Intestinal transplantation: from the laboratory to the clinics

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Abbreviations

Antigen Presenting Cells: APC
Cyclosporine A: CsA
Donor Specific Blood Transfusion: DSBT
Immunosuppression: IS
Intestinal Transplantation: ITx
Total Parenteral Nutrition: TPN
Graft Versus Host Disease: GVHD

Abstract

Intestinal transplantation (ITx) is a valuable treatment for patients suffering from irreversible short bowel syndrome and debilitating complications of total parenteral nutrition. However, the results of ITx remain inferior to other solid organ transplants due to the profound immunogenicity of the organ and the subsequent demand for high levels of immunosuppression with its associated side effects.
Since 2000, 10 consecutive cadaveric intestinal transplantations have been performed in our center. All were treated with the same Leuven Tolerogenic Protocol - an experimentally proven, multifactorial immunomodulatory regimen - which includes the administration of donor specific whole blood, low-dose steroids, low-dose tacrolimus, and limitation of peritransplant intestinal ischemia reperfusion injury. The first patient transplanted in this series was the first successful ITx performed in the Benelux. The 5-year patient and graft survival rate is 90% (follow-up: 11 months – 11 years and 10 months), which compares favorably with the average 50% patient survival rate reported by the Intestinal Transplant Registry. Only one patient (10%) developed an early acute rejection, reversible with immediate steroid treatment. Three patients (30%) faced a later episode of acute rejection (at 4 months, 18 months and 46 months).

Wider application of ITx depends upon the development of these immunosuppressive protocols which prohibit intestinal rejection while reducing the need for immunosuppression.

**Keywords:** Intestinal Transplantation, Tolerance, Immunosuppression, Tolerogenic Protocol, Immunomodulation
INTRODUCTION

Our group initiated a research programme on intestinal transplantation (ITx). First, we developed a new model for ITx in rats and in larger animals (pigs). Some particularities of the immune response elicited by bowel grafts (a vigorous rejection response and a unique capacity to induce a graft versus host disease) have been elucidated using these models. Reliable surgical techniques for transplanting the bowel (as an isolated graft or in combination with other splanchnic organs) were also developed. This preliminary part of our research and preclinical work was performed at the University of Liège, Belgium, at the University of Minnesota, Minneapolis, United States of America, and at the University of Birmingham, United Kingdom.

Starting in 1997 and in collaboration between the laboratories of abdominal transplant surgery and experimental transplantation at the KU Leuven, an immunomodulatory protocol - that is a protocol aiming to reduce the rejection response and to facilitate engraftment - has been designed. This protocol was developed in a rodent model and was then translated to the clinical setting. We report our clinical centre experience with a consecutive series of 10 cadaveric ITx who received this “Leuven Tolerogenic Protocol”, implicating lower maintenance immunosuppression (IS), than normally required for this type of transplant, administration of donor specific whole blood, and limitation of peritransplant intestinal inflammation.

HISTORY OF INTESTINAL TRANSPLANTATION

In the 60’s and 70’s, before the introduction of the anti-rejection drug cyclosporine A (CsA), only 6 ITx have been attempted. No success could be reported. Technical or surgical problems, rejection, infection and suspicion of graft versus host disease (GVHD) were the main causes of failure. This early and disappointing experience demonstrated the difficulty of this transplant procedure (1-3). Of note, at that time, kidney transplants were already performed with some success under steroids and azathioprine-based IS.

The introduction of the powerful anti-rejection calcineurin inhibitor CsA in the 80’s contributed to major improvements in the results of kidney, heart and liver transplantation (4). It was hoped that ITx under CsA would become a “clinical reality”. However, the results remained poor: of approximately 40 cases of ITx performed in the 80’s and 90’s under CsA only 1 graft survived long-term. Interestingly, this recipient had received an intestinal graft from a neonate donor and it was hypothesized that this type of neonatal graft was less immunogenic than conventional adult cadaveric grafts. The recipient is still alive 24 years after ITx and is the longest survivor up to this moment (5, 6).

Finally, it is only with the introduction by Starzl of high-dose tacrolimus-based IS that the short-term
results of ITx have substantially improved (7). Since then, intestinal replacement has become a potentially life-saving treatment for patients with short bowel syndrome and TPN-related life-threatening complications (8 - 10). But the relatively poorer long-term results (versus other organs transplants) have discouraged wider application of this transplant procedure, particularly in Europe (11).

