Transplant International

Transplant International ISSN 0934-0874

REVIEW

Evolution of the immunosuppressive strategies for the intestinal and multivisceral recipients with special reference to allograft immunity and achievement of partial tolerance

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Keywords
immune tolerance, intestinal transplantation, multivisceral transplantation, Recipient preconditioning with Thymoglobulin or Campath (alemtuzumab), weaning of immunosuppression.

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Received: 4 August 2008
Revision requested: 22 August 2008
Accepted: 25 September 2008

doi:10.1111/j.1432-2277.2008.00785.x

Summary

Introduction of new innovative immunosuppressive strategies has been the milestone of the recent evolution of intestinal and multivisceral transplantation. With new insights into the mechanisms of organ engraftment and acquired tolerance, the Pittsburgh tolerogenic protocol was recently introduced and consisted of two main therapeutic principles: recipient pretreatment with lymphoid ablating antibodies and minimal post-transplant immunosuppression with tacrolimus monotherapy. The reported herein improved survival and the striking ability to wean immunosuppression among the intestinal and multivisceral recipients pretreated with a single-dose of Thymoglobulin (rATG) or Campath-1H (alemtuzumab) supports our working hypothesis with successful induction of variable tolerance. It is important, however, that careful monitoring of subtle histologic changes in serial endoscopic-guided mucosal biopsies be carried out for early diagnosis of allograft immune activation with prompt restoration of the baseline immunosuppressive therapy. Future scientific discoveries with better understanding of the mechanisms of immune tolerance and clinical introduction of reliable assays will increase the chance and safety of achieving complete tolerance among the intestinal and other solid organ recipients. This review will focus on the historic evolution of the immunosuppressive and other management strategies utilized for the intestinal and multivisceral recipients at the University of Pittsburgh with special reference to allograft immunity and the successful achievement of partial tolerance.

Introduction

In contrast to other solid organs, intestinal and multivisceral transplantation has challenged, for many decades, both scientists and clinicians because of its immune and functional complexity [1,2]. The profound bidirectional immune interaction between the intestinal allograft and the host immune system delayed its clinical introduction because of the enigma of fatal graft-versus-host disease (GVHD) and the lack of a powerful immunosuppressant to control the destructive effect of the host versus graft response [3]. In addition to the traditional belief of the detrimental allo-immune effect of the gut-associated lymphoid tissue (GALT), the profound immunogenicity of the intestine could also be related to the epithelial innate immunity as well as the facultative capacity of the enterocyte to function as fully competent antigen presenting cells [2,4].

With the advent of tacrolimus in 1990, transplantation of the intestine began to emerge as therapy for end-stage

intestinal failure. However, under a tacrolimus-steroid based immunosuppression, a prohibitive risk of rejection was observed and required the use of acute and chronic high-dose immunosuppression [5,6]. Resorting to heavy maintenance immunosuppression was self-defeating and outlined era I of our experience with high morbidity and mortality that imposed a short-lived moratorium. During the first half of 1995, the moratorium was lifted and the program reopened (era II) with the clinical utilization of induction therapy combined with augmentation of the natural chimeric phenomenon by simultaneous donor bone marrow cell infusion [7–9]. Because of marginal improvement in survival, immune modulation of the visceral allograft with low dose (7.5 Gy) *ex-vivo* irradiation was also added to the protocol [9]. Despite the observed significant reduction in allograft rejection, the use of triple and sometimes quadruple maintenance immunosuppressants eroded the long-term patient and graft survival despite encouraging early outcomes.

At times, it was obvious that a new immunosuppressive protocol with less maintenance immunosuppression is required to increase the practicality and widespread utilization of the procedure [9]. Such a strategy was only feasible after the introduction, with better understanding, of new insights into the mechanisms of organ engraftment and acquired tolerance [10]. With the adoption of two major therapeutic principles namely; recipient pretreatment and minimal use of post-transplant immunosuppression, a tolerogenic protocol was clinically applied in July, 2001 that ushered a new era (III) for the intestinal and multivisceral recipients that was later applied to other abdominal solid organs. With such a novel protocol, attempts were also made to further reduce maintenance immunosuppression, particularly in patients with sustained allograft stability and quiescent allo-immune response [11]. The intestinal and multivisceral patients were the first transplant recipients of any kind to be treated with such an innovative tolerogenic strategy with the aim to achieve partial or near-complete tolerance.

Partial tolerance is defined by long-term allograft acceptance with successfully maintained functions under minimal nontoxic immunosuppression. Such a state of acquired tolerance has been increasingly achieved among liver and kidney recipients utilizing different immunosuppressive protocols including induction with multiple perioperative doses of anti-lymphocyte preparations and low maintenance immunosuppression [12–17]. However, the reported herein Pittsburgh protocol is the first to utilize a single dose of a lymphocyte-depleting agent and that is given prior to transplantation. Above all, it is the first tolerogenic protocol to be applied to the intestinal and multivisceral recipients with the intent to achieve a permanent state of minimal maintenance immunosuppression.

In contrast to partial tolerance, a state of complete tolerance, whether natural or induced, calls for complete sustained discontinuation of immunosuppression with successfully maintained long-term graft functions. Despite incomplete understanding of the true mechanism of immune tolerance, complete tolerance has been reported among liver and kidney allograft recipients with no single example, so far, among the intestinal and multivisceral transplant patients [18–20]. Natural or spontaneous operational tolerance (SOT) has been inadvertently observed in patients in whom immunosuppressants were discontinued because of noncompliance, diagnosis of life-threatening infections or development of drug toxicity [21,22]. With innovative but complex strategies, clinical induction of complete tolerance has recently been achievable in a few kidney allograft recipients [19,20]. The conditioning regimens included myeloablative and non myeloablative preconditioning with co-transplantation of donor hematopoietic stem-cells. In most of these unique recipients, all immunosuppressants were discontinued 6–14 months after transplantation with maintained normal renal allograft functions for up to 5 years. With unsolved disputes, the mechanism of natural and induced allograft tolerance was explained in light of the achievement of persistent mixed cellular chimerism, central deletion, and other mechanisms including regulatory T-cells that reside in the allograft [18–20,23]. It remains to be seen if similar protocols can be successfully applied to the intestinal and multivisceral recipients.

In parallel to the evolution of the immunosuppressive protocols, the field of intestinal and multivisceral transplantation has witnessed the introduction of new innovative surgical techniques and management strategies throughout the three different eras of the Pittsburgh experience [3,7,9]. With the three main prototypes of the intestinal transplantation procedure depicted in Fig. 1, both the donor and recipient operation underwent a few modifications during era II and III as described elsewhere [3,7,9,24]. Of interest, is the inclusion of the donor pancreas with the combined liver-intestinal allograft and preservation of the recipient spleen, pancreas and duodenum in patients who require modified or full multivisceral transplantation for certain disorders such as pseudo-obstruction [25].

