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The Current Status of Hepatic Transplantation at the University of Pittsburgh

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During the last 7 years since February 1989, we have initiated 5 major developments in clinical transplantation. All have prominently (or in one instance exclusively) involved the liver: (a) Clinical trials with the new immunosuppressive drug FK506 (tacrolimus); (b) Weaning of immunosuppressive drugs long after transplantation; (c) Enhancement of whole organ allograft acceptance with adjuvant bone marrow infusion; (d) Intestinal transplantation, alone or as part of hepatic-intestinal and multivisceral allografts; and (e) Baboon-to-human liver xenotransplantation. The early results of these and other clinical studies were described in the 1993 and 1994 editions of this book. All of these clinical trials except xenotransplantation are still active. Here, we will update the 4 active trials and attempt to assess their future prospects.

The patient and graft survival curves for all studies were estimated by the Kaplan-Meier method and the comparisons were done by the log-rank test. Survival time for patients was defined as the time that elapsed from the transplantation date until death, or the date of the last follow-up evaluation. In calculating organ allograft survival, the date of death or the date of graft removal and retransplantation were considered the end points.

Tacrolimus “FK506” and Randomized Trialomania

Fifteen years ago, the introduction of cyclosporine(CyA)/prednisone immunosuppression improved the prognosis of all organ recipients and elevated liver transplantation overnight to practical and widespread use (1). CyA-based regimens were unchallenged until a report appeared in late 1989 (2) describing a pilot clinical experience with tacrolimus (FK506, Prograf®). The drug was first used clinically in replacement of CyA for liver recipients who were undergoing intractable graft rejection. The remarkable 80% rescue rate in the first 10 cases (2) always remained approximately the same as rescue trials expanded to hundreds of cases (3,4) and eventually to thousands, involving all kinds of allografts. Beginning in August 1989, tacrolimus was given as the primary immunosuppressive drug from the time of hepatic replacement (2,5).

Until these trials, kidney transplantation had been the whole organ procedure with which all new immunosuppressive agents and regimens had been developed (6–11). This precedent was broken with tacrolimus because its rescue role was so dramatic and so demonstrative from the outset of its superior therapeutic index. In November 1989, tacrolimus was placed onto the “fast track” of the United States Food and Drug Administration. Multicenter American (12) and European trials (13) were planned, comparing the use of tacrolimus and CyA as baseline agents from the time of transplantation. These began in August and September 1990, respectively. By that time, more than 80 patients already had been enrolled in a prospective “single center”, randomized trial which had begun in Pittsburgh in February 1990 (14). The

evidence of the superiority of tacrolimus for kidney (15) and thoracic organ (5,16) as well as liver (5) transplantation was overwhelming by the time of the Transplantation Society meeting in August 1990. Consequently, all further randomized trials were conducted in the absence of equipoise (17,18).

THE PITTSBURGH RANDOMIZED TRIAL

Study Design

All patients 16–60 years of age referred to the University of Pittsburgh for primary liver transplantation were considered to be potential candidates for randomization to CyA or tacrolimus (14). Exclusion criteria were: hepatitis B virus carrier state, cancer, need for multiorgan transplantation, renal insufficiency (creatinine >2 mg/dl), active infection, stage 4 coma, significant cardiopulmonary disease and previous hepatic hilar reconstructive or portal venous decompressive surgery. Four hours after revascularization of the liver allograft, it was determined if randomization should proceed or be aborted either because of a technically unsatisfactory operation or substandard graft function. Treatment assignment with sealed-envelope implementation was determined with a computer-programmed block randomization technique to assure that the treatment groups remained reasonably balanced. No one was aware of the randomization status during the donor search, and up to the actual randomization. The patient characteristics and indications for liver transplantation for each randomized arm are summarized in Table 1.

Immunosuppressive Therapy

Tacrolimus—The drug was given by constant, 24-hour infusion at a starting dose of 0.1 mg/kg/day. Intravenous treatment continued until oral administration could be started. The planned oral dose was 0.15 mg/kg every 12 hours. The daily adjustments of the intravenous and, later, the oral doses of tacrolimus were guided by evidence of drug toxicity on one hand, graft rejection on the other, and the modifying influence of drug trough plasma levels which were targeted to 1–2 ng/ml (equivalent to whole blood levels of 10–20 ng/ml). Patients were not discharged from the hospital until oral doses were stable, at which time, the 12-hour plasma trough levels usually were around one ng/ml. With further longitudinal follow-ups, these plasma concentrations and the oral doses were allowed to drift down if rejection did not supervene, always taking into consideration drug toxicity and over immunosuppression versus control of rejection (18).

Cyclosporine—A comparable sliding scale strategy was used for CyA, beginning with a continuous infusion of 4 mg/kg/day and optional oral starting doses of 8 mg/kg every 12 hours which were overlapped with I.V. therapy. During the first few weeks, 12-hour whole blood trough concentrations were targeted to the 800–1500 ng/ml range. With further follow-ups, these concentrations were permitted to fall to 600–800 ng/ml if there was no evidence of rejection. The similarity of side effects of CyA and tacrolimus, as well as the fact that dose adjustments were driven by the same 3 inter-related factors of rejection, toxicity and trough levels greatly facilitated management and outcome comparisons.

Prednisone—In the first 81 recipients (CyA n=40; tacrolimus n=41), I.V. methylprednisolone or oral prednisone was started at a daily dose of 20 mg/day (Fig. 1). Because of an overwhelming rate of treatment failure on the CyA arm with the low steroid doses, the protocol was unbalanced after case 81 by adding a 5-day I.V. burst of prophylactic postoperative prednisone to the CyA (n=35) but not the tacrolimus arm (n=38). The bulge in prednisone dosage began with 200 mg on the first postoperative day followed by 40 mg daily decrements until a maintenance level of 20 mg/day was reached on the sixth day. In addition, one gram of intravenous methylprednisolone was given intraoperatively to both cohorts.

Reduction in the maintenance steroid doses was attempted in both arms in the absence of clinical or histopathologic evidence of rejection. Several of the recipients, particularly those on the tacrolimus arm, were taken off prednisone at the end of the first postoperative month.

Conditions for Crossover

The diagnosis of liver rejection was suspected on the basis of clinical and biochemical criteria, but required biopsy and histopathologic confirmation (18). In addition to these specifically indicated biopsies, protocol liver biopsies were obtained before hospital discharge and in most cases at 2 months. Before going forward with biopsy, technical complications were always excluded by either a duplex scan, a cholangiogram or a hepatic angiogram, as indicated.

Treatment failure was defined as the inability of the starting immunosuppression plus an orderly sequence of secondary therapy to control biopsy confirmed rejection (Fig. 2). The treatment of first-time rejection consisted of a one gram bolus of prednisone which could be repeated once, if necessary, and followed in refractory cases by the 5-day burst of prednisone (200 mg/day with 40 mg decrements) and/or a 5–7 day course of OKT3. If these steps failed to control rejection, crossover from CyA to tacrolimus or vice versa was permitted. The reasons for crossover from CyA to tacrolimus are summarized in Table 2. Drug crossover was primarily dictated by treatment failure.

Statistical Analysis

A Cox proportional hazards regression modelling approach (19) was used to analyze the time-to-event data with treatment effects tested for statistical significance using a likelihood ratio test. This approach allows for varying lengths of patient follow-up and is able to simultaneously control for the effect of patient baseline confounders on outcome. The endpoints for statistical analysis were freedom from biopsy-confirmed allograft rejection, retransplantation and death. Crossover was not considered to be an endpoint. The high early rate of unidirectional crossover from CyA to tacrolimus made it impossible to specifically compare the spectrum of drug toxicity of each treatment arm as an important primary endpoint.

RESULTS

Patient Characteristics

The 154 patients were recruited from February 16, 1990 to December 26, 1991, with 79 patients randomized to the tacrolimus arm and 75 to CyA. The patient groups that emerged from the intraoperative randomization were similar with respect to baseline characteristics (Table 1). No patients were lost to follow-up over the course of the study which terminated on May 30, 1995. The mean duration of follow-up was 4 years.

Drug Crossover

A significant crossover from CyA to tacrolimus was triggered during the early postoperative months by the prospectively-defined treatment failure. This trend was observed even after induction prednisone was increased disproportionately on the CyA limb during the second phase of the trial. After randomization, 47 of the 75 (63%) recipients assigned to CyA had been switched to tacrolimus. Seventy percent of these crossovers occurred within the first month of randomization, with another 28% crossing over during the rest of the first year of follow-up. Seven patients crossed over prior to rejection, whereas the other 40 switched to tacrolimus following rejection (Table 2). The remaining 28 CyA patients remained in their assigned treatment arm for the duration of the trial, irrespective of intervening episodes of rejection or retransplantation. In contrast only one recipient who started on tacrolimus from the outset was

changed to CyA. This occurred following retransplantation, preceded by an episode of rejection.

Freedom from Rejection

Cox regression analysis was performed using an indicator variable to represent treatment status and time to rejection as the outcome variable. The freedom from rejection curves (Fig. 3A) showed that patients randomized to the tacrolimus arm were less likely to have acute rejection than those receiving CyA (p=0.003, likelihood ratio test). The freedom from rejection at one year was 36.2% for the tacrolimus patients and 16.8% for patients on CyA. The 95% confidence limits for the 19.4% difference in these rates were 6.9% and 31.9%. A subsequent analysis, which censored the 7 CyA patients (15%) who crossed over prior to an acute rejection (Table 2), yielded similar results. An analysis including age, sex and blood type, indications for transplant and starting dose of prednisone as covariates also produced similar results.

Exploratory Analyses—The unique study design and pattern of crossovers influenced the framework for a set of exploratory analyses. For these analyses, 3 groups were compared: the 79 patients randomized to tacrolimus, the 47 patients who crossed over from CyA to tacrolimus, and the 28 patients who remained in the CyA arm. The results of the comparisons discussed below must be interpreted cautiously since they were not protected by the randomization process.

