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LIVER AND INTESTINE TRANSPLANTATION

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LIVER TRANSPLANTATION

Transplantation of the liver, as originally conceived, was first reported in 1955 as an auxiliary liver transplant in which the native liver was preserved in its normal position and the new liver placed at a heterotopic site, usually the right paravertebral gutter, with portal inflow supplied with systemic blood via the inferior vena cava or iliac vein. Orthotopic liver transplantation is the accepted procedure today. The native liver is removed and replaced by a liver allograft in its normal location. The most common type of transplantation encompasses five anastomoses: the suprahepatic vena cava, the infrahepatic vena cava, the portal vein, the hepatic artery, and the biliary reconstruction.

Indications for Liver Transplantation

The indications for liver transplantation have evolved concomitantly with all aspects of this field and presently include cirrhosis (posthepatic, alcoholic, autoimmune, cryptogenic), cholestatic liver disease (primary biliary cirrhosis, sclerosing cholangitis, biliary atresia), neoplasm, fulminant hepatic failure, and inborn errors of metabolism. There has been progress in the continuing struggle to identify, treat and prevent a

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variety of complications to which the liver transplant recipient is subject. These complications may include postoperative bleeding, technical complications with any of multiple vascular and biliary anastomoses, infections, and graft dysfunction.

Advances Made Possible by FK506 (Tacrolimus)

The struggle to develop clinical liver transplantation to its present status has spanned 3 decades. Advances in organ preservation, surgical techniques, and peri- and postoperative care have been essential components of this success. However, only with improvements in immunosuppressive regimens and treatment of allograft rejection was this parallel evolution translated into acceptable patient survival. With the introduction of cyclosporine in 1980 liver transplantation was expanded to permit universal applicability. It is currently performed in over 100 institutions in the United States alone, as well as Europe; efforts to institute transplantation programs in South America and Asia are well beyond the developmental stage. By 1989, 1,835 patients had been transplanted at our center under cyclosporine and prednisone based immunosuppression.[38, 47] After 3 years of pre-clinical research, a clinical trial of FK506 (tacrolimus) for orthotopic liver transplantation was begun in February 1989: First as rescue therapy for patients with intractable rejection under conventional immunosuppression, then as part of a randomized trial comparing tacrolimus to conventional immunosuppression, and finally as a primary drug.

Initial Clinical Studies with Tacrolimus

The clinical application of tacrolimus began after extensive laboratory research in Chiba, Pittsburgh, and Cambridge.[6, 20, 28, 29, 30, 53, 54, 62, 63, 65] In February 1989 it was used successfully to salvage 8 of 10 liver allograft recipients suffering intractable rejection despite optimal cyclosporine-based immunotherapy.[50] Further use for this indication accrued over 200 patients, and was expanded to include recipients of heart, kidney, lung, and pancreas allografts.[10, 12] In the patients whose graft dysfunction was attributed to acute or the early stages of chronic rejection the success rate has been as high as 90%, and even with chronic rejection of the liver salvage has approached 50%.[12]

Randomized Clinical Trials with Tacrolimus

After the positive experience with the rescue patients, clinical trials using tacrolimus as the primary immunosuppressive agent for recipients of liver, kidney, and thoracic organs were initiated.[46, 55] Although there was clear evidence of drug superiority with our initial cohort of recipients, an Institutional Review Board (IRB) mandated trial was begun in February 1990 which included a total of 79 low risk patients. In Decem-

ber 1991, this trial was discontinued after recommendations from a multi-institutional "Patient's Rights Committee," with the concurrence of the IRB and the Food and Drug Administration (FDA).

In the Pittsburgh randomized trial all treatment variables were equal including a daily dose of 20 mg prednisone. The occurrence of rejection, and the need to treat with added prednisone or other adjuvant therapy directly reflected the efficacy of the competing drugs. With the "intention to treat analysis" (crediting the end-point outcome to the original assigned randomization), the one year patient survival was 94% tacrolimus versus 89% cyclosporine, and graft survivals were 98% tacrolimus versus 80% cyclosporine.[11] However, at the end of 3 1/2 years the composite freedom from rejection, or graft loss (death or retransplantation) was 24% for tacrolimus versus 8% for cyclosporine. Freedom from "adverse events" was 6% for tacrolimus versus 1% for those originally assigned to cyclosporine. Freedom from rejection alone was seen in 33% of patients in the tacrolimus cohort compared to 12% for the cyclosporine cohort. At the end of 1 year 51 of the 75 patients originally assigned to receive cyclosporine had been converted to tacrolimus. It was concluded that tacrolimus was superior to cyclosporine A.[11]

Different although generally congruent information was produced by the European[27] and American[5] multi-center trials which supported commercial release of tacrolimus by the FDA in June 1994. In the European study, although patient and graft survival rates were not significantly different (tacrolimus 82.9% and 77.5% versus cyclosporine 77.5% and 72.6%, respectively), the use of tacrolimus was associated with a significant reduction in acute, refractory acute, and chronic rejection episodes. Although the survival advantage was not statistically significant it was noted that 10% of the surviving grafts credited to cyclosporine had been rescued with tacrolimus. Toxicity variables were similar and comparable.[27] In the American study, actuarial patient and graft survivals were comparable. It was noted that although tacrolimus was associated with significantly lower episodes of acute and refractory rejection there were substantially more toxic events requiring discontinuation of tacrolimus.[5] However, reanalysis of this data reveals that the composite freedom from refractory rejection, retransplantation, and death was 80% for the tacrolimus arm versus 70% for the cyclosporine cohort.[45]

