstrated, that rejection was absolutely dependent on αß but not γδT cells. $CD4^+$ and $CD8^+$ T cells were both necessary for intestinal graft rejection (40).

Various immunosuppressive protocols have been applied in this model as well. Rapamycin and Cyclosporin A were found to be effective to suppress HVGR and GVHR (41). Treatment with MAbs against CD45RB did not have a significant influence on rejection of small bowel grafts in contrast to kidney grafts (42). Using a short course of cyclophosphamide long-term survival (> 80 days) could be induced (43).

In the future, murine intestinal transplantation will be used more frequently due to the increasing microsurgical experience with this model and the enormous immunological potential of this species.

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