# Contribution of large animal models to the development of clinical intestinal transplantation

J. Pirenne

Abdominal Transplant Surgery Department, University Hospitals Leuven, Herestraat 49, B-3000 Leuven, Belgium.

## Abstract

**The intestine has long been seen as a "forbidden" organ to transplant and even nowadays it remains the most challenging abdominal organ to transplant. Large animal experiments have been pivotal, first in developing reproducible and clinically applicable surgical techniques for transplanting the intestine and second, in revealing the unique physiological, immunological, and microbiological challenge that intestinal transplantation (ITx) represents. More recently, large animal models have been used to test new immunosuppressive drugs (FK 506) that have been then successfully used clinically. ITx is no more an experimental endeavor and survival figures of about 70 % can be reached at one year, justifying routine application of ITx to patients who do not tolerate total parenteral nutrition. However ITx remains in 1999 an "unfinished product" and further rssearch will need to be done to allow wider application of ITx to patients without total parenteral nutrition (TPN) related complications. Further research will focus on the following aspects : (1) refined understanding of the factors accounting for the high immunogenicity of the intestine ; (2) development of immunomodulatory strategies to reduce graft immunogenicity and to induce specific hyporesponsiveness ; (3) development of new immunosuppressants, and their usage in combination, to act more specifically on the immune response, and at the price of less toxicity ; (4) development of surgical alternatives to alleviate the organ shortage : graft size reduction, live related ITx. Importantly these questions will need to be addressed in clinically relevant animal models before they are applied to man.** (Acta gastroenterol. belg., **1999**, 62, **221-225**).

**Key words** : intestinal transplantation, rejection.

## Introduction

Total parenteral nutrition is currently the only first line therapeutic option for patients suffering from complete anatomical or functional loss of their intestine (short-gut syndrome). Although parenteral nutrition has made major progress, it is not devoid of complications. These patients would clearly benefit from intestinal transplantation (ITx). The intestine, however, has long been seen as a "forbidden" organ to transplant and even nowadays it remains the most challenging abdominal organ to transplant. Small and large animal experiments have played a crucial role in elucidating some of the obstacles to successful ITx and in making that procedure a clinical reality.

Small and large animal studies are not mutually exclusive but rather complementary. Unlike studies in large animals, rodent studies have the unique advantage to require no particular logistics and to be relatively unexpensive. Availability of genetically well-defined inbred strains and biologically engineered strains (transgenic/knock out), and development of various bioreagents make rodents an ideal animal model to dissect the immune response. Studies in large animals, dogs and pigs, however, are usually more directly clinically relevant.

## Early large animal work demonstrating the unique characteristics of intestinal transplantation

Large animal experiments have been pivotal, first in developing reproducible and clinically applicable surgical techniques for transplanting the intestine and second, in revealing the unique physiological, immunological, and microbiological challenge that ITx represents.

### *Techniques for ITx*

Techniques for accessory ITx were first described by Alexis Carrel in dogs in the beginning of this century (1). Thereafter, multiple models of segmental or entire bowel transplant including or not the colon, and even techniques for multivisceral transplantation have been described (2, 3). The techniques developed in pigs are clinically particularly relevant because the anatomy is very similar to humans. Given the rarity of indications for ITx in humans, it even seems appropriate for transplant surgeons to learn these techniques in large animals before applying them to man.

### *Physiological difficulties*

Autotransplant experiments by Lillehei in the early sixties indicated that, unlike in other organs, there may be physiological obstacles to transplant the bowel (3). Indeed, dogs that received autotransplanted bowels suffered from diarrhea and weight loss. It thus appeared that denervation, lymphatics interruption and ischemia would already disturb the function of the intestinal graft independently of the immune response. However, those animals eventually fully recovered, indicating that these physiological obstacles *per se* were not limiting the applicability of ITx (3). In fact, experiments have

Address : Jacques Pirenne, Abdominal Transplant Surgery Department, University Hospitals Leuven, Herestraat 49, B-3000 Leuven, Belgium.
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shown that the bowel could tolerate some degree of ischemia — up to 5 hours of total ischemia under hypothermia — (3), that lymphatics do regenerate (3, 4), and that the intestinal allograft with its preserved intrinsic motor plexus displays a modified but adequate motor function (5, 6). Interestingly, segmental intestinal grafts have recently successfully been transplanted between two identical triplets, showing that these physiological factors are not limiting the applicability of ITx, at least in the absence of immune mediated injury (7).