Primary Therapy with Tacrolimus

Our clinical experience spans 1,391 consecutive adult and pediatric recipients of primary liver allografts treated with tacrolimus between August 1989 and December 1993.[56] The patient characteristics are summarized in Table 1. Among these 1,391 recipients 85.4% were adults and 14.6% were children. Of the adult patients 26% were over 60 years of age. The most common indication for transplantation was biliary atresia in children and post-necrotic cirrhosis in adults. The severity of disease for the majority of the patients was high as defined by the existing United Network for Organ Sharing (UNOS) criteria: (1) working; (2)

Table 1. CHARACTERISTICS OF THE 1391 TACROLIMUS PRIMARY LIVER ALLOGRAFT RECIPIENTS

Total Patient Population	1391
No. of transplants	1582
Primary transplantation	1391
Retransplantation	191
Median follow-up months (range)	26.6 (3–55)
Pediatric (< 18 yrs)	
Number of patients	203
Mean age ± SD	5.4 ± 5.5
Indications	
Fulminant failure	13 (6.4%)
Postnecrotic cirrhosis	21 (10.3%)
Biliary atresia	99 (48.8%)
Metabolic disease	27 (13.3%)
Primary malignancy	1 (0.5%)
Other	42 (20.7%)
UNOS Status	
1	3 (1.5%)
2	57 (28.1%)
3	72 (35.5%)
4	71 (35.0%)
Adult (> 18 yrs)	
No. of patients	1188
Mean age ± SD	50.1 ± 12.0
Indications	
Fulminant failure	20 (2.5%)
Postnecrotic cirrhosis	749 (63.0%)
Cholestatic disease	200 (16.8%)
Metabolic disease	38 (3.2%)
Primary malignancy	89 (7.5%)
Other	82 (6.9%)
UNOS Status	
1	5 (0.4%)
2	189 (15.9%)
3	476 (40.1%)
4	518 (43.6%)

home (but requiring close medical supervision and/or sporadic hospital care); (3) hospital-bound continuously; (4) ICU-bound.

Clinical Approach to Liver Transplantation

Immunosuppression

Our early clinical experience showed dose related nephrotoxicity, neurotoxicity, and diabetogenicity, which were paralleled with defective metabolism of the drug when there was hepatic graft dysfunction.[1] These observations prompted progressive dose revisions which were guided by a balance between freedom of rejection, toxicity, and trough plasma levels of tacrolimus measured with an enzyme immunoassay

technique and targeted to 1 ng/mL. Our present policy is to give tacrolimus initially intravenously as a continuous infusion at a dose of 0.05 mg/kg/day. The conversion from intravenous to oral therapy is usually made with a small overlap at a starting oral dose of 0.1–0.15 mg/kg every 12 hours.

The steroid administration involved 1 gram of methylprednisolone given intravenously immediately after graft reperfusion, followed by a 5 day burst of methylprednisolone which was started at 200 mg on the first day and reduced to a baseline of 20 mg/day on post-operative day 6. Appropriately lower doses were given in infants and children. In a subsequent phase, the 5 day burst therapy was replaced with a standard 20 mg/day of methylprednisolone, the initial high dose steroid burst being reserved for positive lymphocytotoxic cross matches.[52] With either regimen, steroids were weaned over several months and eventually stopped if there was no evidence of rejection.

Clinical diagnosis of rejection was confirmed by a needle biopsy. Initial therapy for rejection included optimization of maintenance tacrolimus levels and bolus therapy with methylprednisolone or hydrocortisone. Persistence of rejection was treated with 5 day burst therapy using methylprednisolone. Steroid resistant rejection was treated with a 3 to 5 day course of 5–10 mg/day of monoclonal antibody to the T cell receptor (OKT3). Azathioprine (0.5–2 mg/kg/day) was given in cases of recurrent rejection or evidence of clinical toxicity necessitating the reduction of tacrolimus trough levels.

Patient survival was calculated from the date of transplantation until patient death, and graft survival was calculated from the date of transplantation until retransplantation or patient death. Survival curves were generated using the life table method.

Results

With a mean follow-up of 29.6 months (range: 3–55), the overall patient actuarial survival rates in children were 91.1%, 89.6%, 88.5%, 88.5%, and 86.2%, at 3, 6, 12, 24, and 48 months, respectively, and in adults were 89.7%, 86.6%, 82.1%, 77.7%, and 71.4%, at 3, 6, 12, 24, and 48 months, respectively (Fig. 1). There were a total of 1,582 liver allografts. The overall graft survival in children was 81.4%, 80.1%, 79.1%, and 77.0% at 3, 6, 12 and 48 months, respectively, and in adults were 80.3%, 76.5%, 72.0%, and 61.5% at 3, 6, 12, and 48 months, respectively (Fig. 2). The causes for primary graft failure due to rejection were only 18 grafts (1.5%) seen in only the adult population. Greater than 50% of these liver recipients were rejection free. Nearly half of the adult recipients and over 90% of the pediatric recipients were steroid free by 3 months after transplantation. Retransplantation was required in 9.9% of pediatric recipients and 9.8% of adult recipients (Table 2). The difference between patient and graft survival emphasizes the survival benefit of retransplantation.