The milestones in the advancement of the postoperative non immunosuppressive management strategy has been the infectious prophylaxis, monitoring with preemptive therapy of EBV and CMV viremia, and active treatment of bacterial/fungal infections with new antimicrobial drug therapy. During the three different eras, all patients received broad-spectrum anti-bacterial prophylaxis with more prolonged course in high risk patients during era II and III. With the beginning of era II, the

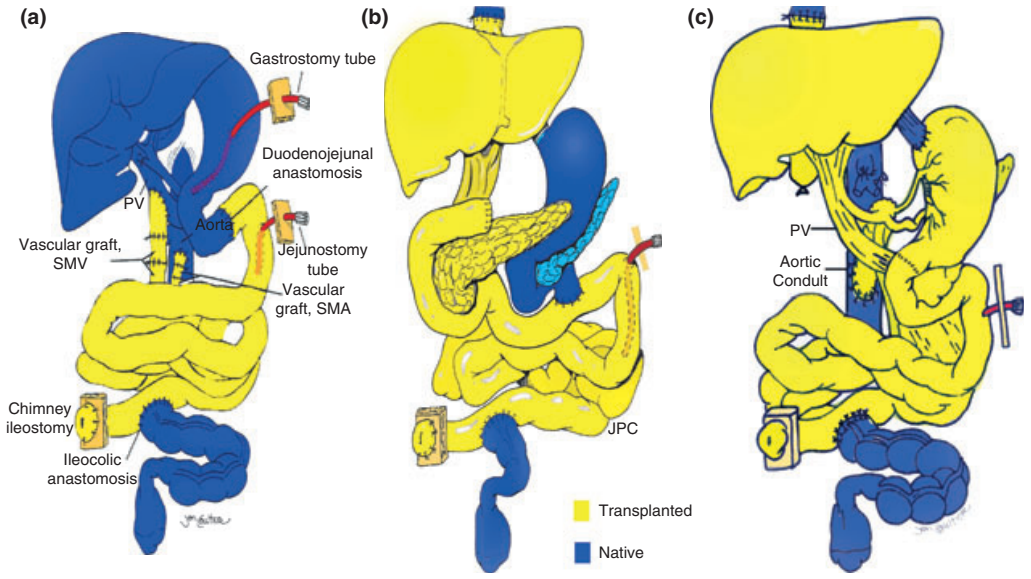

Figure 1 The three main different types of intestinal transplantation. (a) isolated intestine, (b) combined liver-intestine, and (c) multivisceral containing the stomach, duodenum, pancreas, small bowel and liver. The en bloc transplanted organs are yellow and the remaining native organs are blue. Note inclusion of the pancreas with the combined liver-intestinal graft for technical and logistic reasons. A jejunostomy tube was placed for early feeding and chimney ileostomy was created for surveillance ileoscopy and guided mucosal biopsies.

newly developed technique of semiquantitative polymerase chain reaction (PCR) assay of EBV in the peripheral blood has allowed early detection, treatment, and monitoring of EBV infection with reduction in morbidity and mortality of PTLD [9]. Similarly, the PP65 antigenemia test and more recently the PCR assay that has been used to detect early CMV reactivation or *de novo* infection has improved the pre-emptive treatment of this virus. Meanwhile, Gancyclovir replaced acyclovir for early postoperative anti-viral prophylaxis during the second half of era I and CMV-specific hyperimmune globulin (Cytogam) has been added as an adjunct therapy particularly for the high risk era II and III patients. The prophylactic and active anti-fungal therapy has evolved throughout the three eras with the clinical availability of the Liposomal Amphotericin B during era II and Caspofungin as well as Voriconazole as an effective therapy for aspergillosis and other life-threatening fungal infections in era III [26,27].

With similar distribution of donor characteristics and recipient clinical features including age, race, gender, cause of intestinal failure, cold ischemia time, HLA mismatch, lymphocytotoxic cross match, and donor/recipient CMV status, the clinical outcomes during each era are addressed below with special reference to the applied immunosuppressive protocol. Of particular importance, are the improved survival and the achievement of partial tolerance with spaced doses of tacrolimus monotherapy. For better understanding of the allo-immune response associated with intestinal and composite visceral transplantation, the unique documented traits of intestinal immunity are highlighted below with special focus on the central organs included in each of the different visceral grafts (Fig. 1).

Intestinal allo-immunity with special reference to the type of visceral graft

The rich mobile lymphoid content of the intestine with the expected amplification of the natural repopulation phenomenon that takes place immediately after revascularization of any solid allograft was behind conducting the Pittsburgh landmark study tracing the donor- and recipient immune cells in both the allograft and recipient circulation [28]. Such a fascinating dynamic process triggered the search for persistent donor mixed chimerism in our long-term liver- and kidney survivors and its relation to graft acceptance as a prerequisite or associated phenomenon [29]. Despite conflicting experimental data, the observed high risk of intestinal rejection early after transplantation suggests enhancement rather than amelioration of the alloreactivity by the migrating immunocytes [30–32]. However, we believe that the initial acute interaction of the two hematolymphopoietic cell population is central to long-term allo-engraftment [10]. The unexpected observed low risk of clinical GVHD could also be explained by the reciprocal induction of clonal activation that results in exhaustion with deletion of coexisting immune-competent donor- and recipient cells [10,33].

With experimental data demonstrating the inferior tolerogenicity of the intestinal immune cells, it has been our clinical observation that the human intestinal allograft is at a significantly higher risk of failure of engraftment when transplanted alone [7,9,34]. In the meantime, it was evident that the liver has an immunoprotective effect on the concomitantly transplanted intestine as predicted by Calne *et al.* a half century ago with other combined organ transplants [7,9,35]. Despite recently published conflicting results, our current data continues to support the hepatic immunoprotection hypothesis of the visceral allograft [9,36]. The failure to confirm our results by others may reflect the difference in duration of follow-up, sample size effect, and most importantly failure to address the survival outcome in reference to the specific causes of death or graft failure [9]. It remains to be seen whether the multivisceral graft will have immune privilege with better long-term stability as compared with the combined liver-intestine graft with and without preservation of the donor spleen [36]. We postulate that the operational tolerance of these composite visceral allografts is regulated by simultaneous or sequential mechanistic pathways that involve different donor- and recipient tolerogenic immunocytes that circulate or reside in different compartments of the tissue allograft including suppressor and regulatory T-cells [33,37].

The allo-immune response of the intestinal allograft could also be intensified by immunologic and non immunologic risk factors including systemic venous drainage of the isolated intestine, preformed antibodies and HLA mismatch [7,9]. While clinical data failed to demonstrate any immunologic impact of systemic venous drainage, the preformed antibodies with positive T and/or B lymphocytotoxic cross-match seems to increase the risk of intestinal allograft rejection with no significant impact on overall survival [38]. Because of sample size, current published and unpublished data failed to demonstrate the expected impact of HLA mismatch as seen in kidney and other allograft recipients [7,9,38].

Evolution of the immunosuppressive strategies

Era I with conventional immunosuppression

With the advent of tacrolimus in 1989, clinical intestinal transplantation began to emerge as a clinical reality for patients with irreversible intestinal failure and complex abdominal pathology in 1990 [39,40]. However, the early clinical experience was plagued with prohibitive risk of rejection and failure to maintain satisfactory patient and graft survival because of the need for heavy maintenance immunosuppression. During this era, 102 recipients received tacrolimus-steroid-based immunosuppression with azathioprine as an adjunct third agent in selected cases.