The baseline characteristics for these 3 groups are shown in Table 1. Figure 3B shows that the freedom from rejection curves for patients who crossed over from CyA to tacrolimus were identical to those for patients who did not cross over. This is reassuring, as the vast majority of crossovers from CyA to tacrolimus occurred after rejection. There were no statistically significant differences among the groups with regard to freedom from graft failure (Fig. 4, p=0.42), defined as a composite outcome of retransplantation or death. When these outcomes were considered separately, there was a striking difference in terms of freedom from retransplantation for patients receiving tacrolimus in comparison to those who continued on CyA with a p value of 0.02 (Fig. 5). At 3 years, freedom from retransplantation for the tacrolimus patients was 90.3% compared to 70.7% for patients continuing on CyA. Finally, patient survival over the course of the trial was virtually identical for all 3 groups of patients (Fig. 6, p=0.95). At 3 years, patient survival was approximately 84% in each group. Reasons for retransplantation and causes of death are listed in Tables 3 and 4, respectively.

Adverse Events—The adverse events included: central nervous system, genitourinary, cardiovascular system, respiratory and gastrointestinal complications whether or not these were suspected of being adverse drug effects. A spectrum of complications that were not obviously related to drug therapy were also included: technical surgical accidents, worsening of pre-existing cardiovascular disease, degenerative disorders, and traumatic accidents. Two de novo malignancies developed, one in each treatment arm. A patient who died of a squamous cell carcinoma of the larynx after 4.4 years always was on CyA. A patient who always was on tacrolimus died after 2.3 years from a disseminated squamous cell carcinoma of the oropharynx.

It had been learned before the trial began that tacrolimus relative to CyA was associated with similar nephrotoxicity (20,21), diabetogenicity (22) and neurotoxicity (15); an equivalent or lower rate of infection (5,15,23,24); a reduced cumulative prednisone need (5,15,25); a lower incidence of hypertension (5,15,20) and less hyperlipidemia (5,15); and an absence of cosmetic changes (hirsutism, facial brutalization and gingival hyperplasia). The high early rate of unidirectional crossover from CyA to tacrolimus made it impossible to compare these parameters on the 2 limbs because, ultimately, the comparison became one of tacrolimus versus

tacrolimus. In addition, all of the main toxicities were strictly dose related and could therefore be ratcheted up or down at will.

THE MULTICENTER LIVER TRANSPLANT TRIALS: TACROLIMUS VERSUS CYCLOSPORINE

Unlearned Lessons

The comparison of tacrolimus with CyA in 2 multicenter, randomized trials was a prerequisite of government regulatory agencies before tacrolimus could be marketed in pharmacies, and then only for the specific indication of liver transplantation. By the time investigators met in March 1990, in London and Bethesda, respectively, to plan multicenter liver trials, the toxicity profile and clinical pharmacokinetics of tacrolimus had already been delineated and published from Pittsburgh, including valuable management lessons (20–22,24–27).

It had been recognized at an early time that the first Pittsburgh recipients treated with tacrolimus had been overdosed, and one-third reduction of the starting doses (to 0.1 mg/kg/day) already was adopted by February 1990 (21,26,28). This reduction did not prevent toxic drug plasma levels in a significant number of patients, and consequently, the I.V. induction dose was lowered further in August 1991 to 0.05 mg/kg/day. Even more importantly, it had been learned that the elimination of tacrolimus is dependent to a greater extent on the status of liver functions (21,26), making it clear why flexible and individualized dosing was so critical. Irreversible complications had been avoided by using the characteristic side effects from the first day of treatment to determine dose ceilings; the occurrence of graft rejection established the floor (5,15,29). From these observations, the meaning of drug plasma levels was quickly deduced, allowing drug level monitoring to be exploited in subsequent cases.

These management lessons were the same as those which previously had made the use of CyA practical (30), but they were not well understood by many of the participants in the multicenter trials. Three features of these trials presaged problems (17). First, although some of the investigators had used the drug for rescue therapy, no pilot experience was allowed using tacrolimus as the primary immunosuppressive agent from the time of transplantation. Second, on-site plasma trough level monitoring was not available. Although the whole blood trough drug levels (about 10 times that of plasma) were measured, the results were not available for several days after sampling. Consequently, dose adjustment decisions were delayed. Finally, the high starting doses, long since abandoned in Pittsburgh, were used. A predictable epidemic of toxicity reports developed within a few weeks after the beginning of the trial in August (12) and September (13) 1990. Formal dose revisions were not made until 30% and 18%, respectively, of the European and American tacrolimus case enrollment had occurred. The gap between the multicenter study starting doses and those in concurrent use in Pittsburgh never closed, even by trial's end (Fig. 7).

Study Design

The protocols used in the multicenter trials (Fig. 8) resulted in unbalanced therapeutic schedules. The CyA arm was uploaded with twice the induction doses of prednisone in all 12 American centers, a third drug (azathioprine) in 11 centers, and a fourth agent (polyclonal ALG) in one. The 8 European centers also had similar unbalanced and diverse protocols. On both sides of the Atlantic, CyA dose selection and adjustment were at the physician's discretion, whereas the high starting doses of tacrolimus were obligatory. The experimental design resulted in frequent drug switching, but for different reasons on the 2 arms. Crossover away from tacrolimus occurred because of its toxicity at the dangerously high doses being used and crossover toward it occurred because of the comparative lack of CyA efficacy that was still evident despite combining the CyA with high doses of induction prednisone.

European Trial Results

The distorting roles of tacrolimus overdose and a high rate of toxicity were clarified by separate analyses of the early (high dose) and late (reduced dose) phases of the European multicenter randomized trial. The statistical analysis, based on the intent-to-treat approach, showed significantly greater freedom from acute, intractable and chronic rejections. There were a 5% better patient survival and a 5% higher graft survival in the tacrolimus arm (13). The survival advantage was not statistically significant, but the authors noted that about 10% of the surviving grafts credited to CyA were borne by patients who had been rescued with and remained permanently on tacrolimus.

American Trial Results

Critique of Report—The published analysis (12) left the impression, contrary to the European results (13) and our own experience (14,15), that the gain of better tacrolimus efficacy was essentially balanced by its higher toxicity. Critical examination of the original database (17) showed that analytic errors had led to this conclusion. Although the published American analysis (12) was claimed to be according to intent to treat, the data of 155 recipients who lived throughout the year, but were withdrawn from the trial analysis at various times and for different reasons had their data censored for all stipulated end points except patient and graft survival: rejection, intractable rejection, need for retransplantation, steroid need and OKT3 use (Table 5). Thus, the only analyses done by intent-to-treat were patient and graft survival.

In addition to the systematic violations of the intent-to-treat pnnciple, the inappropriate use of the Kaplan-Meier method further eroded the validity of secondary end-point analysis in the American report (12). Instead of using the actual data generated by the patients who survived through the study period, end points subject to censoring were projected thereafter by Kaplan-Meier calculations. An assumption underlying this calculation is that censoring is random with respect to treatment assignment (31). In the published analysis (12), both the number of recipients censored (102 CyA and 83 tacrolimus) and most of the reasons for censoring were distributed among both treatment arms in a non-random fashion (Table 5). While censoring due to adverse events was common in the tacrolimus arm, lack of efficacy and “administrative reasons” were more frequent causes of censoring in the CyA arm.

According to the published data, the death (n=64) and retransplantation (n=52) rates were similar in both treatment arms and nearly equally contributed to the primary end-point results of patient and graft survival (12). However, a portion of patients in both categories were censored in the analysis of secondary end points, as previously mentioned. The listing of only 30 in the “censored by death” category meant that data following withdrawal but preceding death had been omitted from secondary end-point analysis in the other 34 fatal cases. Also, the association of drug treatment with reduction of the retransplantation rate was obscured by the fact that 38 of the 52 second engraftments were ascribed to “technical failures” and censored. In fact, grafts rescued by tacrolimus accounted for 20 (9.5%) of the 210 surviving grafts credited to the CyA arms at the end of the year.

Reanalysis of the American Trial—The conventional intent-to-treat reanalysis was done with the original database (17). With respect to all important prognostic factors, randomization produced groups that were comparable at the outset. Using the “freedom from” formulation of the published study, the numerical results and their statistical significance were different than those published for all end points except for the one-year patient (88%) and graft (80.5%) survival. Consequently, stepwise restoration of all the censored subsets (shown in Table 5 from the published American account) (12) was required.

With reanalysis, freedom from rejection as a single end point was accomplished in 39% versus 32% of the patients randomized to tacrolimus and CyA, respectively (p=0.025) (Fig. 9. left). When data was progressively reinserted from the different categories of censored patients, the "freedom from" curves of both arms progressively descended (Fig. 9). Importantly, the extent of tacrolimus therapeutic advantage was maintained throughout with significant log-rank p values. It was noteworthy in the reanalysis that restoration of freedom from adverse events had no effect on the tacrolimus superiority present before this restoration. This unequivocally corrected the impression left by the published report that the greater tacrolimus efficacy was balanced by increased toxicity.

The most clinically relevant results of the reanalysis are shown in Figure 10. By the end of the first postoperative year, 98% of the tacrolimus-randomized patients were still free of refractory rejection versus only 87% in the CyA arm. Also, the composite freedom from the 3 factors that haunt transplant recipients (refractory rejection, retransplantation and death) was 80% for tacrolimus and 70% for CyA with p value of 0.008.

DRUG-FREE GRAFT ACCEPTANCE

The 2-Way Paradigm

The modern era of transplantation usually is dated from the demonstration of Billingham, Brent and Medawar (32,33) of acquired tolerance in mice after their engraftment with adult allogeneic hematolymphopoietic cells during uterine or neonatal life. The connection between the consequent chimerism in these animals and the successful engraftment of whole organs was not apparent until the demonstration of persistent donor leukocytes (microchimerism) up to 30 years postoperatively in the peripheral tissues or blood of human kidney, liver and thoracic organ recipients (34–39) (Fig. 11, A). When small numbers of donor cells were found using sensitive immunocytochemical and polymerase chain reaction (PCR) techniques, we postulated that they constituted one limb of mutually antagonistic but ultimately reciprocally attenuated or abrogated host-versus-graft (HVG) rejection and graft-versus-host (GVH) reactions (Fig. 12). The disparities in outcome with bone marrow and organ transplantation (Table 6) could be explained by disruption of the leukocyte interaction by the host cytoablation in the first instance, but not the second.

The canceling effect of the coexisting immunocyte populations in postoperatively immunosuppressed organ recipients explained the poor prognostic discrimination of HLA matching in such cases, and the rarity of GVHD after transplantation of immunologically active organs, such as the intestine and liver (or both together). This also was postulated to be the reason for the characteristic cycle of immunologic crisis and resolution (first observed in kidney recipients) (40) that are most commonly reflected by changes in graft function (Fig. 12). The suspicion that bone marrow and organ transplantation were mirror images, resulting from the drastically different treatment strategies (Fig. 11), was strengthened by contemporary reports describing a trace residual population of recipient leukocytes in essentially all human bone marrow recipients who previously were thought to have complete donor cell chimerism (41, 42).