Death was attributable to either technical failure (mostly hepatic

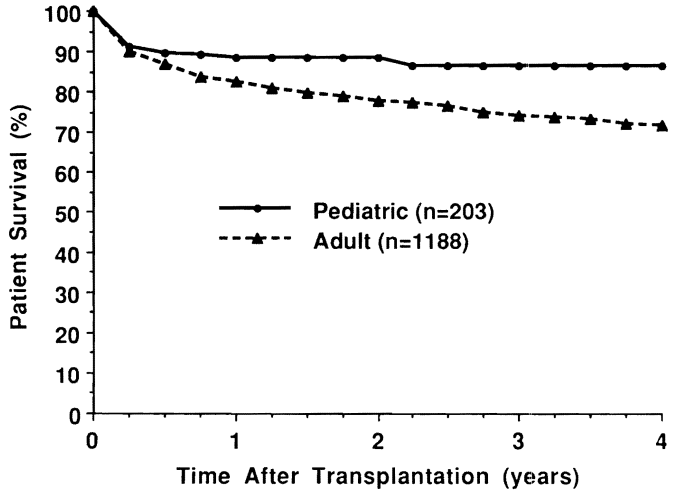

Figure 1. Patient survival for primary liver allograft recipients who received FK506 (Tacrolimus) as the primary immunosuppressive drug therapy.

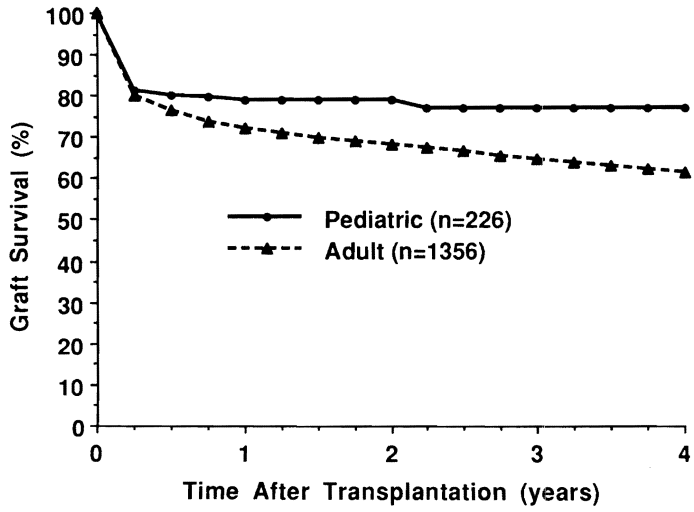

Figure 2. Overall graft survival using FK506 (Tacrolimus) as the primary immunosuppressive drug therapy. The difference between patient and graft survival emphasizes the survival benefit of retransplantation.

Table 2. INCIDENCE AND CAUSE OF RETRANSPLANTATION OF PRIMARY GRAFT

Pediatric Patients	
No. of patients	203
No. of retransplantations	20 (9.9%)
Causes	
Technical failure	6 (3.0%)
Rejection	0
Graft infection	1 (0.5%)
Graft failure	13 (6.4%)
Disease recurrence	0
Other	0
Adult Patients	
No. of patients	1188
No. of retransplantations	116 (9.8%)
Causes	
Technical failure	28 (2.4%)
Rejection	7 (0.6%)
Graft failure	76 (6.4%)
Other	2 (0.2%)
Graft infection	3 (0.3%)
Disease recurrence	0

artery thrombosis, or bile duct complications), sepsis, and immunologically related complications which included rejection, graft-versus-host disease (GVHD), and posttransplant lymphoproliferative disorders (PTLD) (Table 3).

Other aspects of our experience with drug toxicity, drug interactions, opportunistic infections, and other clinical observations have been published elsewhere.[3, 4, 8, 16, 35]

INTESTINAL TRANSPLANTATION

Total parenteral nutrition (TPN) is the standard of care for patients who are unable to maintain a normal nutritional state by the gastrointestinal tract alone. Transplantation of the intestine either alone or accompanied by other intra-abdominal organs (liver, stomach, pancreas, colon) may be beneficial in these patients. Loss of intestinal function may be attributed to loss of bowel length after resections for atresias, infarctions (volvulus, vascular catastrophes, necrotizing enterocolitis), or strictures and fistulas as with Crohn's disease and radiation enteritis. When the anatomic length and gross morphology of the intestine is normal, the functional loss can be attributed to motility disorders (intestinal pseudo-obstruction, Hirschsprung's disease), absorptive insufficiencies (microvillus inclusion disease), polyposis syndromes, and incarcerated tumors.