Era II with induction therapy and allograft immune modulation

To combat the high risk of rejection observed during era I, a more intense pharmacologic approach was taken in 1995 utilizing induction treatment with multiple maintenance drug therapy [7,9,41]. Furthermore, simultaneous donor bone marrow cell infusion was added to enhance donor-cell chimerism [7,9]. Near the end of era II and based on preclinical trials, low-dose *ex vivo* intestinal allograft irradiation was also initiated in adults [9,42].

The Pittsburgh conventional induction therapy protocol initially utilized cyclophosphamide that was replaced with daclizumab after its clinical introduction in 1998. Cyclophosphamide was also given at a dose of 2–3 mg/kg per day for 4 weeks and daclizumab was given in five intravenous doses (1–2 mg/kg) with an initial dose a few hours before surgery and at 2, 4, 6 and 8 weeks following transplantation. With a total of 87 patients, 24 received cyclophosphamide and 63 received daclizumab. In all of these recipients, azathioprine, mycophenolate mofetil (1997), and rapamycin (1998) were also used as an adjunct therapy with a high discontinuation rate of the later two drugs because of associated gastrointestinal drug toxicity and impaired wound healing. Full details of the protocol are described elsewhere [9].

The allograft immune modulation was achieved with a single infusion of 3 to 5×10^8 of unmodified donor cells at the time of transplantation. In April of 2000, the low-dose (7.5 Gy) *ex vivo* irradiation was added to the protocol particularly when the donor bone marrow cells were available [9]. Of the 87 era II patients, 37 received donor bone marrow infusion and 19 underwent transplantation with an irradiated intestinal graft.

Despite significant reduction in early post-transplant rejection (10%), allograft stability continued to decline with marginal improvement in long-term patient and graft survival. These long-term disappointing outcomes with similar results reported by other centers fueled our efforts seeking a new innovative strategy to achieve better long-term allograft stability with safe reduction of maintenance immunosuppression [9,10,23].

Era III with recipient pretreatment and minimization of post-transplant immunosuppression

Based upon better understanding of the seminal mechanisms of graft acceptance, the Pittsburgh preconditioning strategy was introduced mimicking the historic conditioning protocol of hematopoietic stem cell transplantation

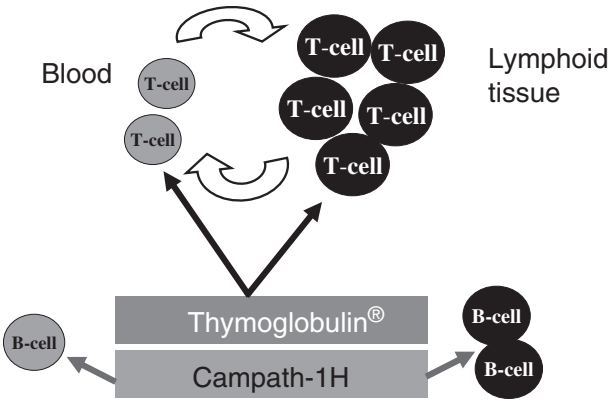

Figure 2 This illustration depicts the dynamics of the lymphocyte depletion by both Thymoglobulin (rATG) and Campath -1H (alemtuzumab). Note that both agents are effective in depleting both the intravascular and tissue T-lymphocytes. However, only Campath -1H is effective against the B-lymphocytes.

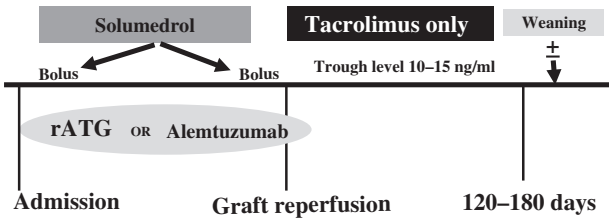

Figure 3 The specific details and different phases of the Pittsburgh preconditioning protocol. The protocol was initiated in July, 2001 and Campath-1H replaced Thymoglobulin in the adult population in November, 2003. In most of the pediatric patients, Thymoglobulin infusion continues for 6–8 h after graft reperfusion.

[23]. As stated in the introduction, the two therapeutic principles of the protocol are simple recipient preconditioning with lymphocyte ablating agents and minimization of the post-transplant immunosuppression. With joint application of both principles, we anticipated reduction of the initial donor-specific immune response and avoidance of the possible erosion of the seminal engraftment mechanism of clonal exhaustion-deletion without high penalty of allograft rejection. Rabbit anti-thymocyte globulin (Thymoglobulin) or alemtuzumab (Campath-1H) were used for recipient lymphoid depletion (Fig. 2) and tacrolimus monotherapy was utilized for post-transplant immunosuppression with avoidance of maintenance steroids. With 3–6 months of allograft stability, attempts were made to reduce, in a stepwise fashion, the frequency of tacrolimus dosage. The intestinal and multivisceral recipients were the first transplant patients of any kind to be treated with such an innovative tolerogenic strategy. The protocol was initiated in July, 2001 and first used among the adult population. After approval of the Hospital Committee on Innovative Practice and of the Pharmacy and Therapeutic Practice Committee, a total of 206 consecutive adult ($n = 122$) and pediatric ($n = 84$) patients underwent transplantation under perioperative lymphoid depletion. The different phases of the protocol are depicted in Fig. 3 and full details are summarized below.

Recipient pretreatment. Because of the time constraint inherited with cadaveric transplantation, patients were admitted to the hospital upon organ acceptance and pretreatment was promptly initiated particularly with Thymoglobulin. After the first 46 adult patients, Campath-1H replaced Thymoglobulin and was used for a total of 76 recipients. Until recently, Campath-1H was not utilized for the pediatric population with no single example in the reported herein series.

With partial depletion of both circulating and tissue lymphocytes, the Thymoglobulin peripheral effect was systematically studied in the first 11 intestinal and multivisceral recipients. As shown in Fig. 4, the peripheral cell count for the different T-cell subsets dropped to zero at day 1 with repopulation beginning within a few weeks and completed by the first 6 months following transplantation. Similar but more profound effect with depletion of B-cells was noticed with Campath-1H [43,44]. In one of our pretreated multivisceral recipients, the Thymoglobulin lymphoid tissue-depleting effect was captured in a para-aortic lymph node that was sampled 6 h after initiation of therapy and before allograft implantation (Fig. 5).

Based upon published experimental data and personal communications, Thymoglobulin was given in a single dose of 5 mg/kg that was infused over 4–6 h and completed before allograft reperfusion in all adult patients [23,45]. Because of legitimate concerns, the protocol was applied in children a few months later with a slow infusion rate that required continuation of therapy for 4–6 h after allograft reperfusion [33]. Intravenous dexamethasone (1 g in adults and 0.4 mg/kg in children) was given for prophylaxis against cytokine release syndrome during anti-lymphocyte antibody infusion and prior to allograft implantation. With an estimated half-life of 7 days, Thymoglobulin, both active and inactive, was detected in trace amounts in the recipient circulation at the end of the first postoperative week With such low levels of circulating active Thymoglobulin, we postulate, but have not proven, that recipient pretreatment has minimal depleting effect on the donor immunocytes and thus does not significantly impair the subsequent development of the donor mixed chimerism required for long-term allo-engraftment.