Discontinuance of Drugs: Historical Experience

The concept that successful organ transplantation involves, in essence, the engraftment of a fragment of donor extramedullary bone marrow explains why immunosuppressive drugs can sometimes be stopped after human organ transplantation, especially when the organ is the liver (36,43). By October 1995, 12 (28%) of our 42 longest surviving liver recipients (13.5–26 years) were drug free. The nearly equal cumulative duration of these 12 patients off immunosuppression and under treatment is evident in Figure 13.

The foregoing collection does not include cases in which drugs were stopped because of life-threatening infections. In May 1993, Reyes, et al (44), reported to the American Society of Transplant Surgeons that the 10 liver recipients shown in Table 7 had their immunosuppression stopped as early as 6 months posttransplantation because of EBV-associated B-cell lymphomas, HIV or hepatitis C virus. After an average drug-free time of more than 4 years, 8 (80%) of these 10 patients remain well, including 5 of the 6 with posttransplant lymphoproliferative disease (PTLD).

The Prospective Weaning Trial

A prospective weaning trial begun in 1992 for liver recipients who were 5–10 years posttransplantation has complemented these observations (45). Candidates were limited to those who had been rejection free for at least 5 years. A pre-weaning work-up including baseline biopsy was necessary to rule out hepatitis or ongoing rejection as well as occult complications of the allograft vascular and biliary tract systems.

Most of the patients entered (97%) had experienced one or more of the long-term adverse effects of one or more of their immunosuppressive drugs, including impending renal failure (24/80), skin cancer, verruca vulgaris of skin, significant osteoporosis and arthropathy (n=12), morbid obesity (n=3), refractory systemic hypertension (25/80) and recurrent opportunistic infections. Weaning gradually was done in 2 controlled steps; first, gradual reduction of the daily steroid doses with total withdrawal guided by the results of the corticotropin stimulation test and second, gradual stepwise withdrawal of azathioprine, CyA or tacrolimus. Further reductions in immunosuppression were made on a monthly basis and biochemical monitoring of liver cell injury was done every 1–2 weeks and when it was clinically indicated. Specific details of the weaning protocol and management policy have been published elsewhere (45).

Rate of Success—In the first 80 cases, 44 (55%) of the starting patients have come off drugs completely or have moved uninterruptedly in that direction. Twenty-two (28%) have been completely off drugs for an average of 2.5 years (Fig. 14, right upper quadrant). Weaning of these recipients was accomplished over 18 months, at a rate which we now believe was too fast. Another 22 patients are still weaning over a period of 2 years (Fig. 14, right lower quadrant). The slower weaning approach was prompted by a 30% incidence of rejection (Fig. 14, left lower quadrant), involving 24 patients to be discussed further below. The final 15% (n=12) had weaning frozen at a lower than starting level for a variety of reasons. In 4 of these cases, medical surveillance could not be maintained closely enough to proceed safely. In 3 more, panic by the referring physician foreclosed the weaning. Other patients had weaning frozen when they were discovered to have biliary tract complications, pregnancy, recurrent PBC, or recidivism of alcoholism after weaning had started. The overall impression was that the vast majority of these patients had been arbitrarily kept at a level of immunosuppression that they no longer needed. In the total collection of 80 cases, including those who flunked weaning, the ultimate daily doses of CyA and prednisone were essentially reduced by half, and the azathioprine doses even more so (Fig. 15).

The Threat of Rejection—The benefits of weaning are too obvious to dwell upon. However, it is important to emphasize that there was a 30% risk of histopathologically verified rejection (n=24). The rejections occurred from 1–29 months after starting the weaning. Of interest, 4 rejections were in patients known from prior study to have microchimerism. The rejections were classed histopathologically as minimal to mild in 20 cases necessitating only restoration of the previous baseline treatment. However, the rejection was moderate or severe in 4 cases and one of these patients became jaundiced with a peak serum bilirubin of 12 mg/dl. All 4 were rescued by switching to tacrolimus.

The clinical course of the liver recipient with the worst rejection, and the only example of jaundice is shown in Figure 16. Another patient whose kidneys were failing from CyA toxicity had this drug abruptly stopped. Azathioprine and prednisone also were stopped much too quickly. However, the patient did well without drugs for almost 2.5 years before developing acute rejection. After rescue with low-dose tacrolimus, liver function returned to normal over several weeks. He was started on renal dialysis and is waiting for a cadaver kidney.

The circumstances in this case were similar to those in a very unsatisfactory weaning trial involving 12 patients at the Mayo Clinic (46). Like our patients, the Mayo recipients were on triple-drug therapy from which they were weaned rapidly because of CyA-associated renal failure. The Mayo patients also were earlier in their course, only 3.1 years posttransplantation, and prior stability of hepatic graft function was required for only one year. Six of the 12 patients developed rejection, and 2 of the 6 died.

Our earlier (45) and expanded weaning experience as well as that from the Mayo Clinic (46) has suggested that patients coming off complex CyA-based regimens have a high incidence of rejection. When our patients were on any of the CyA-based cocktails shown in Figure 17, they either could not be weaned or had a very low success rate. However, when monotherapy with CyA preceded weaning, the success rate rose to over 30%. Successful weaning was achieved regularly only in the patients being weaned from an azathioprine-prednisone regimen or from tacrolimus.

DONOR LEUKOCYTE AUGMENTATION

Donor leukocyte infusion to facilitate organ allograft acceptance was initially an eagerly anticipated natural extension of the neonatal tolerance models of Billingham, Brent and Medawar (32,33). The momentum carrying organ and bone marrow transplantation on a common current was lost between 1959 and 1963 when the preparatory recipient cytoablation plus donor bone marrow caused lethal GVHD in the MHC-disparate, outbred, large animals used to test the strategy (47,48, reviewed in 49). In addition, donor leukocytes were shown not to be necessary for successful human kidney transplantation using either total body irradiation (50–52) or immunosuppressive drugs (40,53). Because it was not suspected that the leukocytes contained in the organs were capable of engraftment, chimerism was seemingly irrelevant to an explanation of organ allograft acceptance. A secondary dogma evolved that cytoablation to “make microenvironmental space” was a necessary condition for leukocyte engraftment (reviewed in 54) in spite of early (55,56) and recent evidence (57) to the contrary.

A Historical Perspective

Despite these incorrect assumptions, the strategy of adjuvant donor bone marrow for organ transplantation was never completely abandoned, largely because of its expenmentally grounded advocacy by Monaco, et al (58–61). In an extensive clinical trial of the Monaco protocol in which cryopreserved donor bone marrow cells were given 3 weeks after cadaveric kidney transplantation under conventional conditions of continuous immunosuppression, Barber, et al (62), detected PCR evidence of donor DNA in the blood of many of these patients. However, the same finding in some of the non-marrow controls aroused suspicion that this was an artifact until the discovery in 1992 of spontaneous chimerism (34–38). Late follow-up studies of Barber’s patients confirmed the presence of chimerism in both control and study patients, but far more frequently in the augmented cohort in which the long-term results were better (63). In addition, sporadic reports (summarized in 64) of the benefit of donor-specific blood transfusion, first reported by Salvatierra, et al (65), prompted van Twuyver, et al (66), to speculate that transfusions (either donor-specific or from haplotype-matched, third party donors) could be causing stem cell engraftment and persistent microchimerism.

The Pittsburgh Bone Marrow Trial

When it was realized that these leukocyte augmentation procedures were, in effect, iatrogenic amplifications of a natural posttransplant event (see previous section), we began a prospective clinical trial in December 1992 to enhance chimerism by perioperative infusion of 3–6 × 10^8/kg unmodified donor bone marrow (BM) cells in cadaveric liver, kidney, and thoracic organ recipients (67). The trial was subsequently expanded to include whole pancreas and intestine. Conventional tacrolimus/prednisone immunosuppression was given, without any kind of recipient preconditioning.

Global Results—Table 8 summarizes the current status (to February 1, 1996) of the first 150 patients treated between December 1992 and November 1995, compared with 95 contemporaneous control cases in which permission for BM harvest could not be obtained. BM infusions did not cause complications. Trivial skin manifestations of GVHD similar to those which occur at about a 5% incidence after conventional liver transplantations (36) were seen in 2 of the augmented liver recipients. These resolved spontaneously in one case and after a temporary increase in prednisone dose in the other. It was noteworthy that none of the BM-augmented intestinal or multivisceral recipients developed any evidence of GVHD.

The high patient (92.7%) and graft survival (88.6%) was similar to that in the control cases (90.5% and 89.5%). All of the surviving recipients still receive tacrolimus, but 40% of the study and 36% of control patients have had prednisone stopped. The nearly identical cumulative incidence of rejection in the 2 groups is shown in Figure 18. Of interest, serial immunological monitoring revealed a 48% incidence of induction of stable donor-specific hyporeactivity in evaiuable study patients as compared to 32% of controls. In a study excluding patients with follow-up less than 6 months, donor-specific hyporeactivity was almost 2-fold higher in donor BM-augmented liver, heart and lung transplant recipients than that in the control group. Of interest, this trend has not yet been seen in kidney recipients.

While these parameters (including clinical course) have been similar in the 2 groups, peripheral blood mononuclear cell (PBMC) chimerism has been consistently detectable by flow cytometric analysis (Fig. 19) and/or PCR in 117 of 121 (97%) of the evaluable BM-augmented patients compared to 36 of 73 (49.5%) of the controls. The level of chimerism has been estimated to be greater than 1000 fold that of control patients (67,68). Its multilineage character was confirmed in selected patients of the BM group by detection of donor DNA in the sorted T ($CD3^+$), B ($CD14^+$) and NK ($CD56^+$) cells in both myeloid and erythroid colonies. The presence of donor dendritic cell progenitors was demonstrated in GM-CSF and IL-4-enriched cultures of the PBMC.

The foregoing results have conformed precisely to expectations engendered by the 2-way paradigm. The eventual effect of the greatly enhanced chimerism in the study group remains speculative. However, the cumulative evidence from a variety of rodent models (69–71) suggests that these recipients will be selectively spared chronic rejection and can aspire to a drug-free state after a period of 5–10 years.