A multi-disciplinary team must assess the baseline disease as well as the severity of other co-morbid states, particularly liver failure and its accompanying gamut of complications. Appropriate selection of candidates and optimization of preoperative morbid conditions (infection,

Table 3. INCIDENCE AND CAUSE OF MORTALITY

Pediatric Patients	
No. of Patients	203
No. of mortalities	18 (8.9%)
Causes	
Technical failure	6 (3.0%)
Sepsis	1 (0.5%)
Immunusuppression related	1 (0.5%)
Extrahepatic event	3 (1.5%)
Disease recurrence	0
Graft failure	7 (3.4%)
Other	0
Adult Patients	
No. of Patients	1188
No. of mortalities	119 (10.1%)
Causes	
Technical failure	22 (1.9%)
Sepsis	33 (2.8%)
Immunosuppression related	9 (0.8%)
Other	1 (0.1%)
Graft failure	33 (2.8%)
Disease recurrence	1 (0.1%)
Extrahepatic event	20 (1.7%)

malnutrition) can have a major impact on outcome. Critically ill patients should be excluded. Multiple previous abdominal surgery is not a contraindication for transplantation.

Early Experimental and Clinical Studies

Experimental and clinical intestinal transplantation have been through several developmental phases over the last 30 years.[21, 48] Success remained blurred because of a high incidence of graft loss due to infection, technical complications and rejection. Rejection and the predicted counterpart of GVHD were two problems that were expected with intestinal transplantation.

Two events helped bring intestinal transplantation to the forefront of clinical practice. First was the demonstration in clinical liver transplantation of the greater efficacy of tacrolimus as described in the previous section. Then, recent laboratory and clinical research with intestinal transplantation played a critical role in establishing a generic bi-directional paradigm of transplantation and immunology that is relevant to all organs.[17, 24, 48, 59]

This reassessment began after a series of human applications of the modified form of this operation, which began in 1987 when a 3-year-old girl received a multivisceral abdominal graft that contained the stomach, duodenum, pancreas, small bowel, colon, and the liver. She had an extended survival of 6 months with good intestinal graft function with-

out developing either rejection or GVHD.[49] The cadaveric organs of this recipient were depleted of T lymphocytes by infusing the donor with OKT3 treatment before procurement and by ex-vivo irradiation after their removal. This type of preconditioning was suspected to have contributed to the wide spread B-cell lymphoma that caused the death of this child. Subsequently 4 more patients achieved functional cadaveric intestinal grafts when transplanted alone,[14] as liver/intestinal composite graft,[15, 23] or as multivisceral allografts.[39] Only the intestine-alone recipient of Goulet and Revillon[14] is alive with a functioning graft, while two liver/intestinal recipients of Grant[15, 23] survived for 58 and 66 months. Also, a living related donor intestinal segment was transplanted by Deltz[7] in February 1988 which supported nutrition for 61 months. All the above transplants were performed under cyclosporine based immunosuppression.

Present Patient Population

We have performed 71 intestinal transplantations in 67 patients between May 1990 and February 1995.[57] There were 3 types of intestinal allografts which are shown in Figure 3: isolated intestinal (n = 23), combined intestine/liver (n = 32), and multivisceral (n = 11). Those patients with satisfactory liver function without evidence of portal hypertension received an isolated intestinal allograft. Patients with inborn errors and also TPN induced cholestatic liver disease received a liver plus intestinal allograft. The multivisceral type allograft was indicated in patients who had extensive abnormalities of the entire gastrointestinal tract which included absorptive/secretory, motility, or vascular disorders. The principles and various modifications of these procedures have been described elsewhere.[18, 51, 58, 60, 61] The colon was included as part of the allograft midway through our experience with intestinal transplantation in all 3 recipient cohorts. This was prompted by high post-operative stomal outputs requiring frequent admissions for dehydration.

Our more recent experience also involves including bone marrow infusion at the time of intestinal transplantation. Bone marrow cells were recovered from the same donor as the intestine and were infused intravenously into the recipient in the immediate post-operative period in 4 recent cases (3 primary and 1 retransplantation). The rationale for this approach of including simultaneous bone marrow infusion after solid organ transplantation was derived from experimental and clinical evidence that the existence of "chimerism" is critical for graft acceptance.[9, 26]

There were 37 children, 16 male and 21 female with a mean age of 4.3 ± 4.2 years. Twenty-nine patients were adults, 16 male and 13 female, with a mean age of 33.3 ± 9.5 years. The original diseases leading to transplantation are as listed in Table 4. All patients have been followed through April 1995. Median follow-up was 21 months ranging