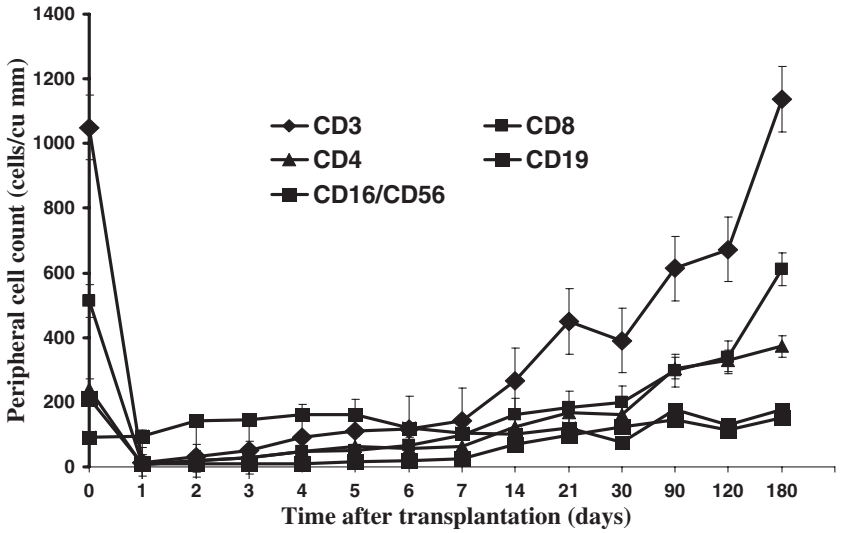

Figure 4 The depleting effect (mean ± SD) of a single 5 mg intravenous Thymoglobulin dose in 11 intestinal recipients. Complete depletion occurred within 24 h from infusion with no effect on B-lymphocytes. Note gradual and full recovery of all of the T-lymphocyte subsets including natural killer cells within the first 180 days following treatment.

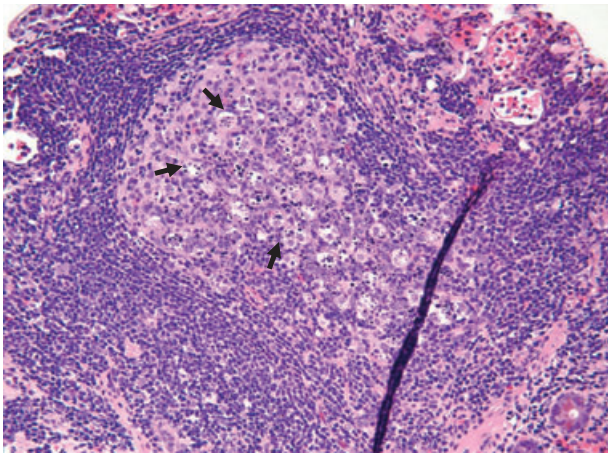

Figure 5 The tissue-depleting effect of Thymoglobulin captured in a para-aortic lymph node of a patient who underwent multivisceral transplantation because of pseudo-obstruction with liver failure. The recipient lymph node was sampled 6 h after initiation of the Thymoglobulin infusion and before allograft implantation. Note the impressive apoptosis of the central germinal lymphocytes (400×).

Nearly 2 years after initiation of the pretreatment protocol, Campath-1H, the humanized alemtuzumab (anti-CD52 mAb), substituted Thymoglobulin for adults and was infused in a single dose of 30 mg under the same umbrella of steroid prophylaxis. Unbound Campath-1H remains in the circulation longer than Thymoglobulin and for 1–2 weeks after infusion with more protracted biological effect lasting for 6–12 months [33,43].

In contrast to Thymoglobulin, Campath-1H can be infused within a 2-h period thereby shortening or completely avoiding any delay in performing a cadaveric organ transplant [43,44,46]. With either Thymoglobulin or Campath-1H, it is not uncommon to observe intraoperative coagulopathy particularly in liver failure patients because of platelet destruction/dysfunction and fibrinolysis. Accordingly, intraoperative serial monitoring of platelet count, fibrinogen level, and other clotting factors must be performed with judicious replacement of the defective factors as indicated.

Recipient lymphoid depletion with either Thymoglobulin or Campath-1H pretreatment is not recommended in patients with history of malignancy and viral C hepatitis because of the potential risk of disease recurrence during the prolonged state of impaired immune surveillance [43,47]. The protocol should also be cautiously used for retransplantation and in patients with severe pretransplant coagulopathy and extensive portomesenteric venous thrombosis because of the additional risk of intraoperative hemorrhage. The pretransplant diagnosis of immunoglobulin deficiencies should also preclude lymphoid depletion of the recipient because of the prohibitive risk of GVHD.

Post-transplant immunosuppression with a steroid-free regimen. Tacrolimus alone and without maintenance steroids was given after transplantation with an initial intravenous or oral dose to achieve 12-h tacrolimus trough level of 10–15 ng/ml by the third postoperative day. The same level was targeted for the next 3 postoperative months after which levels of 5–10 ng/ml were sought. Dexamethasone or hydrocortisone was added only in patients with significant rejection and those who developed adrenal insufficiency. For reasons that are not clear, it is our observation that a state of adrenal insufficiency and/or steroid dependency develops among the intestinal recipients, particularly of the multivisceral graft, who

received Thymoglobulin or Campath-1H with increased long-term risk of bone resorption and osteoporosis (K.M. Abu-Elmagd, unpublished data). Such a postoperative state of steroid dependency could be possibly masked during era I and II due to routine use of high-dose maintenance steroids as part of the immunosuppressive protocols. In a few patients, temporary steroid treatment was also given in response to unexplained persistent fever, severe arthralgia, failure to thrive, and GVHR. The use of mycophenolate mofetil, rapamycin or azathioprine was considered for patients with tacrolimus-related complications particularly among the pediatric population [33].

Weaning of immunosuppression. During the early phase of the trial, the weaning process was initiated 3–4 months after transplantation. This practice has been recently modified to only include patients who are beyond the 6th postoperative month. Of the inclusion criteria, for tacrolimus weaning, are allograft stability and a rejection-free state of more than 60 consecutive days. Of the exclusion criteria are the pretransplant diagnosis of autoimmune disorders and retransplantation due to irreversible acute or chronic allgraft rejection. All recipients who are potential candidates for weaning must undergo baseline endoscopic guided intestinal mucosal biopsy. The process of weaning was only initiated after mutual agreement between the transplant physician and the patient with full acceptance of the potential risk of allograft rejection and possible graft loss. The twice per day tacrolimus dose was initially changed to a single daily dose, often with small total dose reduction, and later spaced to every other day, three times per week and ultimately to two times per week (Fig. 6). Throughout the weaning process, there was a tendency for gradual reduction of the total weekly dose with the aim for a 24-h tacrolimus trough level of 5 ng/ml and undetectable 48- and 72-h trough levels. Meanwhile, the hydrocortisone daily doses, wherever maintained, were slowly reduced or discontinued as tolerated. With a slow stepwise weaning process, all patients were subjected to close follow-up with 1–2 per week mucosal biopsies and more frequently if clinically indicated. After 4–6 weeks from last dose reduction, the follow-up visits were reduced to 1–2 per month. With the exception of the early cases, prompt reversal of the weaning process was initiated upon development of any unexplained clinical and endoscopic changes with histopathologic features suggestive of allograft immune activation. The Cylex immune cell function assay and monitoring of serum levels of class I and class II donor-specific antibodies are more recently utilized to assess candidacy and guide safe weaning [48].