The Liver Transplantation Results—Forty-four of the 150 BM-augmented patients were primary liver recipients. This subgroup is considered separately in Table 9. Although the differences in results versus the non-augmented controls are not yet significant, the exceptionally high patient and graft survival and the 100% incidence of blood chimerism augers well for the future of these recipients. The 3 deaths in the 44 recipients were unrelated to marrow infusion. Two were caused by fulminant sepsis on postoperative day 22 in one case and on day 35 in the other. The third death was caused by metastatic recurrence of a malignant melanoma 18 months after transplantation.

Three of the 44 augmented recipients were infants or children. The survivors of both study and control groups have excellent graft function (Table 9). About 50% of liver recipients in each cohort are currently off steroids. With a minimum follow-up of one year, the cumulative risk of rejection, the histopathologic grade of rejection, and the response to treatment were similar in both groups (72). Immunological monitoring performed by MLR and supplemented by responses to mitogens and recall antigens revealed a 2-fold higher incidence of induction of donor-specific hypo-reactivity in study recipients as compared to controls.

This experience indicates that adjuvant infusion of bone marrow for liver and other organ recipients is safe and that it augments the level of natural chimerism. Although a trend towards more frequent development of stable donor-specific hypo-reactivity has been noted, it remains to be seen whether there will be better long-term allograft survival and a reduced (or absent) need for immunosuppression.

LIVER-INTESTINAL AND ABDOMINAL MULTIVISCERAL TRANSPLANTATION

Case Material

During the period from July 24, 1990 until September 8, 1995, a consecutive series of 46 patients were given liver allografts in continuity with the intestine (n=35) or as part of a multivisceral composite graft (n=11). Tacrolimus immunosuppression was used in all cases (73–77). Thirty of these recipients were infants or children with a mean age of 3.5 years (range, 0.5–15.5). The 16 adults had a mean age of 32 years (range, 19–55). The indications for the 2 different transplant operations are shown in Table 10. In most cases, the liver failure was secondary to the cholestatic effect of long-term total parenteral nutrition (TPN). However, patients with liver-based inborn errors of coagulation (see footnote a. Table 10) or metastatic hepatic disease tended to have better hepatic function.

Three of the patients had previously undergone transplantation procedures. One of the multivisceral recipients came to this operation after an isolated intestinal allograft had been rejected (footnote b, Table 10); another had a prior liver/intestinal operation (footnote c, Table 10); and a third had undergone liver transplantation 50 months previously (footnote d, Table 10). The second of these 3 exceptional cases was left on the liver/intestine list because this was the category that accounted for almost all of the survival.

Liver-Intestinal Graft Recipients

All 35 of these recipients (9 adults and 26 children) had irreversible intestinal and hepatic failure. The lymphocytotoxic crossmatch was positive in 3 recipients. One is still alive at 19 months. The other 2 died of chronic rejection and PTLD at 24 and 30 months after transplantation, respectively. The colon was included in 9 of the 35 liver/intestinal grafts shown in Table 10, and in one pediatric patient with Hirschsprung's disease, it was used for rectal reconstruction by a pull-through technique. Fourteen of the 35 allografts were obtained from cytomegalovirus (CMV)-seropositive donors.

Multivisceral Recipients

There were 7 adults and 4 children (Table 10), excluding the one accounted for in footnote c. Although combined liver and intestine grafting was initially considered for most of these 11 patients, multivisceral transplantation was chosen at the time of surgery because of a significantly ischemic or diseased native stomach and/or pancreas. In one patient with Budd-Chiari syndrome and mesenteric venous thrombosis, fatal hemorrhage caused by portal hypertension was prevented during the multivisceral operation by temporarily occluding the celiac axis and the superior mesenteric artery with intra-aortic balloons placed under

radiographic guidance preoperatively. This recipient survived the operation with a blood loss of 26 units (77). In another pediatric patient who had renal insufficiency following a failed liver-intestinal transplantation, both kidneys were included enbloc with the multivisceral graft (77). A lymphocytotoxic crossmatch was strongly positive in one patient who died of intractable cellular rejection after 58 days. The colon was part of the multivisceral graft in 8 of the 11 recipients and 4 grafts were from CMV-seropositive donors.

Donor Considerations

All grafts were obtained from ABO blood type-identical cadaveric donors. HLA matching was random and uniformly poor. Immunomodulation of donors or grafts by either irradiation, antilymphocyte preparations (ALG, OKT3), or other modalities was studiously avoided. Recognition of the risks that this kind of manipulation introduced, including the promotion of B-cell lymphomas (78), was one of the key steps in bringing intestinal transplantation to reality. The principles of composite graft harvesting have been described elsewhere (78). Modifications were frequently required to accommodate anatomic and pathologic circumstances in the recipient (73–79).

In recent cases, BM cells have been removed from the donors for infusion into recipients of whole organs, including those containing intestine (see preceding section). The dose of the unaltered BM cells was $3–6 \times 10^8$/kg, given over 30–60 minutes, 2–12 hours after revascularization of the organ allograft.

Postoperative Management

The complex management of these unique recipients is described in detail elsewhere (80,81). Immunosuppression was the same as described under the FK506 trials for liver transplantation, using the high-dose steroid induction. In a few cases, azathioprine or mycophenolate mofetil (RS61443, Cellcept®) was given as a third drug from the outset. In long-term survivors of the muitivisceral operation, blood glucose and c-peptide levels were determined after intravenous injection of 0.5 g/kg glucose.

Intestinal graft rejection was diagnosed by clinical findings, endoscopic examination and histopathological study of endoscopic-guided biopsies. Intestinal graft function was assessed by body weight, volume of stomal output, frequency and nature of the stools, and degree of dependency on TPN versus enteral feeding and/or oral diet. In addition, absorptive functions were directly measured by d-xylose tests and by 72-hour fecal fat excretion. Gastrointestinal motility was evaluated by measurements of gastric emptying after radiolabeled test meals, intestinal transit time of a barium meal, and manometric measures of contractile activity.

Systemic antibiotics were given prophylactically for the first 5 days, as well as subsequently, if indicated by the results of blood and body fluid cultures. Selective gut decontamination was used for 4–6 weeks after transplantation and resumed later during moderate to severe rejection episodes. Chronic viral and protozoal prophylaxis was with acyclovir for CMV and bactrim for Pneumocystis carinii. Because of the high incidence of CMV disease among most of the early recipients who received CMV-positive grafts, ganciclovir also was given prophylactically for 2–3 weeks in children and for 3 weeks to 3 months in adults based upon the CMV status of both donors and recipients. If severe CMV infection occurred despite prophylaxis, Foscarnet, CMV immunoglobulin or both, were added to or replaced the ganciclovir treatment (82,83).

Donor leukocytes circulating in the recipient peripheral venous blood were identified as donor with donor-specific anti-HLA Class I monoclonal antibodies and by fluorescence-activated cell sorter analysis (67). The results were confirmed using probes directed against HLA Class

II chromosomes by PCR and by in-situ hybridization techniques with a Y-chromosome-specific probe.

Survival

Patients—With potential follow-ups of 4–65 months (to January 1996), 25 (54%) of the 46 patients have died. The actuarial survival rates for the 2 types of transplantation combined (n=46) were 71%, 65%, 52% and 41% after 6, 12. 24 and 60 months, respectively (Fig. 20A). There was no significant survival difference with the 2 kinds of operations (Fig. 20B). Survival was similar for adults and children (Fig. 21). Interestingly, there was no mortality after 3 years among recipients who survived this long.

The causes of the 25 deaths, (19/35 liver plus intestine and 6/11 muitivisceral) were variable (Table 11). Fatal infections were responsible in 11 recipients. Graft rejection (n=5) or PTLD (n=5) accounted for 10 more. The remaining 4 patients died of technical (n=3) and management errors (n=1).

Grafts—Because all but 2 grafts were lost by patient's death, the actuarial survival for the 48 grafts in the 46 patients closely mirrored patient survival (Fig. 22). Only 2 grafts (both liver/intestine) were removed and replaced at retransplantation - with another liver-intestinal graft in one case and a muitivisceral graft in the other. Although the retransplant procedures were technically successful, the children died 60 and 57 days later from refractory rejection and PTLD, respectively.

Two other recipients lost parts of their composite grafts. Severe preservation injury of the pancreas in one instance necessitated pancreatectomy. Hepatic artery thrombosis in the other allograft required replacement of the allograft liver (postoperative day 11), but the intestine was spared. These patients died 197 and 29 days, respectively, after their primary transplantation.

Risk Factor Analysis

The practical and widespread use of intestinal transplant operations appeared from our early experience to be close at hand (73–75). Two correctable factors eroded these optimistic expectations. One was inclusion of the colon with the small bowel (76,84) and the other was acceptance of the grafts from CMV-seropositive donors (76,82–84).

The Colon Question—The survival rate for grafts which did not include the colon (n=29) was 75%, 62% and 49% at one, 2 and 3 years, respectively. Allografts that contained the colon had 42% survival at one year, 32% at 2 years and 26% at 3 years (p=0.04) (Fig. 23). The adverse effect of colon was more pronounced in adults than in children. Increases in plasma tumor necrosis factor-alpha were higher in recipients given colon as opposed to those who were not (76). This supported the possibility that development of augmented endotoxemia in the colon recipients may have been a reason for the increased risk.

The CMV Factor—Recipients of CMV-positive liver-intestinal and multivisceral grafts (n=18) had 2- and 5-year survival rates of 40% and 20% compared to 60% and 52% when the donors were seronegative (n=28, p=0.03) (Fig. 24). Although the immediate cause of death often was something other than CMV, the pervasively harmful influence of the transplanted virus, and the necessity for its intensive treatment, particularly in preoperatively CMV-negative recipients, have been well documented (76,82,83).

Bone Marrow Augmentation

We discussed earlier the insight about mechanisms of organ allograft acceptance that resulted from the discovery of donor leukocyte microchimer-ism in long-surviving organ recipients. Intestinal and multivisceral recipients were initially excluded from the attempts in kidney, liver and thoracic organ recipients to augment this spontaneous chimerism with donor BM infusion. It was feared that adding BM leukocytes to the immunologically active intestinal graft might push the recipient into GVHD. However, the absence of any significant complications after infusing 3–6 × 10^8/kg BM cells in more than 100 recipients of all of the other vital organs prompted us to extend this treatment to bowel recipients whenever permission could be obtained from the donor family for BM removal. This was possible for 10 of the 15 intestinal transplantations performed during 1995 and up to December 31 (Table 12).