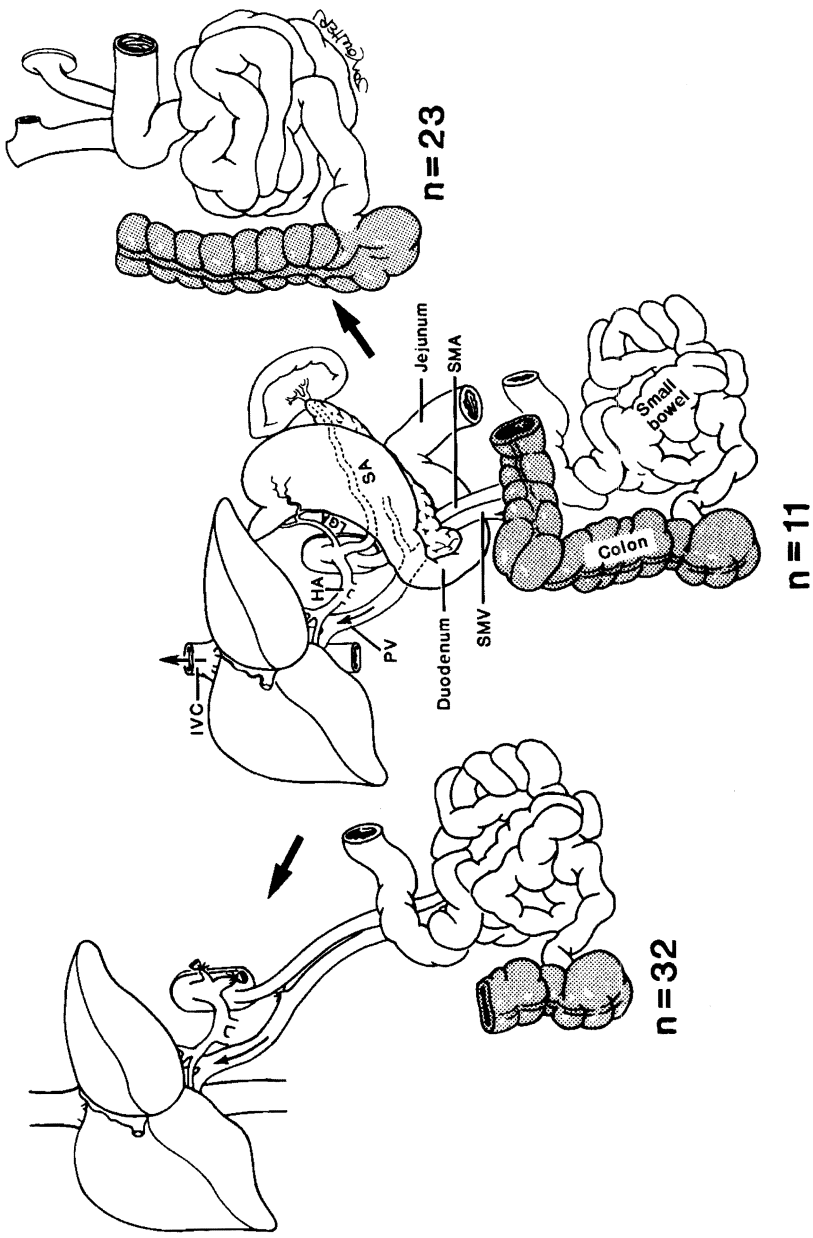

Figure 3. *See legend on opposite page*

Table 4. CAUSES OF INTESTINAL FAILURE

Children		Adults	
Volvulus	9	Thrombotic disorder	8
Gastroschisis	8	Crohn's disease	7
Necrotizing enterocolitis	6	Desmoid tumor	5
Intestinal atresia	6	Intestinal trauma	4
Pseudo-obstruction	3	Intestinal adhesions	2
Microvillus inclusion disease	3	Pseudo-obstruction	1
Intestinal polyposis	1	Malignant gastrinoma	1
Hirschsprung's disease	1	Volvulus	1
Total	37	Total	29

from 1.5 to 57 months. All but 4 of our most recent cohort of recipients have been followed for 1 year post-transplantation.

All donors were cadaveric of the identical ABO blood type as the recipients. Matching of human leukocyte antigen (HLA) was random and uniformly poor. The lymphocytotoxic cross match was positive in 7 patients. A history of normal intestinal function in a potential liver donation referral is adequate for possible intestinal donation. The procurement of multiple visceral organs focuses on the isolation and cooling of these organs, preserving their vascular and parenchymal anatomy. The organs are flushed with University of Wisconsin (UW) solution.[13] The cold ischemic time averaged 7.7 ± 2.4 hours.

Immunosuppression

Immunosuppression consisted of tacrolimus and low dose steroids, to which prostaglandin E1 was added briefly during the early post-operative stage. Adjustment of tacrolimus dosing and/or supplemental use of steroids, OKT3, and azathioprine were given for the treatment of rejection episodes. This was based on severity and/or tacrolimus related toxicity as previously described.[3] Other aspects of toxicity as well as infectious disease complications and complex nutritional management are as described elsewhere.[2, 36]

Figure 3. The three types of intestinal allografts: intestine alone (right), liver/intestine (left), and multivisceral (middle). IVC = portal vein; HA = hepatic artery; SMA = superior mesenteric artery; SMV = superior mesenteric vein; SA = splenic artery; LGA = left gastric artery. Colonic segments (shaded) were included in 29 recipients scattered through the three cohorts. (*From* Todo S, Reyes J, Furukawa H, et al: Outcome analysis of 71 clinical intestinal transplantations. Ann Surg 222:270–282, 1995; with permission.)

Results of Clinical Trials

During the potential follow-up of 1 to 5 years and as of April 1995, 32 patients (48.5%) are still alive. The actuarial survival rate for the 63 patients at 3, 6, 12, and 24 months was 84.1%, 80.9%, 72.8%, and 57.5%, respectively. The estimated actuarial survival for all types of grafts was 73.5%, 70.5%, 62.8%, and 48.1% at 3, 6, 12 and 24 months, respectively (Fig. 4). There was no survival difference between the 3 types of transplant procedures or between the pediatric and adult age groups (Fig. 5). Of the 32 surviving patients, 28 still bear their primary allografts which are functional. Only 1 patient requires intermittent night time intravenous hydration. The remaining 4 patients returned to TPN after graft removal and 1 recently underwent a successful retransplantation. Graft survival was similar in adults and children.