Immunologic, infectious and survival outcomes

Rejection. The use of induction therapy and allograft immune modulation (era II) significantly reduced the risk of acute rejection during the first 90 days after transplantation (10%) compared with that was observed during era I (78%). However, the risk has increased during era III to 50% with the development of moderate to severe steroid-resistant rejection episodes in one third of the cases that required treatment with muromonab CD3, Thymoglobulin or Campath-1H. Such an increase in host-versus-graft reaction with early donor-specific

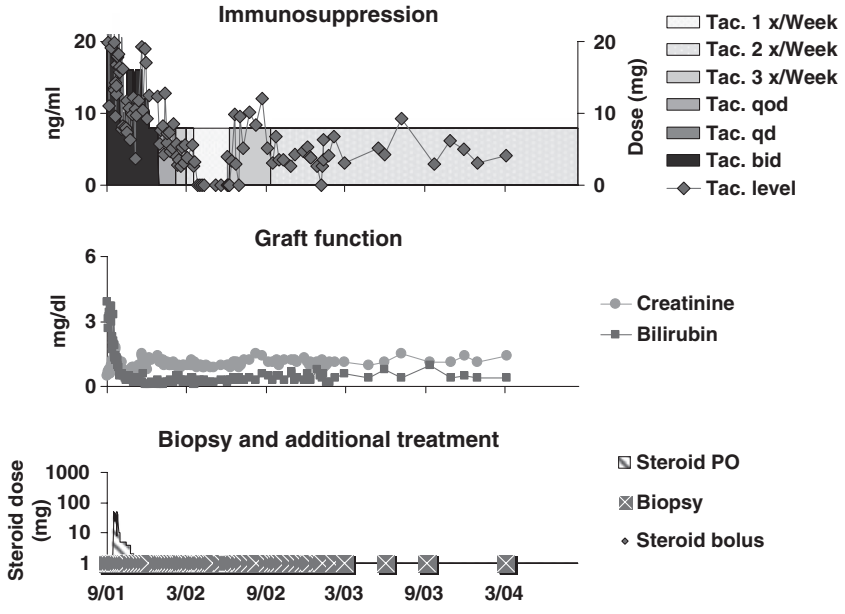

Figure 6 The clinical course of a multivisceral recipient pretreated with Thymoglobulin. The patient was transplanted in September, 2001 because of pseudo-obstruction and TPN-induced liver failure. The clinical and immunologic postoperative course was uneventful and the step-wise weaning process was successful as shown here. The patient is currently alive with fully functioning graft nearly 7 years after transplantation with a single 5 mg tacrolimus dose twice per week. Tacrolimus is the only medication that the patient is currently receiving with an excellent quality of life.

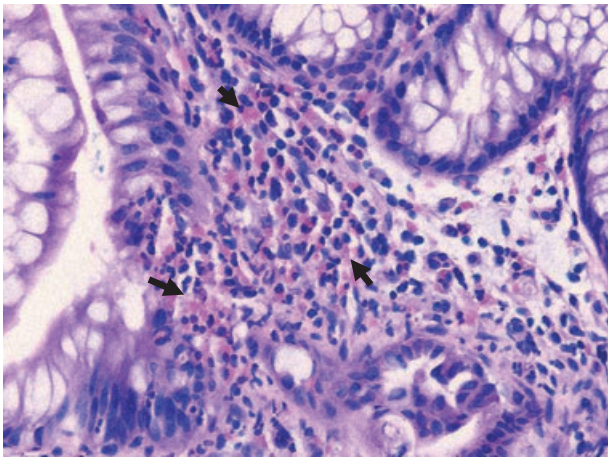

Figure 7 Endoscopic mucosal biopsy of an isolated intestinal graft in a recipient pretreated with Thymoglobulin. Note extensive eosinophilic infiltrates of the submucosa that is commonly observed in the preconditioned recipients with avoidance of post-transplant maintenance steroids (400×). A few patients were treated with steroid bolus because of the development of significant eosinophilic cryptitis.

immune activation reflects the minimal use of post-transplant immunosuppression 'tacrolimus monotherapy' on the basis of the protocol premise to allow the seminal engraftment mechanism of clonal exhaustion-deletion. The avoidance or minimal use of maintenance steroid could also be responsible for the significant eosinophilic infiltrates observed in most of the surveillance intestinal mucosal biopsies (Fig. 7).

Chronic rejection of the intestinal allograft was observed during the three different eras of the Pittsburgh immunosuppression protocols. During era I (tacrolimus plus steroid), the overall risk was 11% with a mean (SD) follow-up of 57 ± 63 months (range 0–205). With induction therapy (era II), and anti-lymphocyte pretreatment (era III), the incidence was 20% and 14%, with mean follow-ups of 61 ± 43 (range: 0–138) and 30 ± 20 (range: 0.3–72), respectively. Because of the relatively shorter duration of follow-up with era III, it remains to be seen whether recipient preconditioning will change the long-term risk of intestinal and multivisceral chronic allograft arteriopathy.

Success of weaning

Minimization of long-term immunosuppression was attempted with each of the immunosuppressive protocols. In eras I and II without recipient preconditioning, 18 (24%) out of 74 current survivors with a mean follow-up of 82 ± 20 months are receiving tacrolimus in a single or every other day dose with undetectable to less than 5 ng/ml trough blood levels. There is a single example of rapamune only immunosuppression in an isolated intestinal recipient. Reduction of immunosuppression in most of these patients was clinically indicated because of development of opportunistic infections and other immunosuppressant side-effects including neurotoxicity, renal dysfunction, diabetes and hypertension. A similar single example of partial 'prope' clinical tolerance has also been reported in a noncompliant living related intestinal transplant recipient after successful treatment of severe rejection [49]. Such a success without preconditioning may reflect a state of spontaneous operational tolerance that has been exceptionally observed with other long-term abdominal solid organ allograft survivors despite conventional immunosuppression [21].

As described earlier and illustrated in Fig. 3, the intent of the preconditioning protocol in era III was the attempt to wean all patients in whom graft stability was achieved. Thus, we tried to wean 136 (66%) out of the 206 pretreated patients. Of these attempts, 57% have been successful (Fig. 8). In the remaining 43% of the patients, rejection of the intestine (Fig. 9a) and/or the liver (Fig. 10) occurred at variable times during the tacrolimus dose weaning process. These patients required treatment with dexamethasone and/or one of the anti-lymphocyte monoclonal or polyclonal antibodies including muromonab-CD3, Thymoglobulin and Campath-1H. In every case, the rejection process was reversed and the graft was salvaged (Fig. 9b, 10). Interestingly, we have observed a unique histopathologic feature of weaning-induced acute rejection characterized by severe crypt loss (cryptopenia) despite intact villous surface epithelium (Fig. 11). Full details of the different histopathologic features that characterize acute intestinal allograft rejection in patients pretreated with Thymoglobulin or Campath-1H were fully described by Wu at al [50]. The clinical course of one of the isolated intestinal recipients, during the early phase of the study, was illustrated in Fig. 12. The patient was weaned to a bi-monthly single oral dose of tacrolimus (8 mg) and developed severe rejection 4 weeks later that was successfully reversed with muromonab-CD3 as shown in Fig. 9. A re-weaning attempt was made few months later with successful long-term outcome on a single every other day 5 mg dose of tacrolimus.