The results in the 10 BM-augmented patients have been at least as good as in the 5 non-augmented contemporaneous controls (Table 12). The greatly augmented chimerism is construed to be an investment in the future of these patients, rather than a strategy to prevent or control perioperative acute rejection. Of extreme importance, none of the BM-augmented recipients developed any evidence of GVHD.

Long-term Rehabilitation

This experience has established the feasibility, although not the practicality, of both combined liver-intestinal and multivisceral transplantation. Successfully treated patients had gratifying rehabilitation with stable graft function for 5 years or longer in several cases. Although gut absorption was never completely normal, 20 of the 21 current survivors are free of intravenous nutritional support. The exceptional patient (multivisceral) requires intermittent intravenous nutrition at night because of dysmotility of the gastrointestinal graft. Several other patients take special dietary precautions to avoid diarrhea.

These were the results in the pioneer era of 1990–1993. After acquiring this experience, we declared a nearly one-year moratorium in order to develop strategies with which to circumvent the lethal risk factors. The improved results in 1995 after resuming activity are evident from Table 12, and have been particularly encouraging in the long view because of the safety of the BM augmentation protocol.

SUMMARY

Tacrolimus is a more potent and satisfactory immunosuppressant than CyA for combination therapy with prednisone. In randomized trials comparing the 2 drugs, the ability of tacrolimus to rescue intractably rejecting grafts on the competing CyA arm allowed equalization of patient and graft survival on both arms when the intent-to-treat analytic methodology was applied. The ability of tacrolimus to systematically rescue the treatment failures of CyA suggested, as a matter of common sense, that it is the preferred baseline drug for hepatic transplantation. This conclusion was supported by analysis of secondary end points, including the ability to prevent rejection.

Hepatic-intestinal, multivisceral and isolated intestinal transplantation became feasible on a practical basis only after the advent of tacrolimus. Nevertheless, better management strategies must be devised before intestinal transplantation, alone or with other abdominal viscera, will meet its potential. One such strategy is based on the discovery of the presence of previously unsuspected, low-level donor leukocyte chimerism in long-surviving allograft recipients. We believe that this chimerism is the essential explanation for the feasibility of organ transplantation and a link to the acquired neonatal tolerance demonstrated by Billingham. Brent and Medawar (32). The hematolymphopoietic chimerism in organ recipients explains why

weaning to a drug-free state in selected long-term survivors is frequently feasible and particularly if the allograft is a liver. Weaning should never be attempted without a stepwise protocol and careful monitoring of graft function.

Recognition of the natural chimerism that develops after whole organ transplantation has led to efforts to augment it with perioperative donor BM infusion. This procedure has been shown to be free of significant complications (including GVHD) in all kinds of whole organ recipients, including those given intestine.

The prospects of clinical xenotransplantation must be evaluated in the same context of chimerism as that delineated for allotransplantation with the discovery of spontaneous chimerism. Before addressing chimerism-related questions in xenotransplantation, the additional barrier of the complement activation syndromes that cause hyperacute rejection will have to be surmounted. Although measures to effectively transplant xenografts have so far eluded us, the availability of the more potent drug, tacrolimus, and recognition of the seminal basis of allograft (or xenograft) acceptance via chimerism has inserted an element of reality into the largely wishful thinking that has been evident in discussions about the future of xenotransplantation.

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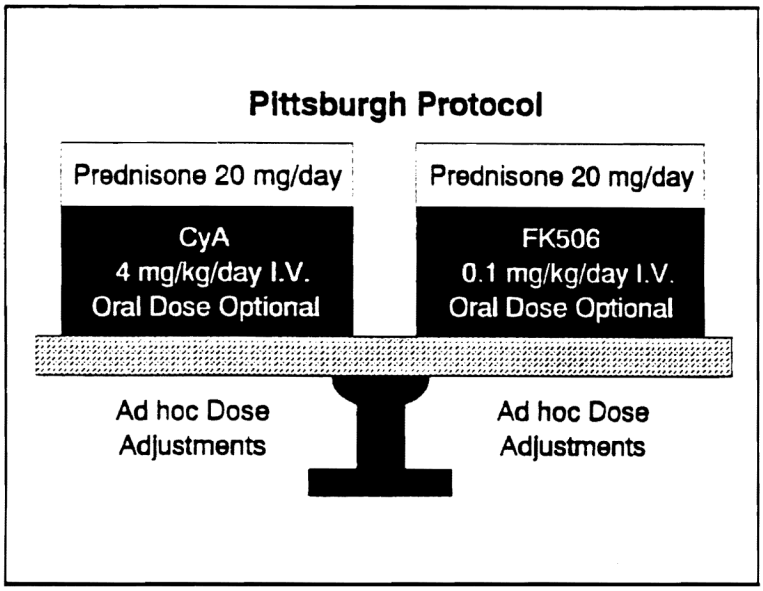

Figure 1.
The experimental design of the Pittsburgh randomized trial. Treatment variables were equal except for the competing drugs. (CyA and tacrolimus)
[From: Fung J, Eliasziw M, Todo S, et al. The Pittsburgh randomized trial of tacrolimus vs cyclosporine for liver transplantation. J Am Coll Surg (in press)].

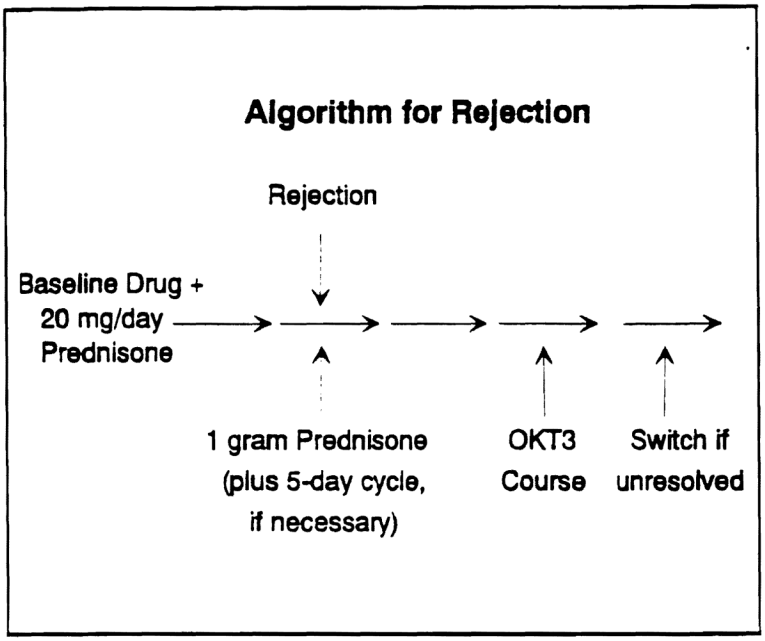

Figure 2.
Algorithm used to determine treatment failure, with the option of crossing over to the competing immunosuppressant drug. The diagnosis of rejection required biopsy proof [From: Fung J, Eliasziw M, Todo S, et al. The Pittsburgh randomized trial of tacrolimus vs cyclosporine for liver transplantation. J Am Coll Surg (in press)].

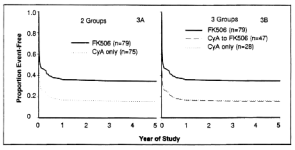

Figure 3.

A) Freedom from rejection (acute and chronic) comparing the 2 randomized treatment arms. B) Freedom from rejection comparing patients randomized to tacrolimus with those randomized to CyA and subsequently crossed over with those who continued on CyA for the duration of the trial

[From: Fung J, Eliasziw M, Todo S, et al. The Pittsburgh randomized trial of tacrolimus vs cyclosporine for liver transplantation. J Am Coll Surg (in press)].

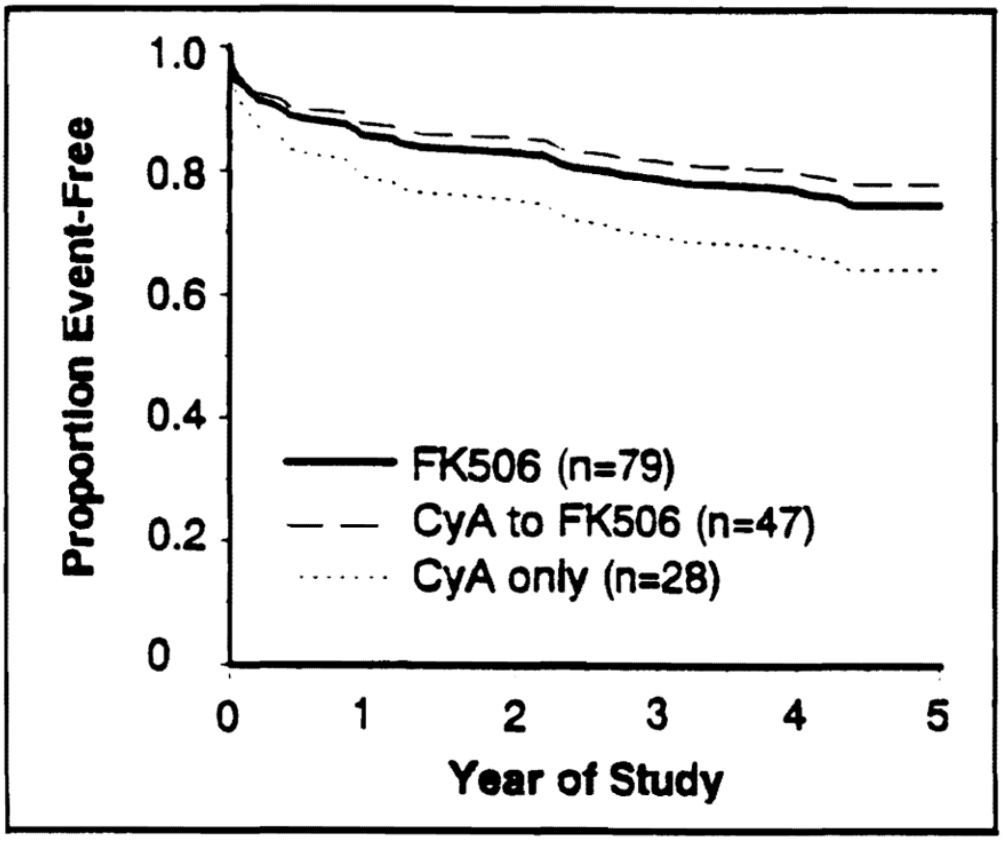

Figure 4.
Freedom from retransplantation or death in the Pittsburgh randomized trial of tacrolimus versus CyA
[From: Fung J, Eliasziw M, Todo S, et al. The Pittsburgh randomized trial of tacrolimus vs cyclosporine for liver transplantation. J Am Coll Surg (in press)].