*Immunological characteristics*

Bowel transplantation causes a two-way immunological conflict, not only a standard rejection response, but also a graft-versus-host disease (GVHD), similar to that observed after bone marrow transplantation ; this reaction is caused by the lymphoid tissue conveyed within the bowel graft. These unique immunological characteristics of the intestinal allograft were early suspected by Lillehei (3). Recipients of entire ITx allografts died without gross evidence of intestinal graft rejection but presumably due to an immune reaction directed against the recipient. On the other hand, heterotopic segmental grafts were rapidly rejected. It thus became apparent that the immune response elicited by ITx was dependent upon some balance between two opposite immune responses (3). The notion that some degree of GVHD may mitigate rejection and that the fate of intestinal grafts may eventually depend upon a balance between rejection and GVHD was further substantiated by Cohen in a canine model : Recipients of unirradiated grafts died early from GVHD whereas recipients of irradiated grafts (50 rads) had extended survival ; however, excessive irradiation of the bowel graft (150 rads) resulted in limited survival prolongation, probably resulting from complete suppression of a potentially beneficial GVH effect (8).

*Microbiological issues*

By definition, the intestine is a septic organ ; thus, its transplantation causes major infectious problems. This was early recognized by Toledo-Pereyra who showed that ITx induces endotoxemia (9). This was confirmed later by others and it appears that endotoxinemia occurs particularly at two time points, first early postoperatively as a consequence of ischemia-reperfusion injury, and then later on at the time of rejection (10). Endotoxin is an immune adjuvant and it is possible that endotoxinemia would promote rejection after transplantation (11). In addition to bacterial and endotoxine translocation, ITx recipients are susceptible to systemic infection due to the profound immunosuppression regimen they receive to control the immune response. In fact, infection has been the limiting factor to long-term survival in many large animal models.

## Special issues explored in large animal models

*Importance of GVHD versus rejection*

From small animal experiments it had been anticipated that GVHD may be a major problem in ITx recipients (12). However, in large animal experiments, rejection and not GVHD has been the major obstacle. However, it would be equally wrong to underestimate the importance of GVHD in both large animal and in human ITx. Rejection usually predominates over GVHD and thus destroys the effector cells of GVHD but clear cases of GVHD have been unequivocally observed both in fully allogeneic large animal models and in man (13, 14, 15). Work by Starzl has actually reappraised the importance of graft versus host immune response after solid organ transplantation and particularly after transplantation of the liver and other organs containing hematopoietic cells ; for example, the incidence of clinical GVHD after liver transplantation may reach 5% and the incidence of subclinical GVH responses has probably largely been underestimated (16, 17).

*Absorption studies*

Although not completely normal, absorption of carbohydrates, proteins and lipids is reestablished after ITx and autotransplants do provide complete nutritional independence. Absorption of nutrient entirely depends on the integrity of the grafted intestine and thus will be impaired in case of immune and non immune mediated mucosal injury (ischemia-reperfusion, rejection, graft infection) (for a review see 5, 6).

*Motor function*

This has been extensively studied by Kelly and Sarr in canine models (5, 6). In summary, migrating motor complex (MMC) do persist after ITx because they are controlled by the preserved nervous system. However, the close temporal coordination of the MMC between the native and the transplanted segment is disrupted by ITx and is only partially restored with time. In addition, hormonally mediated postprandial disruption of fasting motility is maintained after ITx in large animals (for a review see 5, 6).

*Preservation*

One particularity of intestinal allografts seen to a lesser extent with other organs is that cold storage, independently of reperfusion can induce substantial damage to the mucosa, probably because of enzymes that are still active at low storage temperature (18). In addition, ischemia-reperfusion as with other organs will induce activation of proinflammatory pathways, including the expression of cytokines, chemokines, adhesion molecule and this may increase graft susceptibility to rejection. It results that storage and ischemia-reperfusion lead to mucosal damage, increased mucosal

permeability and bacterial translocation. Preservation studies have shown University of Wisconsin (UW) to be superior to Eurocollins and lactate ringer's in a 24 hour preservation model (19). The exact time limit for preservation of the bowel is not known but there is no doubt that one should aim at an ischemia time that is as short as possible. Recently, lazaroids have been shown to improve preservation and to limit ischemia-reperfusion induced damage (20).