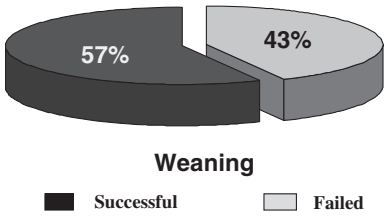

Figure 8 The clinical outcome of the weaning protocol in 136 recipients. Note failure of weaning in less than half of the patients.

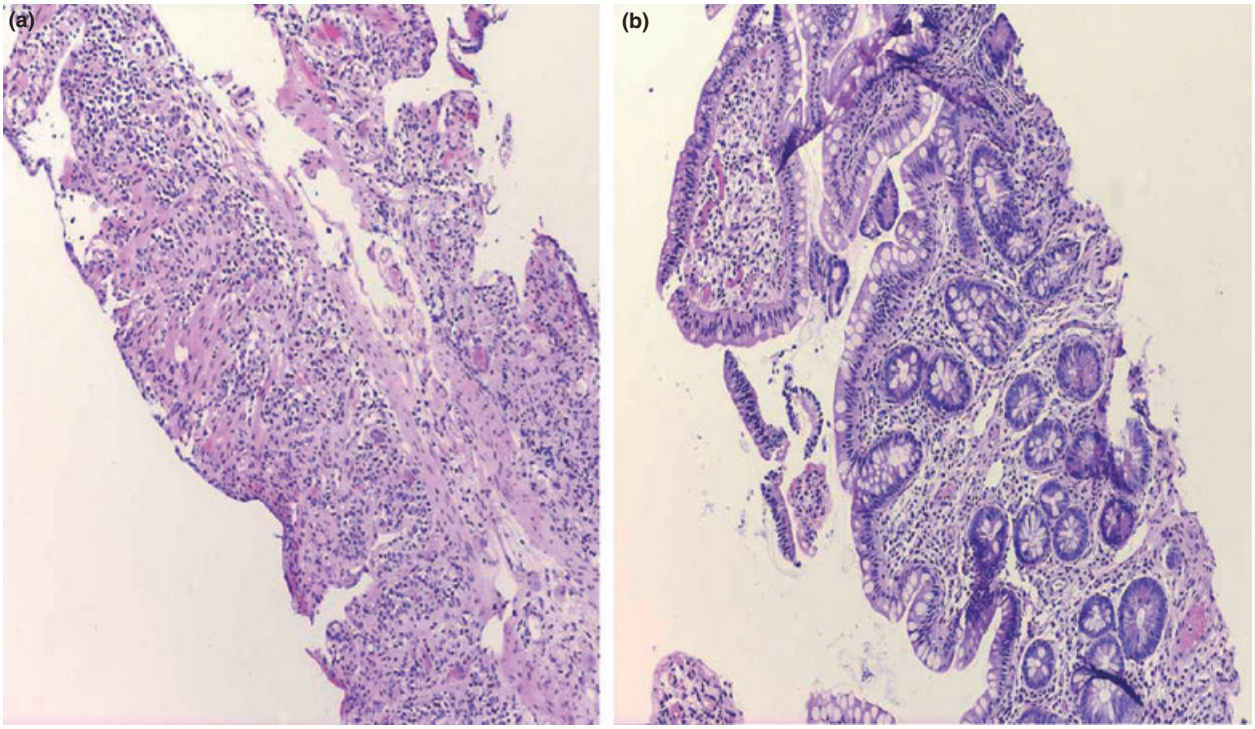

Figure 9 Severe intestinal allograft rejection in one of the early isolated intestinal recipients who was quickly weaned to a single 8 mg dose of tacrolimus every 2 weeks. (a) Break through rejection diagnosed 4 weeks after initiation of the bi-monthly dosage (100×). (b) Reversal of the tissue damage with full allograft recovery after 10 days course of muromonab-CD3 and twice per day doses of tacrolimus (100×).

Univariate and multivariate statistical analyses failed to identify any significant predictors of success of weaning. The analyzed variables included age, type of allograft, lymphocytotoxic cross-match, HLA typing, cold ischemia time, allograft immune modulation, type of anti-lymphocyte depleting agent, maintenance steroids, and development of post-transplant infections. The age factor was skewed by the fact that the adult recipients were the primary focus of the weaning protocol with small number of children being subjected to the weaning trial. There was a tendency for better success of weaning among recipients of liver contained visceral allografts compared with those who received intestine only. Unexpectedly, positive lymphocytotoxic cross-match and degree of donor/recipient HLA match/mismatch did not influence the success of weaning. Cold ischemia time was also not a significant predictor as well as allograft immune modulation. The success of weaning was similar among the Thymoglobulin and Campath-1H pretreated recipients. Finally, there was no correlation between the success of weaning 'partial tolerance' and the early or late development of bacterial, fungal or viral infections. The lack of a statistical power among some of these clinical and immunologic variables may reflect a small sample size or a discrepancy in the follow-up periods.

When compared with eras I and II, the overall 173 (84%) current survivors of era III recipients achieved a higher percentage of minimal immunosuppression with better net state of tolerance. Despite a shorter duration of follow-up with a mean of 43 ± 22 months, 66 (38%) recipients are on a spaced single dose of tacrolimus. In addition, the dosage was less frequent with a twice per week dose in 3 (5%), three times per week in 18 (27%), every other day in 14 (21%), and a single daily dose in the remaining 31 (47%) patients (Fig. 13). The trough tacrolimus blood level is generally undetectable in patients who are on a dosage schedule of every other day to two times per week. With a single daily dose, the targeted 24-h tacrolimus trough level is at or less than 5 ng/ml. The relatively large scale achievement of these unprecedented results supports the underlying immunologic principles of the protocol. A detailed pharmacokinetic study and *in vivo* immunologic assay are underway to rest the hypothetical dispute of therapeutic indifference between daily and less frequent dosage of tacrolimus. The long-term safety of the protocol has been tested in a total of 25 recipients who showed no evidence of chronic allograft arteriopathy (Fig. 14) after 1–2 years of spaced doses of tacrolimus. Nonetheless, additional long-term follow-up is required to further assess the durability of allograft stability.