Death was the cause of primary graft loss in 35 recipients. Surgical removal of the graft followed by immediate attempted retransplantation occurred in 4 recipients, and there was surgical removal of the graft and return to hyperalimintation in another 4 recipients. The principal reasons for primary graft losses included surgical or clinical management failures which caused the death of the recipient. In 8 recipients the grafts were functioning at or shortly before the time of death. Technical surgical failures were more common in children and included intestinal anastomotic leaks, hepatic artery thrombosis, biliary anastomotic leaks, and cerebral infarction associated with intraoperative cardiac arrest. There was 1 recipient who was non-compliant and developed chronic rejection of the intestinal allograft.

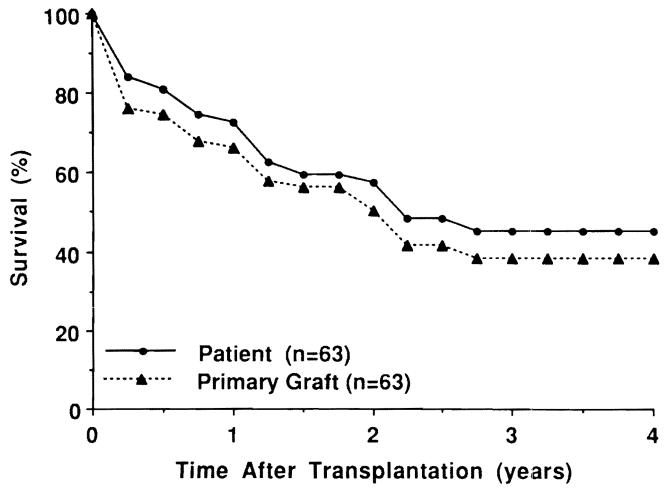

Figure 4. Actuarial patient and allograft survival of the first 63 recipients. (*From* Todo S, Reyes J, Furukawa H, et al: Outcome analysis of 71 clinical intestinal transplantations. Ann Surg 222:270–282, 1995; with permission.)

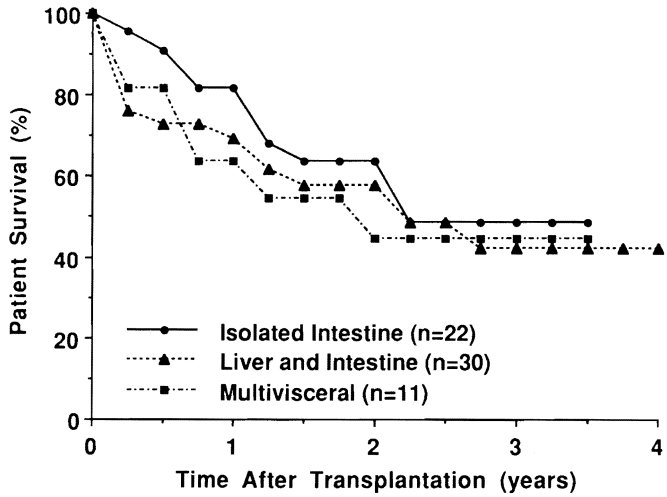

Figure 5. Allograft survival with the three different types of procedures. (*From* Todo S, Reyes J, Furukawa H, et al: Outcome analysis of 71 clinical intestinal transplantations. Ann Surg 222:270–282, 1995; with permission.)

Reasons for the loss of the other 25 allografts included an array of factors which were interlocking and complex. The principal diagnosis was rejection in only 6 cases, whereas infection as a complication attributable to the immunosuppressive therapy was the major diagnosis in 19 recipients. Bacteria and fungi were accountable for 6 such complications; however, cytomegalovirus (CMV) (n = 5) as well as Epstein-Barr virus–associated post-transplant lymphoproliferative disease (PTLD) (n = 12) were responsible for significant morbidity. Eight of the 12 patients who developed PTLD died due to this complication.

Retransplantation was performed in 4 patients on the same day as primary graft removal (2 liver/intestine) or 1 to 2 months post removal of the primary allograft (2 intestinal recipients only). All retransplantated patients died between 47 and 147 days after the second transplant, with the diagnosis of rejection (n = 2), PTLD (n = 1), and sepsis (n = 1). Also, an isolated liver retransplantation was performed in a pediatric recipient who developed thrombosis of the hepatic artery after a combined liver/intestinal graft. A fifth intestinal only retransplantation was carried out in a recipient who had received an intestine only 11 months after graft enterectomy. This patient tolerated retransplantation and was given bone marrow with the second intestinal allograft.

Analysis of these cases has yielded 6 statistically significant risk factors for graft loss and death: high tacrolimus blood trough levels, bolus steroid therapy, OKT3 use for treatment of allograft rejection, length of operation, CMV positive status of donor and recipient, and an inclusion of a segment of colon with the transplanted allograft. The survival of serologically CMV-negative recipients when they receive a

CMV-negative donor allograft was 62% versus 47% when the donor allograft was CMV positive. Also, an adverse effect was noted when the recipient was already CMV positive at transplantation, with a survival of 54% if the donor allograft was CMV negative, but only 14% when the donor allograft was CMV positive. The predominant site of CMV disease was the intestinal allograft, and therapy for this disease was marginally effective in these intestinal transplant recipients. Inclusion of a segment of donor colon in the allograft in 29 cases showed a significantly inferior survival than when the colon was not included.