WHY IS IT SO DIFFICULT TO TRANSPLANT THE INTESTINE?

Is the difficulty physiological in nature? Of note, normal growth and nutritional status have been observed in rat recipients of a syngeneic (genetically identical) transplant. In addition, ITx has been performed with success between identical twins in humans (12). These observations clearly prove that - in the absence of an immune response - the lymphatics interruption, the denervation and the unavoidable period of ischemia that accompany ITx are not an obstacle to transplant the bowel.

The difficulty to transplant the bowel is immune in origin. The bowel is simultaneously a highly immunocompetent and immunogenic organ (13). Because it contains immunocompetent hematopoietic cells, the intestine is capable of inducing a GVHD in the recipient (like after bone marrow transplantation) (14). GVHD after ITx is not frequent, but is associated with a high mortality. In contrast, the incidence of mild and/or subclinical graft-versus-host responses may be frequent (up to 20%) (15). But the largest immunological obstacle to transplantation of the bowel is the rejection response which is extremely vigorous and which may rapidly lead to complete disappearance of the normal mucosal architecture, secondary bacterial/endotoxin translocation and gram-negative sepsis (16).

ITx is characterized by a high incidence and severity of early acute rejection, frequent late acute rejection, a high incidence of chronic rejection and for these reasons dependence of the patient upon profound and chronic IS with its attending complications (infection, malignancies and drug toxicity). These are the main reasons for the poorer short- and long-term results of ITx versus other organ transplants (17 - 19).

Why is the rejection response after transplantation of the bowel so vigorous? Alloimmune responses in general are initiated by “Antigen presenting cells” (APC) of donor and recipient origin. The bowel contains high numbers of APC and these APC are permanently activated by ‘danger signals’ like bacterial products and endotoxin which are largely present in the lumen of the bowel. The innate immune system of the bowel is thus chronically activated and may stimulate adaptive immunity against the graft. In addition, endotoxin has immunoadjuvant properties and can also accelerate immune responses. Finally the lymphoid nature of the bowel makes it also more immunogenic than other organs. All this results in the development of a larger number of effector, cytotoxic T cells and a vigorous graft rejection (17). The severe and complex immune response triggered by the
bowel can be controlled by tacrolimus-based profound IS, but this leads in turn to a higher incidence of infection and malignancies.

RESULTS OF THE INTESTINAL TRANSPLANT REGISTRY (www.intestinaltransplantassociation.com)

Between 1985 and 2011, the Intestinal Transplant Registry has collected data from 78 centers worldwide who have transplanted approximately 2569 transplants. This corresponds to a yearly activity worldwide of approximately 100 transplants. ITx activity represents less than 0.5% of all organ transplant activity worldwide. 49.7% have been children, 50.3% adults. 1114 have been isolated bowel transplants, 841 combined liver-bowel transplants and 598 multivisceral transplants (the three types of ITx are represented in figure 1).

The average 1-year patient survival is between 70% and 80%. The 5-year patient survival is 50% and thus remains poorer compared to other organ transplants (ITR 2011 report). Rejection and infection remain the leading causes for graft failure and death (8, 9) and Ojo et al. have demonstrated that among all solid organ transplants, ITx recipients have the highest risk of developing renal dysfunction posttransplant due to the higher amounts of nephrotoxic calcineurin inhibitors generally administered (18). From the Intestinal Transplant Registry it appears that the following factors are predictors of better graft survival (2006-2011): patient at home (versus hospital-bound) at time of transplantation, an induction course with ATG or anti-IL 2 receptor antibodies, center experience and first transplant.

Figure 1. Intestinal transplantation: 3 types. 1A: type 1: isolated intestinal transplantation; 1B: type 2: combined liver and intestinal transplantation; 1C: type 3: multivisceral transplantation (defined by inclusion of the stomach).
versus second transplant. In patients who have survived one year posttransplant, the combined transplantation of the liver improves the outcome.

An encouraging message from the Registry is that the intestinal function in a large majority of the patients who survive more than 6 months after the procedure is normal (TPN-free). In addition, Abu-Elmagd et al. have shown that nutritional autonomy could be achieved in 90% of the survivors and that most of them were reintegrated to society with self-sustained socioeconomic status (19).

NEED FOR IMMUNOMODULATION

It is clear from these data that the improvement of the long-term results (and therefore the wider application) of ITx depends upon the development of so called “tolerogenic” strategies (= methods to promote engraftment while reducing IS and its attending complications). Several tolerogenic protocols have been described and will be shortly reviewed (17, 20).