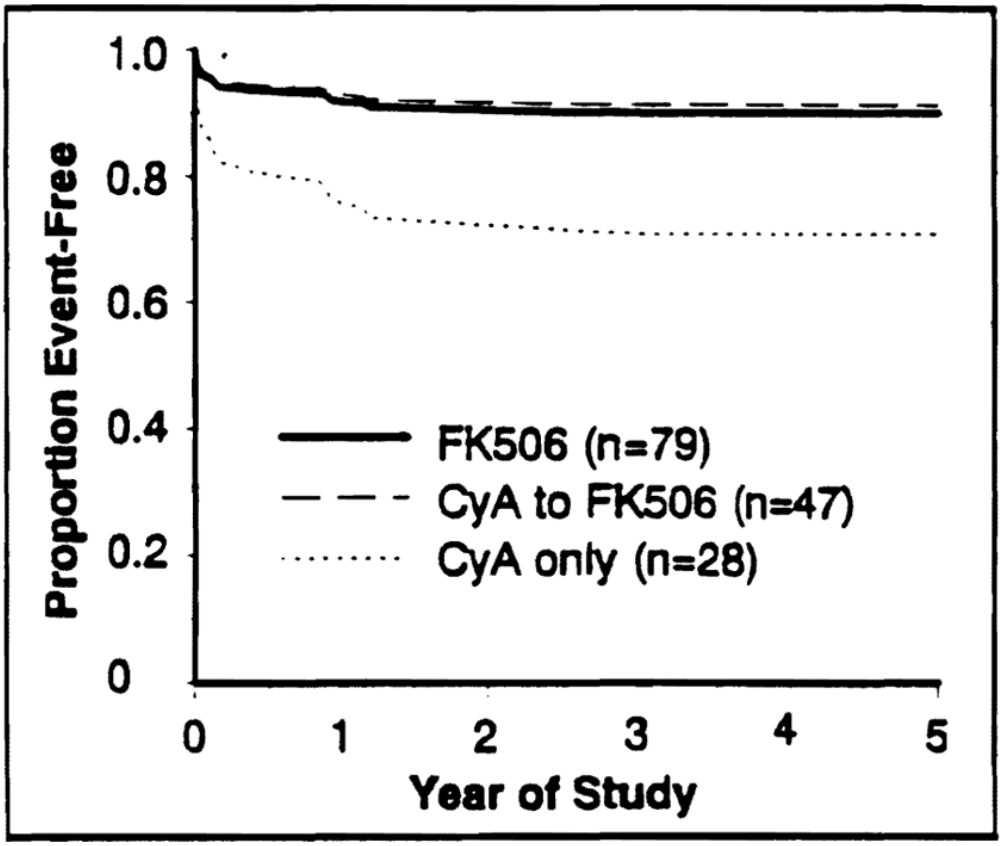

Figure 5.
Freedom from retransplantation in the Pittsburgh randomized trial of tacrolimus versus CyA [From: Fung J, Eliasziw M, Todo S, et al. The Pittsburgh randomized trial of tacrolimus vs cyclosporine for liver transplantation. J Am Coll Surg (in press)].

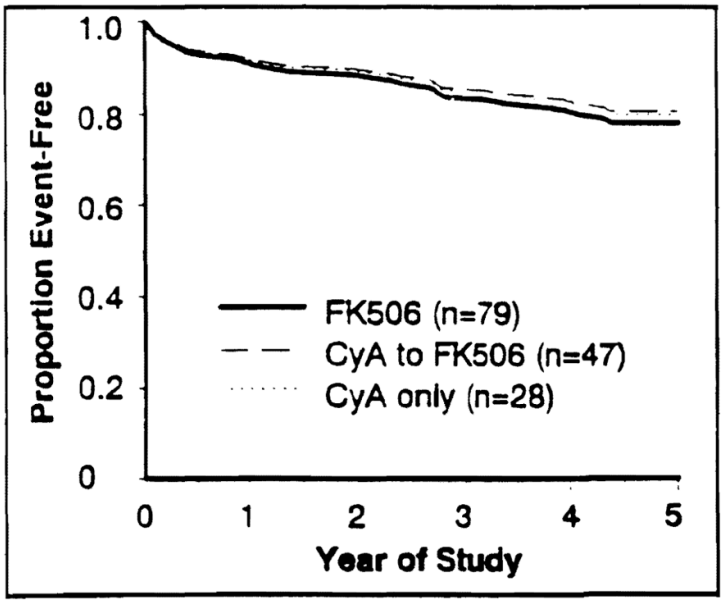

Figure 6.
Freedom from death in the Pittsburgh randomized trial of tacrolimus vs CyA
[From: Fung J, Eliasziw M, Todo S, et al. The Pittsburgh randomized trial of tacrolimus vs cyclosporine for liver transplantation. J Am Coll Surg (in press)].

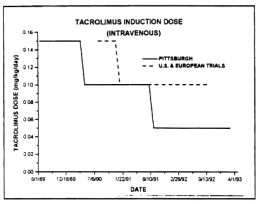

Figure 7.

Starting intravenous doses used in Pittsburgh (solid line) and in the US and European multicenter trials (dotted line). Note that the I.V. induction dose was already reduced by one-third by the time the Pittsburgh randomized trial was begun in February 1990 and that this was still too high.

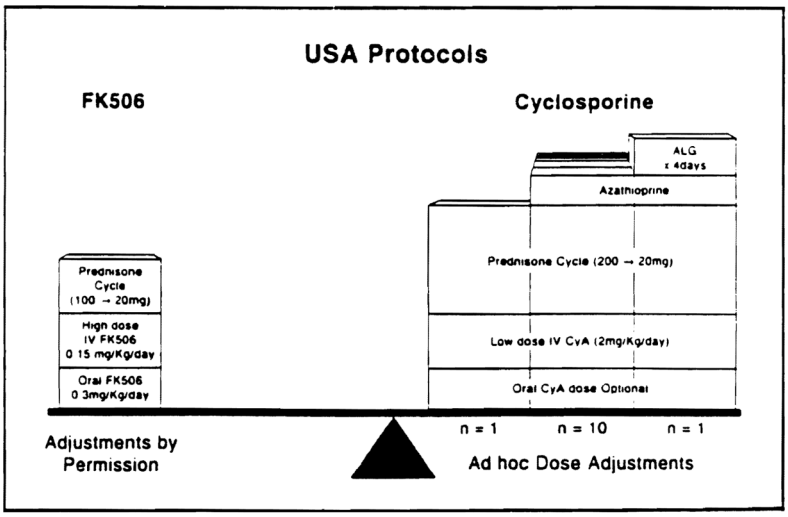

Figure 8.
"Unbalanced" experimental design in multicenter trials (American details shown) *n = number of centers* (From: Starzl TE, Donner A, Eliasziw M, et al. Randomized trialomania? The multicenter liver transplant trials of tacrolimus. Lancet, 1995:346:1346).

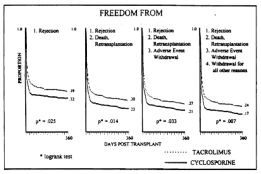

Figure 9.

Freedom from various undesirable end points using tacrolimus versus CyA in reanalysis of American trial (log-rank test)

(From: Starzl TE, Donner A, Eliasziw M, et al. Randomized trialomania? The multicenter liver transplant trials of tacrolimus. Lancet, 1995; 346:1346).

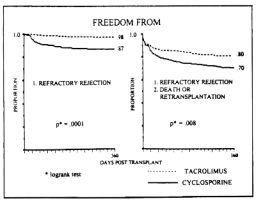

Figure 10.

Freedom from refractory rejection (left), and refractory rejection plus graft loss (right)(from death or retransplantation) in reanalysis of American randomized trial

(From: Starzl TE, Donner A, Eliasziw M, et al. Randomized trialomania? The multicenter liver transplant trials of tacrolimus. Lancet, 1995; 346:1346).

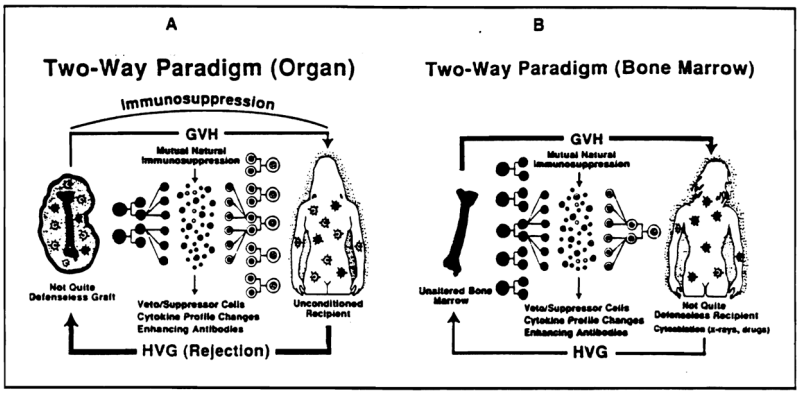

Figure 11.
The mutual engagement of migratory immunocytes from the graft (A: organ, B: bone marrow) and the recipient under potent pharmacological immunosuppression “2-way paradigm”.

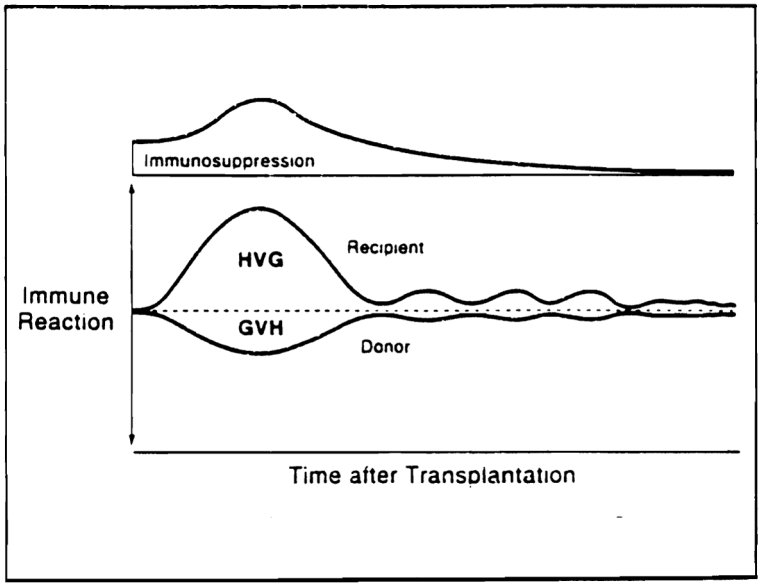

Figure 12.
Dualistic immune reactions of the HVG and GVH in the 2-way paradigm of transplantation immunology. The evolution of tolerance of each leukocyte population to the other is seen as a low-grade stimulatory state that may wax and wane rather than a deletional one.