Two of the augmented patients received an isolated intestinal allograft and 2 were given liver plus intestine. One recipient was an isolated intestinal retransplant recipient who had a previous history of PTLD that had been in remission since discontinuance of the immunosuppression and removal of his primary allograft 11 months previously.

Evidence of donor cells was detected in all 4 bone marrow augmented bowel recipients by either PCR and/or flow cytometry. In the female recipient who received a male allograft, presence of donor cells was confirmed by fluorescence in situ hybridization for the Y chromosome. No evidence of GVHD was found in any of the 4 recipients who were given bone marrow cells.

CHIMERISM AND THE INDUCTION OF GRAFT ACCEPTANCE

Until recently, insights into the acceptance of a transplanted organ by the recipient immune system were focused on manipulations of the recipient cell population with the use of immunosuppressive drugs. This experience allowed for improved survival after organ transplantation and the development of therapeutic dogmas to which we have adhered tenaciously. Interestingly, most patients progressively require less and less immunosuppressive therapy, and some have discontinued therapy altogether.[37]

Since 1969 it was noted that Kupffer cells and other tissue leukocytes became predominantly of the recipient phenotype within 100 days after transplantation while the hepatocytes retain their donor specificity permanently.[19] Twenty-two years later other insights emerged regarding allograft acceptance, which were based on the observation that donor leukocytes from transplanted organs had migrated and survived throughout the body of the recipient for as long as three decades.[39, 40, 43, 44] This process occurred in all successfully transplanted organs, the liver being the most tolerogenic transplanted organ because of its much larger total leukocyte load as well as its lineage profile of the migratory leukocytes. The events following transplantation were then seen as a two way cellular action—graft versus host (GVH) and host versus graft (HVG). Under the cover of immunosuppressive drugs (traffic directors) the graft as well as the recipient become genetic composites composed of cells of both parties (Fig. 6).

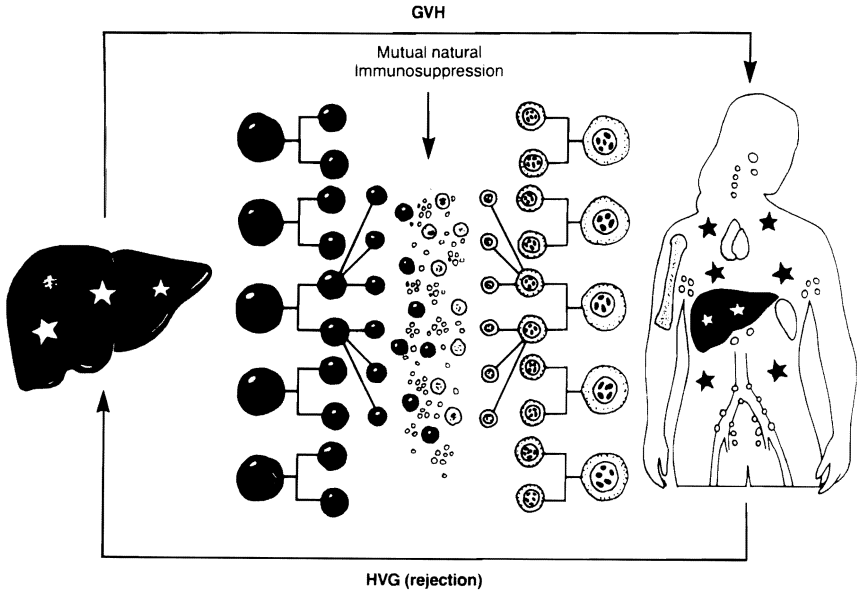

Figure 6. Under the cover of immunosuppressive drugs (traffic directors) there is a mutual engagement of migratory multilineage immunocytes between the graft and the recipient, which become genetic composites composed of cells of both parties. GVH = graft versus host; HVD = host versus graft. (*From* Starzl TE, Demetris AJ, Murase N, et al: Donor cell chimerism by immunosuppressive drugs: A new view of organ transplantation. Immunol Today 14:326, 1993; with permission.)

This bidirectional migration has been particularly dramatic in all successfully transplanted intestines, and was also observed at different proportions in the liver, kidney, and heart.[17, 25, 31, 33] Here, since neither the recipient nor the graft is leukocyte depleted, it is possible to routinely perform intestinal and multivisceral transplantation without an exorbitant risk of graft versus host disease (GVHD). This spontaneous "chimerism" after whole organ transplantation differs from bone marrow transplantation in that the treatment strategy involved empirically leaving both cell populations intact. This reciprocal interaction (mutual natural immunosuppression) may blindfold the major histocompatibility complex effect, thus removing tissue matching as a crucial requisite for success, and largely eliminates the threat of GVHD.[41]

Evidence supporting chimerism was found in retrospective studies of long-term survivors of kidney allografts (30 years post-transplantation), liver allografts (10–21 years post-transplantation) and recipients of thoracic organs.[42, 64] The identity of donor and recipient cells was established after special staining procedures (immunostaining or sex identification after fluorescence in situ hybridization [FISH]), and polymerase chain reaction [DNA fingerprinting]).