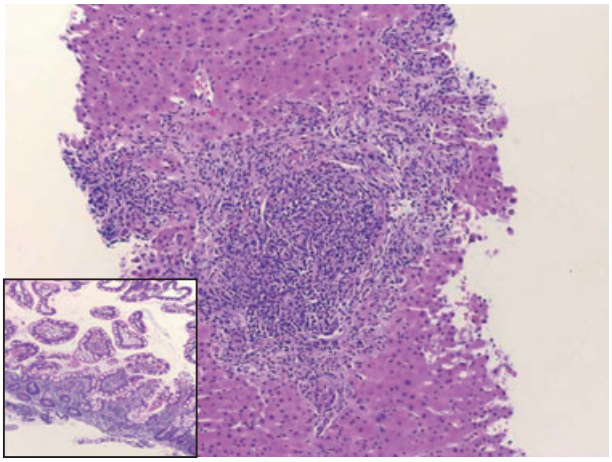

Figure 10 Rejection of the liver allograft in a patient with Crohn's disease who underwent combined liver-intestinal transplantation and pretreated with Thymoglobulin. Hepatic enzymes elevation was noticed after spacing of the tacrolimus dose to three times per week. The shown histopathologic findings of extensive lymphocytic infiltrates, hepatocyte necrosis, and bile duct injury established the diagnosis of moderate to severe liver rejection (100×). The patient was treated with steroid bolus and 10 days course of muromonab-CD3 as well as reversal of the weaning process. Note normal histology of simultaneous intestinal mucosal biopsy (left lower corner). A year later, the patient succumbed to chronic rejection despite reversal of the acute rejection episode and use of conventional maintenance immunosuppression after failure of weaning.

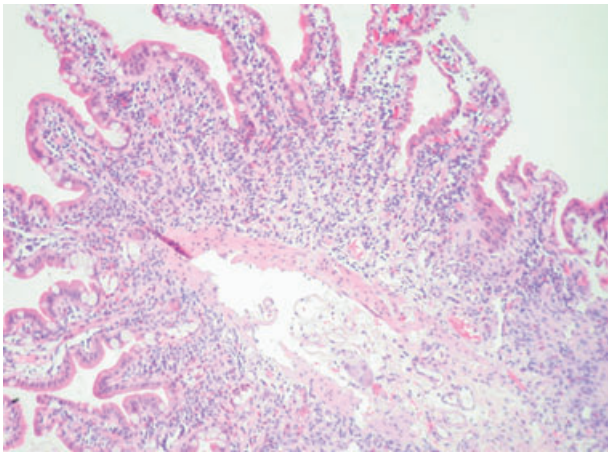

Figure 11 Intestinal allograft rejection induced by stepwise spacing of the tacrolimus dose to three times per week. Note severe crypt loss (cryptopenia) despite intact villous surface epithelium (100×).

Graft-versus-host disease

Histologically proven GVHD was documented with similar frequency among the three immunosuppressive protocols with an overall incidence of 5%. However, the clinical syndrome of graft-versus-host reaction (GVHR) developed in a higher percentage (8%) among the pretreated recipients (era III), particularly among children. The GVHR diagnosis was suspected with the transient development of significant skin rash, fever, and other constitutional symptoms [33]. Despite lack of specific histopathologic features in the recipient skin biopsy, the diagnosis was supported in all cases by simultaneous transient detection of circulating donor lymphocytes (2–22%) in the recipient peripheral blood utilizing the in situ hybridization technique and/or flow cytometric studies. In all of the GVHD patients, donor chimerism in the peripheral blood was persistent with a variable percentage ranging from 5% to 100%. Of great interest is the development of donor-derived multilineage complete chimerism in four of these recipients with Campath-1H pretreatment in three (unpublished data). Asymptomatic patients were not routinely tested for chimerism.

Infections

Era I was plagued with a high risk of life-threatening viral, bacterial, and fungal infections [7,51]. With fewer incidences during era II compared with era I, era III was associated with a significant reduction in opportunistic infections particularly the development of post-transplant lymphoproliferative disorder (PTLD) (9, unpublished data). The serial monitoring of cytomegaloviral (CMV) and Epstein Barr viral (EBV) peripheral blood load with prompt pre-emptive anti-viral therapy most probably contributed to the favorable outcomes during era II and III [9,52]. Of added benefits were the concomitant adoption of prolonged anti-viral prophylaxis and avoidance of the use of CMV positive donor to CMV negative intestine alone or modified multivisceral recipients [7,9,53].

Regardless of the applied immunosuppression protocol, bacterial, and/or fungal infections continued to occur at a relatively higher rate among the intestinal and multivisceral recipients compared with other solid organs [40,51,54]. Intestinal and multivisceral transplantation is a potentially contaminated procedure because of the nature of the operation and the commonly associated complex abdominal pathology. In addition, significant injury to the intestinal mucosal barrier, because of severe preservation injury and/or rejection, is associated with a prohibitive risk of bacterial and fungal translocation with subsequent development of life-threatening systemic infections. Above all, most patients are at a continuous high risk of line infections during the relatively protracted postoperative recovery. With learned lessons from era I, recipients of era II and III experienced fewer incidences of bacterial and fungal infections. Of particular importance is the introduction of technical innovations, prolonged use of post-transplant anti-microbial prophylaxis, and more recently the availability of new effective

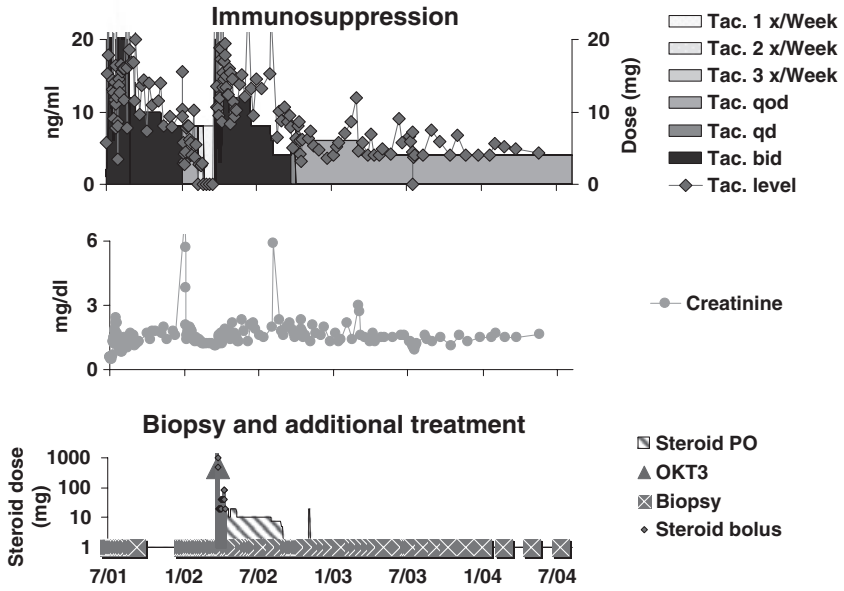

Figure 12 The postoperative course of a Thymoglobulin pretreated isolated intestinal recipient with failure of initial weaning. After full recovery of the intestinal graft, a second weaning trial was attempted with successful outcome.