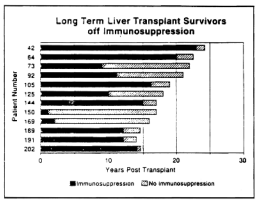

Figure 13.
Time of discontinuance of immunosuppressive therapy among 12 drug-free long-term liver transplant survivors (13.5–26 years).

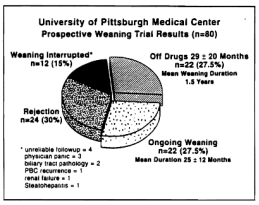

Figure 14.
Update of the outcome analysis of the Pittsburgh prospective weaning trial results. (n=80)

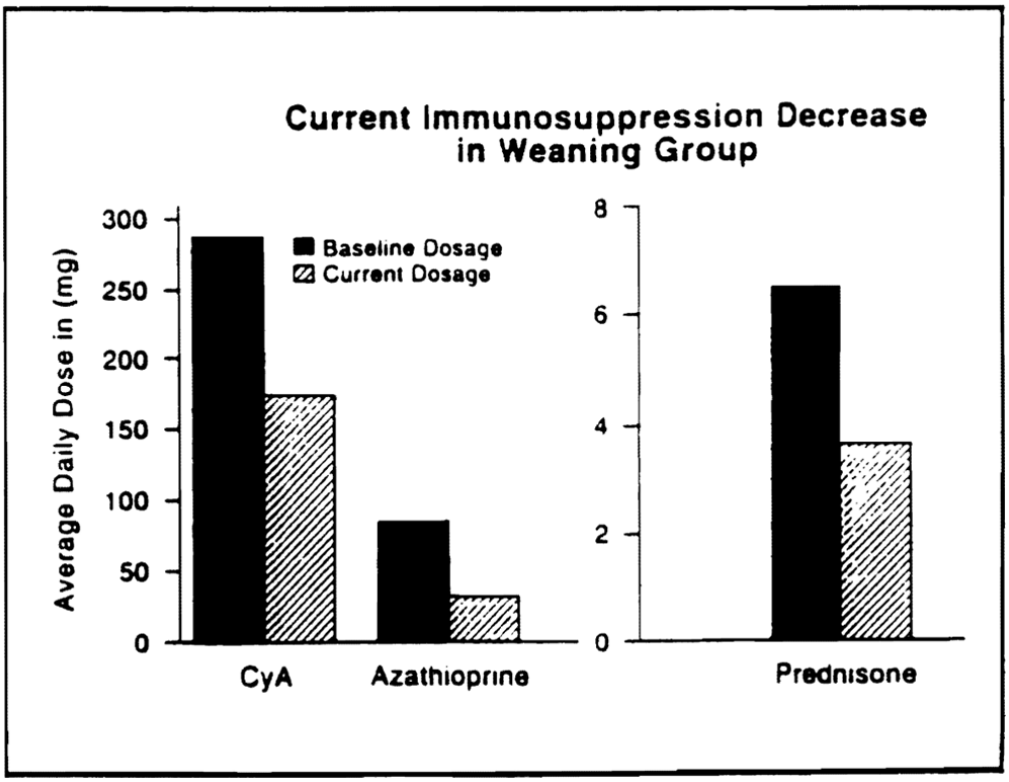

Figure 15.
The current reduction in the baseline immunosuppressive dosage among the weaning liver recipient group.

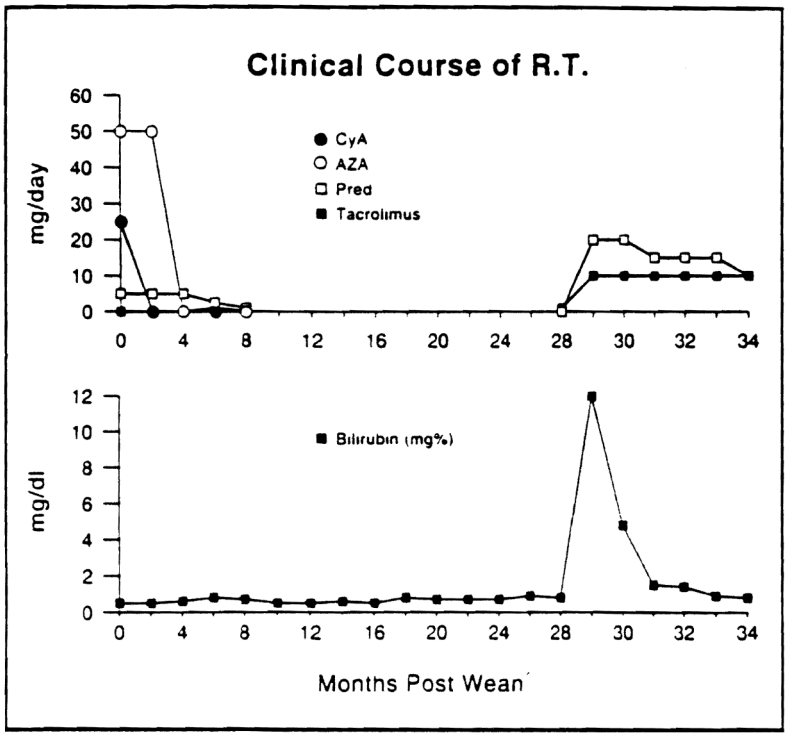

Figure 16.
The clinical course of the liver recipient who failed the immunosuppressive weaning trial. Note the sudden increase in serum bilirubin 28 months post-wean which returned to normal value after rescue with low-dose tacrolimus.

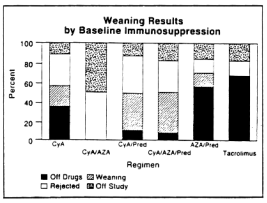

Figure 17.

The results of the Pittsburgh weaning trial according to the baseline immunosuppression. Note the better success with tacrolimus and azathioprine/prednisone.

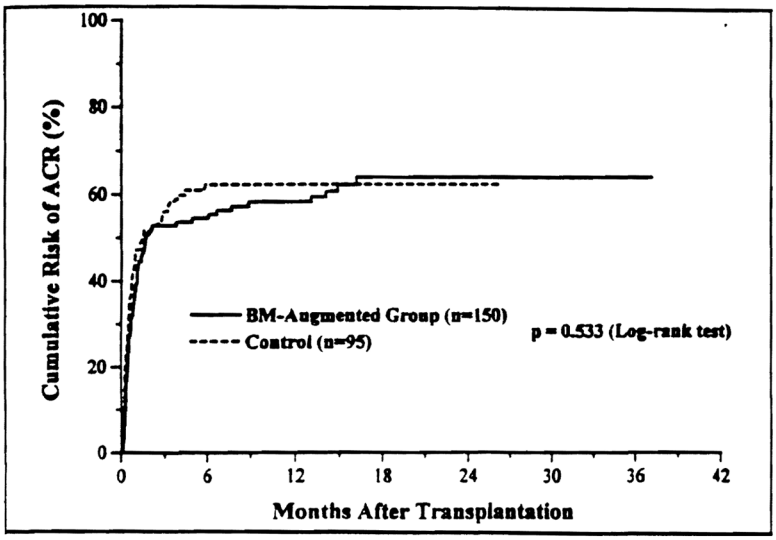

Figure 18.
The cumulative risk of acute cellular allograft rejection among the BM-augmented and control groups.

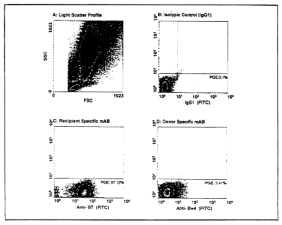

Figure 19.

Detection of donor (HLA-BW4+) cells in the PBMC of a BM-augmented liver recipient by flow cytometry. Density plot histogram suggests that at 883 days posttransplantation, 3.4% of lymphocytes within the recipient's circulation were of donor origin (D). A: Light scatter profile highlighting the gated events (in box); B: Isotype control for antibodies specific to donor HLA (IgG); C; Profile of cells stained with antibodies specific for the recipient HLA (B7). All analyses were performed on an EPIC Elite Flow Cytometer (Coulter Corporation, Hialeah, FL).

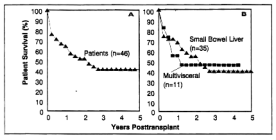

Figure 20.
The actuarial survival of the combined liver-intestinal and multivisceral recipients: A) all 46 patients, and B) according to procedure.

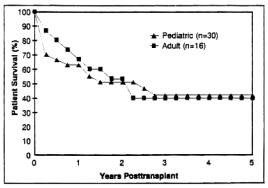

Figure 21.
The actuarial patient survival after combined liver-intestinal and multivisceral transplantation among both adult and pediatric recipients.

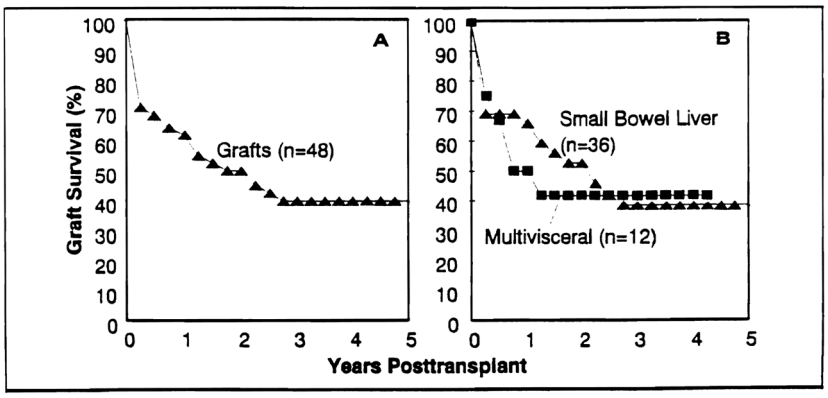

Figure 22.
Survival of the combined hepatic-intestinal and multivisceral grafts: A) all 48 attempts including 2 retransplantation and B) according to procedure.