A. COMBINED LIVER TRANSPLANTATION

It is known that the liver has a protective (anti-rejection) effect on the organs that are simultaneously transplanted. This liver protective effect is based on a regulational mechanism by induction of T-regulatory cell responses and a deletional mechanism by apoptosis of cytotoxic T-cells, also known as activation induced cell-death. However, because the bowel is highly immunogenic, the degree of protection offered to a bowel graft by the liver is less overt than for other solid organs (kidney or heart). Indeed, although the incidence of acute rejection after combined liver-ITx does not seem to differ very much from isolated ITx, the severity of rejection seems to be less pronounced. In addition, the incidence of chronic rejection of the bowel seems also to be reduced after a combined liver transplantation (21, 22).

B. BONE MARROW AUGMENTATION

This technique consists of infusing bone marrow-derived cells at the time of - and in addition to - transplantation of the bowel. This strategy has been tested by the team of Pittsburgh, sometimes in combination with irradiation of the bowel graft (in an attempt to reduce its immunogenicity) (7). Results of these studies have however been inconclusive and bone marrow augmentation has been abandoned since then. In addition, we showed in a large animal model that this technique of bone marrow augmentation can potentially sensitize the recipient (resulting in a higher incidence of rejection) and can also lead to, or aggravate GVHD (23).

C. BONE MARROW TRANSPLANTATION

Cases of true tolerance (complete absence of IS) have been described after combined kidney and bone marrow transplantation (24) and also after combined liver and bone marrow or stem
cell transplantation (25). These protocols, albeit efficient, require important preconditioning in the recipient and are toxic. Theoretically they could be applied to ITx and in fact have been applied successfully to ITx in rodents (26), but are probably not applicable to human ITx due to the exceeding risk of infection that these procedures entail.

D. TOLERGENIC IMMUNOSUPPRESSION

The teams of Pittsburgh and Miami have reported the use of respectively ATG or alemtuzumab in combination with tacrolimus in order to induce a tolerogenic response and promote engraftment of the bowel (9, 10, 15). Short-term results have been excellent. These protocols, however, have been accompanied by an incidence of acute rejection of more than 30% and cases of late acute rejections have been described. Longer follow-up is warranted.

THE “LEUVEN TOLERGENIC PROTOCOL”

The Leuven pro-regulatory regimen is an experimentally-proven multi-step protocol that promotes tolerance via the development of regulatory cells (17). Regulatory cells have been shown to play a major role in protecting from autoimmunity and rejection (27). They are either naturally occurring or induced by allo-antigens (27). The protocol that was developed in the experimental transplantation laboratory creates an environment favorable to the development of these regulatory cells by 4 steps (17, 28):

1- administration of Donor Specific Blood Transfusion (DSBT), a technique already used in the 70’s in organ transplantation but abandoned with the introduction of CsA in part because the mechanisms of action were unknown. Our lab and other labs have shown that DSBT operates via the development of regulatory cells (29);
2- avoidance of high-dose of steroids which can block the positive effect of DSBT and paradoxically trigger rejection (30);
3- avoidance of high-dose of calcineurin inhibitor (tacrolimus) which can block the generation of regulatory cells whereas low dose promotes their expansion (31);
4- reduction of the amount of the so-called ‘danger signals’ like endotoxin translocation and inflammation at the time of transplantation. The rationale for this last step is the fact that the endotoxin translocation and the inflammatory environment at the time of reperfusion activate APC and aggravate rejection. Endotoxin can also block the tolerogenic effect of DSBT (32). Reducing the amount of endotoxin translocation and inflammation at the time of transplantation is obtained by (i) selecting only excellent ischemia-free donors and harvesting perfect intestinal grafts, (ii) applying small bowel decontamination to donors and pursuing it in recipients in order to reduce the amount of endotoxin, (iii) administrating of glutamine to donors and recipients to protect the mucosal barrier and finally (iv) reducing the period of cold and warm ischemic times.
Minimal IS was used and consisted in (i) induction IS (with either anti-IL 2 receptor antibody at day 0 and 4 or with thymoglobulines at 1.5 mg/kg for 4 days); (ii) azathioprine tapered to 0.5 mg/kg by 3 to 6 months, (iii) tacrolimus levels of 15 µg/ml early posttransplant and reduced to 6-8 µg/ml (for isolated transplants) or less than 5 µg/ml (in combination with liver transplantation) by 6 months posttransplant and (iv) steroids were given at a low-dose of 16 mg posttransplant and tapered to 4 mg by 3 to 6 months posttransplant.