*Development of new immunosuppressive regimens*

Before the era of calcineurin inhibitors, "conventional" immunosuppressive regimens such as steroids, azathioprine, antilymphocytes globulins have not been able to control the alloimmune response elicited by ITx in various canine and pig models. In general, survival was prolonged from one week to maximum 3 or 4 weeks (21).

Introduction of cyclosporine A (CsA) gave new hope and significant survival prolongation was noted by Ricour, Revillon and co-workers (22). However, the anti-rejection effect of CsA, even given iv to avoid absorption problems, was not as dramatic and reproducible in ITx as it was for other organs (23).

It then became clear that the intestine was by far more immunogenic than other organs and that successful grafting of the intestine would require significantly higher immunosuppression. At that time, Grant showed that rejection of pig intestinal allograft could be controlled by using a CsA based immunosuppressive regimen, providing that high dosage of CsA were used (25 mg/kg iv followed by 20 mg/kg oral) (24). But such high levels would not be clinically applicable due to CsA nephrotoxicity. Those experiments however were crucial in that it was possible for the first time to obtain long-term survival in large animal recipients of an intestinal allograft. Other experiments by Gruessner also showed that rejection of intestinal allografts could be prevented, by using a quadruple CsA based immunosuppressive cocktail (25). Thus, those various experiments were pivotal in indicating that the immune reaction elicited by ITx could in fact be overcome but at the price of profound immunosuppression. They set up the ground for human trials of ITx in various Tx centers worldwide.

Another calcineurin inhibitor FK 506 — Tacrolimus — was then introduced by Starzl. It appeared very early that FK 506 was superior to CsA in small animal models in preventing rejection, GVHD, and thus in prolonging graft survival (26). These early observations were then confirmed in preclinical studies in pigs where it appeared that FK 506 was superior to CsA in controlling rejection (27).

Substantial progress has since been made by introducing FK 506 in the clinical arena and FK 506 based immunosuppression is now the standard in ITx. Experiments by Alessiani and d'Alessandro have shown the additional protective effect of MMF plus FK 506 or CsA in ITx (28, 29). However, the gastrointestinal toxicity of MMF (diarrhea, vomiting) may limit its application clinically. Many studies comparing FK 506 versus CsA have actually used the old formulation of CsA (Sandimmun). The new formulation of CsA (Neoral), however, has an increased bioavailability, compared with the oral sandimmun after ITx in a pig model (30). The role of Neoral in ITx should thus be reassessed. Interestingly, Cohen showed that the combination of Rapamycine and CsA prolonged intestinal allograft survival (31). As may have been expected, the association of FK 506 and Rapamycine was not effective, presumably because of the known competition between these two drugs that bind to the same receptor (31).

*Influence of HLA matching*

Unfortunately, the HLA matching has not been reported in the international registry and it is not known whether HLA matching reduces rejection in clinical ITx. There are indirect reasons to believe it does. First clinical experience with kidney and particularly with highly immunogenic organs such as the pancreas does indicate a positive effect of matching. Second, there is one pivotal study in dogs indicating that DLA (Dog Leukocyte Antigen) matching reduces rejection and improves survival of segmental intestinal allografts (32).

*Transplantation of the colon*

Diarrhea has often been observed in recipients of ITx despite adequate function of their graft and this has been particularly a problem in those patients who have no residual native colon. Colon Tx has thus been proposed to solve that problem. Using a preclinical model in pigs, it has been shown, however, that addition of the colon to the small bowel considerably increases the incidence of posttransplant complications, rejection and infection (27). Similar observations have been made in human ITx. Thus, only a small segment of colon, if any, should be added to small intestinal allografts. It has been shown by Nakhleh that one cannot rely on colonic biopsies only to assess presence or absence of rejection in other parts of the graft. In fact based on pigs studies there seems to be a hierarchy of susceptibility to rejection : ileum > jejunum > colon (33).

*Portal versus caval drainage*

Certain small animal experiments have shown the superiority of portal versus caval drainage in terms of graft acceptance and metabolic side effects. In large animals, however, like in man no clear difference has been documented (34, 35). If technically possible, it seems logical to prefer portal drainage because it is more physiological. But in case the portal system is not readily accessible one should not hesitate to perform a systemic drainage of the graft.