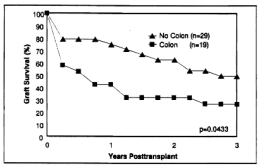

Figure 23.
Survival of 19 primary grafts containing a colon segment versus 29 grafts not including colon (p=0.04).

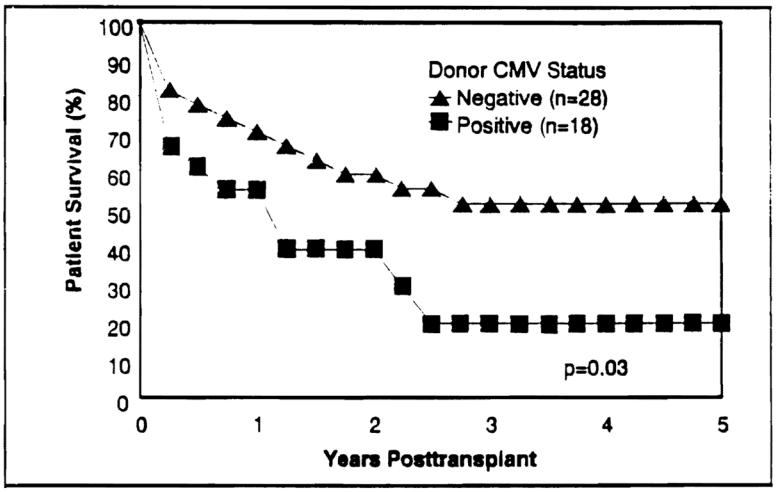

Figure 24.
The detrimental effect of CMV-positive grafts on combined liver-intestinal and multivisceral patient survival (p=0.0302).

Table 1

Patient characteristics of the Pittsburgh liver transplant randomized trial.[a]

	CyA n (%)	FK506 n (%)	CyA to FK506 n (%)	CyA Only n (%)
Number of patients	75	79	47	28
Age (mean year)	42.5	43.2	42.3	42.9
Sex (M/F)	46/29	49/30	26/21	20/8
Indication for transplant				
Cirrhosis				
Alcoholic	21 (28)	28 (35)	9 (19)	12 (43)
Post-hepatic	14 (19)	17 (22)	10 (21)	4 (14)
Cryptogenic	9 (12)	8 (10)	6 (13)	3 (11)
Cholestatic disease				
Primary biliary cirrhosis	8 (11)	8 (10)	6 (13)	2 (7)
Primary sclerosing cholangitis	6 (8)	7 (9)	3 (6)	3 (11)
Autoimmune hepatitis	5 (7)	2 (3)	4 (9)	1 (4)
Biliary atresia	1(1)	2 (2)	0 (0)	1 (4)
Metabolic disorders	4 (5)	2 (3)	4 (9)	0 (0)
Others	7 (9)	5 (6)	5 (11)	2 (7)
Blood type				
A	41 (55)	29 (37)	23 (49)	18 (64)
O	27 (36)	34 (43)	18 (38)	9 (32)
B	4 (5)	11 (14)	4 (9)	0 (0)
AB	3 (4)	5 (6)	2 (4)	1 (4)

[a]Study terminated on May 30, 1995.

Table 2

The Pittsburgh liver transplant randomized trial and crossover from CyA to tacrolimus.

Reason for Crossover	Prior to Rejection(n=7)	Following Rejection (n=40)
Steroid resistant rejection	0	26
Refractory rejection	0	13
CyA nephrotoxicity	0	1
Ischemic injury	5	0
Hemolysis	1	0
Family insistence	1	0

Table 3

The Pittsburgh liver transplant randomized study and retransplantation.

Reason for Retransplantation	Tacrolimus (n=8)	CyA to Tacrolimus (n=4)	CyA Only (n=8)
Primary failure	1	0	4
Ischemic injury	3	1	0
Graft rejection	1	1	0
Vascular	1	0	4
Recurrent disease	2	1	0
Infection	0	1	0

Table 4

The Pittsburgh liver transplant randomized study: causes of death.

Cause of Death	Tacrolimus (n=16) n (%)	CyA to Tacrolimus (n=10) n (%)	CyA Only (n=5) n (%)
Infection	8 (47)	5 (50)	2 (40)
Vascular	4 (24)	2 (20)	1 (20)
Graft failure	1 (6)	0 (0)	1 (20)
Multiorgan failure	1 (6)	0 (0)	0 (0)
Respiratory failure	1 (6)	0 (0)	0 (0)
Malignancy	1 (6)	0 (0)	1 (20)
Recurrent liver disease	0 (0)	3 (30)	0 (0)

Table 5

Reasons for withdrawal (censoring) from American multicenter study for secondary end point analysis.[a]

	Tacrolimus n (%)	CyA n (%)
Total number randomized	263	266
Total censored	83 (31.6)	102 (38.3)
Reason for censoring		
Death	14 (5.3)	16 (6.0)
2nd transplantation for technical problems	17 (6.5)	21 (7.9)
Adverse event	37 (14.1)	13 (4.9)
Lack of efficacy	6 (2.3)	32 (12.0)
Administrative[b]	9 (3.4)	20 (7.5)

[a] From Table 3: a comparison of tacrolimus (FK506) and cyclosporine for immunosuppression in liver transplantation. N Engl J Med 1994; 331:1110–1115.

[b] Explanation (loss to follow-up, declination of further study, and failure to meet candidacy criteria after transplantation) not compatible with text.

Table 6

Historical differences between bone marrow and organ transplantation.

Bone Marrow		Organ
Yes ←	Recipient Cytoablation	→ No
Critical ←	MHC compatibility	→ Not important[a]
GVHD ←	Principal complication	→ Rejection
Common ←	Drug-free state	→ Rare
Tolerance ←	Term for success	→ "Acceptance"[b]
Yes ←	Donor leukocyte chimerism	→ No

[a] Except when perfect

[b] "Operational tolerance"

Table 7

Tolerance with infections.[a]

	PTLD	HIV	HCV
n	6	2	2
Time drugs stopped posttransplant	Median 1 year (0.8 to 8 years)	6, 7years	0.5, 2 years
Survival rate (80%)	5/6	1/2	2/2
Time drug free (years)	4.1	6.1	4.2

[a]This is a follow-up to October 1995, of a series reported to the American Society of Transplant Surgeons in May 1993 (44).

Table 8

Patient and graft survival in BM-augmented and control primary organ allograft recipients.

	BM-Augmented Survival (n)			Controls Survival (n)		
Organs Transplanted	**n**	**Patient**	**Graft**	**n**	**Patient**	**Graft**
Liver	44	41	41	29	25	25
Kidney	64[a]	64	59	36[b]	34	33
Heart	15	12	12	18	17	17
Lung	15	12	12	9	8	8
Small bowel	9[C]	7	6	3[d]	2	2
Multiorgan	3[•]	3	3	0	0	0
Total	150	139	133	95	86	85

[a] Kidney alone (n-32); +pancreas (n=25); +islets (n=7)

[b] Kidney alone (n= 17); +pancreas (n=17); +islets (n=2)

[c] Small bowel alone (n=4); +liver (n=3); +pancreas (n=2)

[d] Small bowel alone (n=2); +liver (n=1)

[•] Heart+lung (n=2); liver+kidney (n=1)

Table 9

Clinical features of BM-augmented and control primary liver allograft recipients.

	BM-Augmented	Control
Number of patients	44	29
Age (mean/year)	50±12	51±12
Follow up (mean/months)	17±8	22±3
Graft survival (%)	93	86
Bilirubin (mean/mg/dl)	0.7±0.5	0.7±0.3
Off steroids (%)	49	56
Rejection (%)	43	56
GVHD(%)	5[a]	0
Chimerism (%)	100	50

[a] Trivial skin rash requiring no treatment for one patient and small transient increase in prednisone for the other.

Table 10

Causes of intestinal failure and indications for liver-intestinal and multivisceral transplantation.

Cause	Liver-intestine	Multivisceral
Adults (n=16)		
Crohn's disease	1	0
Abdominal trauma	3	0
Celiac A occlusion	0	3[a]
SMA thrombosis	4	0
Desmoid tumor	1	1
Metastatic gastrinoma	0	1
Budd-Chiari syndrome	0	1
Pseudo-obstruction	0	1[b]
Children (n=30)		
Gastroschisis	8[c]	0
Necro-enterocolitis	6	0
Volvulus	5	1
Intestinal atresia	4	1
Microvillus disease	2	0
Psudo-obstruction	0	2[d]
Hirschsprung's disease	1	0

[a] These patients developed short-gut syndrome due to concomitant superior mesenteric artery (SMA) thrombosis because of protein S defciency (n=1) antithrombin III deficiency (n=1) or unknown (n=1).

[b] The patient received the multivisceral graft after failure of the primary isolated intestinal graft due to refractory rejection.

[c] One patient required multivisceral retransplantation 15 months after receiving the liver-intestinal graft because of graft dysfunction.

[d] One patient had pseudo-obstruction after birth that was not diagnosed, and received isolated liver allograft 50 months before the multivisceral graft.

Table 11

Causes of death among liver-intestinal and abdominal multivisceral recipients.

	Total (n=25)	Liver-Intestinal (n=19)	Multivisceral (n=6)
	n (%)	n (%)	n (%)
Infection	11 (44)	8 (27)	3 (50)
Viral	5	3	2
Fungal	2	2	0
Bacterial	4	3	1
Rejection	5 (20)	3 (16)	2 (33)
Acute	4	2	2
Chronic	1	1	0
B-cell lymphoma	5 (20)	4 (21)	1 (17)
Technical	3 (12)	3 (16)	0 (0)
Management error[a]	1 (4)	1 (5)	0 (0)

[a] Hypernatremia.

Table 12

Patient and graft survival in BM-augmented and control intestinal allograft recipients.

	BM-Augmented Survival (n)			Control Survival (n)		
	n	**Patient**	**Graft**	**n**	**Patient**	**Graft**
Isolated intestinal	5	5	4	2	1	1
Combined liver-intestinal	3	1	1	3	2	2
Multivisceral[a]	2	2	2	0	0	0

[a]Liver, stomach, duodenum, pancreas, and intestine.