RESULTS FROM THE UNIVERSITY HOSPITALS LEUVEN

Under this protocol, 10 consecutive cases of ITx from deceased donors have been performed in our center between October 2000 and October 2013. The technique used for combined liver and small bowel transplantation was described by Sudan et al. and consists in a combined en bloc procurement of the liver, pancreatic head and small bowel (33). It is a procedure which is technically easier than a combined liver and bowel procurement (without the pancreas) because it avoids the need for a separate biliary anastomosis in the recipient. A combined liver and intestinal graft was transplanted in five patients. The other five patients received an isolated small bowel graft with additional kidney transplantation in two for enteric hyperoxaluria, secondary to short bowel syndrome (34).

Eight of the recipients were female and two were male, with a median age of 38.5 years (range: 2 years 8 months - 57 years) and median weight and body mass index (BMI) of respectively 55.5 kg (range: 10.9 – 62.9 kg) and 19.3 kg/m² (range: 10.9 – 24.9 kg/m²). Two patients (20%) were under 18 years of age at time of transplantation. ABO blood group was identical in 8 patients (80%) and compatible in 2 (20%). Indications were anatomical or functional short bowel syndrome: volvulus (4), intestinal ischemia (3), Morbus Crohn (2) and chronic intestinal pseudo-obstruction (1). All patients were TPN-dependent with multiple infections as a consequence of catheter sepsis. Median waiting time between listing on the transplant list and transplantation was 385 days (range: 10 – 907 days). At time of transplantation, 2 patients (20%) were hospitalized and 8 (80%) were at home. The median cold ischemia time (CIT) was 5 hours and 11 minutes (range: 3 hours 45 min – 6 hours 37 min).

The 5-year patient and graft survival rate is 90%, according to the Kaplan-Meier analysis (figure 2). Up to this moment, 8 of the 10 patients (80%) are clinically well and rejection-free with a median follow-up of 2142 days (254 days – 4321 days). We saw only one early rejection (within the first three months posttransplant), which accounts for 10% of our series. The recipient was a patient who suffered from Morbus Crohn. 18 months after transplantation he also developed a late acute rejection, which was reversible with high dose of steroids. Retrospectively we found that not only the recipient was affected by a mutation in the NOD2 gene.
Figure 2

![Survival Function Graph](image)

(missence mutation 2104C -> T {SNP8; R702W}) but also the donor graft (missence mutation 2104C -> T {SNP8; R702W}). Both findings correspond to the argument pointed out by Fishbein et al. that Crohn’s disease-associated polymorphisms in the NOD2/CARD15 gene represents a critical immunological risk factor for intestinal allograft rejection (35). We documented two additional late rejections in two other patients. One was diagnosed 4 months after transplantation. It was an acute cellular rejection followed by an Aspergillus’ sepsis, to which the patient succumbed. The other late rejection occurred 3 years and 10 months posttransplant and was due to therapy withdrawal secondary to non-compliance. With immediate treatment, the rejection could be reversed. The longest survivor succumbed 11 years and 10 months posttransplant due to self administration of non-steroidal anti-inflammatory drugs that caused diffuse small bowel enteropathy and bacterial translocation. No arguments of a fulminate acute or an underlying chronic rejection could be withheld.

In our series, no GVHD or posttransplant lymphoproliferative disorder was diagnosed and in only one patient an EBV infection was reported. None of the patients developed kidney failure. Seven out of the eight survivors were performing very well at last follow-up with a Karnofsky performance score of 90-100%.
One child was not able to resume all her activities and had a score of 61-89%.

**CONCLUSION**

In conclusion, the obstacle to ITx is the difficulty to control rejection without resorting to profound and chronic IS with its attending side effects (infection, cancer, drug toxicity). For this reason, long-term results remain poorer compared to other organ transplants. This difficulty is caused by the vigorous antigen presentation capacity of the bowel and the vigorous rejection that ensues. ITx is currently proposed to physically fit patients with short bowel syndrome and severe life-threatening TPN complications for whom ITx could be life-saving. The immunomodulatory protocol described herein seems to promote graft acceptance and long-term survival at the cost of low levels of IS. Results obtained with this Leuven tolerogenic protocol seem comparable with other solid organ transplants.

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