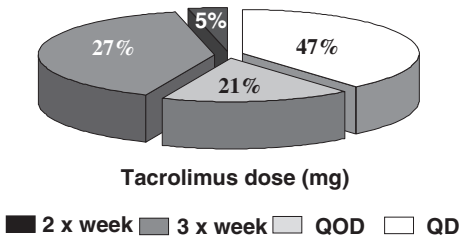

Figure 13 The frequency of tacrolimus dosing among 66 long-term survivors with Thymoglobulin or Campath-1H pretreatment. Note that more than half of the patients are receiving a spaced single dose of tacrolimus ranging from 2 to 8 mg and given two times per week to every other day.

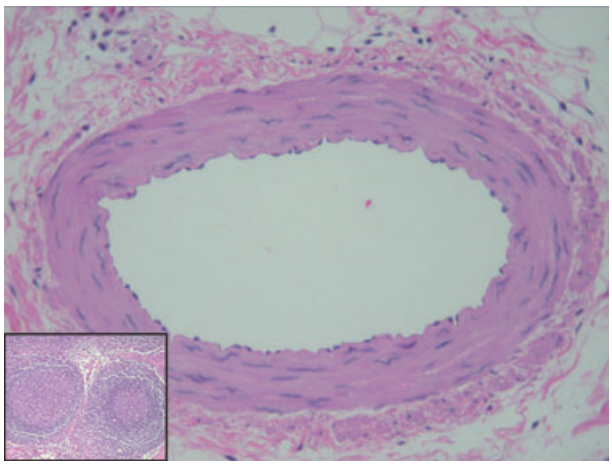

Figure 14 Full thickness biopsy of a mesenteric artery and lymph node (left lower corner) sampled from a resected segment of a terminal ileal allograft during stoma closure. Note the absence of chronic arteriopathy or any histopathologic evidence of immunologic injury with tacrolimus monotherapy at a dose of 5 mg twice per week with undetectable trough level for more than 1 year (400×). The allograft mesenteric lymph node architecture was also preserved with no evidence of sclerosis.

anti-bacterial and anti-fungal drug therapy. Nonetheless, the net state of immunosuppression particularly with the need to treat significant episodes of acute rejection and the associated potential risk of translocation influences the risk of infection regardless of the initially adopted immunosuppression protocol. It is our strong recommendation to initiate prophylactic anti-microbial and anti-viral therapy during any episode of intestinal allograft rejection that precipitates mucosal ulceration or requires heavy augmentation of immunosuppression.

Other morbidities

Despite the intent of minimizing post-transplant immunosuppression during era III, the reduction in the risk of chronic renal insufficiency, hypertension and diabetes was insignificant. It is important to emphasize that the intestinal allograft recipients, regardless of the utilized immunosuppressive protocol, are at a significantly higher risk of such morbidities compared with other abdominal solid organ transplant patients. This unique population with a long-standing history of short gut syndrome, TPN dependence, excessive fluid losses, and recurrent line infections has, from the outset, marginal renal reserve because of

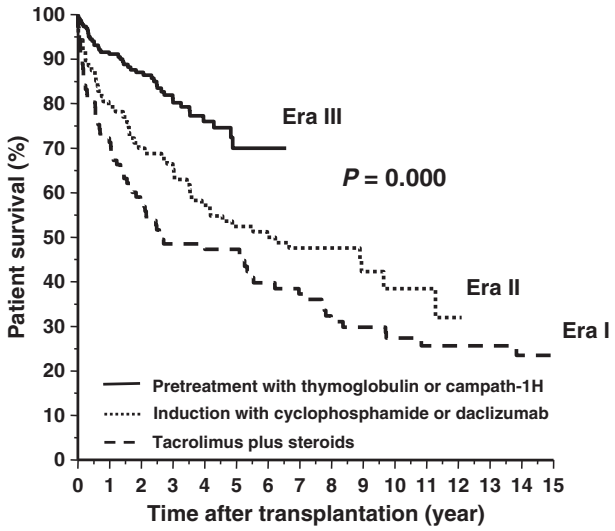

Figure 15 Kaplan Meier survival curves of the Pittsburgh intestinal and multivisceral recipients during the three different eras and according to the received immunosuppressive regimen. The survival of the Thymoglobulin or Campath-1H pretreated patients (era III) was significantly better than those who received induction therapy (era II) or tacrolimus plus steroids only treatment (era I). Note the continuous decline in survival among the era I and II recipients with long-term follow-up.

nephrolithiasis, frequent episodes of dehydration, and antibiotic drug nephrotoxicity. In addition, the patients in this population are at a higher risk of post-transplant tacrolimus nephrotoxicity particularly during the first three postoperative months with the need for a relatively high 12-h trough levels (10–15 ng/ml). In addition to the diabetogenicity of tacrolimus and steroids, some of the multivisceral patients could develop diabetes due to pancreatic allograft insufficiency.

Survival

Despite the initial increase in early survival during era II with utilization of cyclophosphamide or daclizumab induction therapy, the associated heavy maintenance immunosuppression slowly eroded long-term patient survival with rates approaching that observed with our early era I experience (Fig. 15). During era III, the tolerogenic immunosuppressive strategy have been associated with significant improvement in survival outcomes with a relatively low long-term attrition rate. In era II and III, the risk of patient death and graft loss because of rejection and/or opportunistic infections particularly PTLD were significantly reduced compared with era I. With Thymoglobulin or Campath-1H recipient pretreatment (era III),, patient survival was 91% at 1 year and 75% at 5 years with a functional graft survival rate of 86%, and 61%, respectively. These survival rates are comparable to other solid abdominal organs including the liver. The reported herein survival advantages should also be addressed in the milieu of other innovative surgical and management strategies that were discussed earlier and introduced at sequential and more commonly at overlapping periods. Nonetheless, it remains to be seen whether these achieved survival benefits will be further maintained beyond the first and second decade after transplantation.

Summary

Despite the high immunogenicity of the intestine because of the associated massive lymphoid tissue and possible role of the epithelial innate immunity, the field of visceral transplantation has recently evolved with long-term survival comparable with other solid abdominal organs. In addition to innovative surgical techniques and better postoperative management strategies including efficient prophylactic anti-microbial and anti-viral therapy, the introduction of novel immunosuppressive protocols, as described herein, has been the milestone in the establishment of better long-term allograft stability. Under Thymoglobulin or Campath-1H pretreatment and post-transplant tacrolimus monotherapy, the patient survival has reached 91% at 1 year and 75% at 5 years with functional graft survival of 86% and 61%, respectively. Achievement of a better net state of partial tolerance with successful minimization of long-term immunosuppression has been also possible with spaced doses of tacrolimus in more than half of the patients with attempted weaning. The minimization process, however, should be carefully monitored and considered only in patients with clinical, endoscopic, histopathologic, and immunologic evidences of sustained allograft stability. In addition, the lessons learned herein and the described histopathologic features characteristic of early and breakthrough rejection must be utilized to guide recipient management with similar protocols and any other future weaning strategies. Nonetheless, minimization of immunosuppression should be individualized and limited to a twice per week dosage in highly selected patients until more reliable immunologic tolerance assays are available to safely monitor further reduction or even discontinuation of therapy [55]. Furthermore, more innovative tolerogenic protocols may be required to optimize the success of weaning and possible achievement of complete allograft tolerance.

Acknowledgements

The authors would like to thank Mrs. Dolly Martin and Mrs. Darlene Koritsky for their assistance in preparing the article.

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