*Protective effect of combined liver ITx*

Experimentally and clinically, the liver is known to induce tolerance or at least hyporesponsiveness to simultaneously or sequentially transplanted organs from the same donor ; for example, kidneys transplanted with the liver are less rejected. Small animal studies have indicated that this phenomenon may apply to combined liver and ITx (36, 37). Interestingly, one of the first successful human cases of ITx under CsA was a combined liver and ITx (38). Because the bowel is so immunogenic, it was proposed at that time to transplant the liver even in patients who only suffer from bowel insufficiency, in order to induce hyporesponsiveness and to help the bowel to engraft.

To better study that question in a clinically relevant model, combined liver and ITx was done in pigs under FK 506 based immunosuppression (39). No immune advantage was seen in recipients of liver bowel versus bowel alone with regard to incidence and severity of rejection. However this was an acute model and long term survival was not obtained mainly due to infection. Recent data from human indicate that in the long run, there is some advantage to transplant the liver with the bowel, in terms of reduced incidence of rejection. But this protective effect does not justify to transplant the liver in addition to the bowel in patients with preserved liver function, given the marginality of that effect, the increased short term morbidity/mortality of the combined procedure, and the shortage of liver grafts.

*Immunomodulatory strategies to promote intestinal engraftment*

Lymphoid tissue conveyed with an intestinal allograft may induce GVHD but paradoxically, donor derived hematopoietic tissue may have protective effects (16, 40). In certain experimental models, transplanting an organ with donor derived lymphoid tissue can protect the graft from rejection through mechanisms that remain to be elucidated (16, 40). This, plus the observation of stable microchimerism in stable long-term recipients of liver and kidney grafts led to the concept that leukocyte enriched organ, in place of stimulating the immune response, may induce some degree of hyporesponsiveness, possibly through the establishment of stable microchimerism (16, 41). On that basis, some clinical centers have started programmes of combined solid organ plus donor specific bone marrow transfusion. Experimentally, however, infusion of donor bone marrow on the day of transplantation has induced neither tolerance nor hyporesponsiveness in unmodified outbred pig recipients of an intestinal graft (42, 43). On the contrary, this strategy has led to more pronounced GVHD and to increased rejection. This strategy i.e. donor specific transfusion of bone marrow, will have to be refined with regard to type, timing, and quantity of cells injected, type of recipient preconditioning regimen and immunosuppression that is needed to promote the tolerogenic effect of those manipulations.

One recent experimental finding by Gruessner, in a portally drained ITx model in pigs is that intraportal administration of donor specific blood transfusion at the time of surgery increases survival under FK 506 immunosuppressive therapy (44).

## Future prospects

Lessons learned, in part in large animal models and subsequently applied to the clinical arena have improved the results of ITx. Administration of FK 506, a policy consisting in profound antibiotic prophylactic regimens, decontamination protocols in both donors and recipients, and vigourous anti-viral protection of the recipient (against cytomegalovirus and Epstein-Barr), have significantly improved the results. ITx has recently reached more standard clinical application and the one-year survival rate of intestinal allografts reaches now 70% i.e. figures roughly similar to lung transplantation (45). However, due to its physiological, microbiological and immunological characteristics, the small bowel will remain the most challenging abdominal organ to transplant. One is still confronted with various problems. First, late graft loss to rejection (after one year), albeit rare in other solid organ transplants, frequently occurs after ITx. Second, the profound immunosuppression that is currently needed to control the alloimmune response causes a high rate of sepsis, cytomegalovirus infection, and a relatively high incidence of posttransplant lymphoproliferative disorders. Third, one is confronted to a profound organ shortage and many pediatric recipients die on the waiting list before a suitable donor organ can be found.

Thus, it appears that ITx remains in 1999 an "unfinished product". Further research should focus on the following aspects : 1) refined understanding of the factors accounting for the high immunogenicity of the intestine ; 2) development of immunomodulatory strategies to reduce graft immunogenicity and to induce specific hyporesponsiveness ; 3) development of new immunosuppressants, and their usage in combination, to act more specifically on the immune response, and at the price of less toxicity ; 4) development of surgical alternatives to alleviate the organ shortage : graft size reduction, live related ITx. Importantly these questions will need to be addressed in clinically relevant animal models.

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