The observation of surviving donor multilineage passenger leuko-

cytes being associated with organ graft acceptance permits us to view the engraftment of any whole organ in the same context as a "mini" bone marrow transplantation. The acceptance of this concept inherently brings about the question of eventually stopping immunosuppressive medication altogether. Many non-compliant patients have stopped their medications sporadically and then completely when all liver functions remained normal. Other patients have had their immunosuppression withdrawn because of infectious complications.[37] Because complications of immunosuppression have been the principal cause of late death in our long-term surviving recipient population, a prospective, physician-directed weaning trial was begun. This involved the systematic decrease in the baseline immunosuppression in patients suffering complications of long-term immunosuppression.[32] Complete weaning was accomplished in 16 patients (27.1%) with 3–19 months drug-free follow-up, is progressing in 28 (47.4%), and failed in 15 (25.4%). Patients who failed the weaning trial were restored to their baseline immunosuppression regimen. There were no grafts lost or significant loss of graft function from the rejections. This and our previous experience with patients off immunosuppression indicates that cautious weaning can be performed safely and under careful surveillance 5 to 10 years after transplantation.[32]

The strategy of augmenting this natural migratory traffic would supplement the minimal dose of the passenger leukocytes (which are of bone marrow origin) and thus enhance the natural chimerism. Since March 1993 such a prospective trial has been under way at the University of Pittsburgh by infusing 3×10^8 unaltered donor bone marrow cells (obtained from the thoraco-lumbar vertebrae of the cadaveric donor) perioperatively into an unmodified recipient.[9, 34] This trial presently includes 64 patients that have been simultaneously transplanted with donor bone marrow and liver (n = 28), liver and islets (n = 1), kidney (n = 17), kidney and pancreas (n = 2), kidney and islets (n = 6), heart (n = 8), and lungs (n = 8). All patients were maintained on routine immunosuppression with tacrolimus and prednisone. Also, 53 recipients of whole organ allografts in whom consent to retrieve donor vertebral column was not available were monitored as contemporaneous controls.

No complication of bone marrow infusion was observed in any of the patients. Four of the 9 kidney/bone marrow recipients were able to come off steroids versus none of the kidney transplant alone recipients. The liver allograft recipients show similar graft function in both groups. All 3 recipients of pancreatic islet cells are currently being maintained on exogenous insulin. Similar episodes of rejection were seen in 58% of bone marrow-augmented and 64% of control patients. Asymptomatic GVH reaction in the skin was seen in only 2 patients (recipients of liver/bone marrow), which completely regressed with slight increase in baseline steroid therapy in 1 patient and no therapy in the other. Evidence of circulating donor cells by flow cytometry, PCR, or fluorescence in situ hybridization was present in 18 of 18 study patients and in 9 of 21 control patients. Using in vitro testing by MLR it was possible to

show evidence of evolving donor-specific hyporeactivity in 41% of bone marrow augmented patients and 18% of control patients.

Although all patients are still receiving immunosuppressive therapy, these data suggest that simultaneous infusion of donor bone marrow at the time of whole-organ transplantation may lead to augmentation of chimerism. The timing and eventual progress to a drug-free state remains speculative.

SUMMARY

The evolution of tacrolimus immunosuppression for recipients of liver allografts has substantiated our belief in the clinical superiority compared to cyclosporine. This was shown in our initial cohort of patients, as well as randomized liver transplant recipients. Results of the multi-center trials conducted in Europe and the United States are congruent with this conclusion. In these randomized trials, crossover from cyclosporine to tacrolimus because of intractable rejection (but not vice versa) was a common event that frequently prevented death or the need for retransplantation. We believe tacrolimus will supplant cyclosporine as the principal baseline immunosuppressive drug for transplantation of the liver and other organs. Not only was there improved patient and graft survivals, but also observed was an improvement in the associated quality of life due to a lower need for steroids, and fewer cosmetic side effects which is of particular importance in the pediatric population.

There is a significantly better understanding of not only the mechanisms of tacrolimus but also of a previous epiphenomenon such as "chimerism," which at the present time we believe is the central event responsible for graft acceptance. The observation of surviving donor multilineage passenger leukocytes being associated with organ graft acceptance implies a persistent engagement of donor and recipient immunocytes with consequent development of various degrees of donor specific non-reactivity. This is of particular importance in recipients of intestinal grafts, where recipient-specific non-reactivity of the chimeric donor cells must occur if the patient is to escape the complication of GVHD.

We are presently armed with a new potent immunosuppressive drug, tacrolimus, and an understanding that the migration and grafting of "passenger leukocytes" of bone marrow origin is the seminal explanation for allograft acceptance. The next forefront will involve manipulation of this process not only for the transplantation of already successful whole organs such as liver, kidney, pancreas and heart, but also in the development of the intestinal transplantation program. Thus, augmentation of the leukocyte traffic of unconditioned recipients of cadaver whole organ allografts by the concomitant intravenous infusion of donor bone marrow cells and under the same conditions of immunosuppressive management of tacrolimus/prednisone treatment will be our path into the future.

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