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REVIEW

The evolving role of alemtuzumab (Campath-1H) for immunosuppressive therapy in organ transplantation

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Summary

Alemtuzumab is a monoclonal anti-CD52 antibody, which has been used extensively off label in solid organ transplantation. Its primary use has been as an induction agent at the time of organ transplantation, although there is limited experience using it to treat steroid-resistant rejection. Prolonged lymphocyte depletion can be expected following alemtuzumab treatment even with one dose of 30 mg intravenously. The nature and kinetics of lymphocyte repopulation depend on the maintenance immunosuppression being administered. In comparison with Thymoglobulin, a polyclonal depleting antibody preparation, alemtuzumab offers significant practical benefits with lower cost, fewer side effects in administration, and no specific issues with i.v. access. The risks and benefits of depleting induction agents, such as alemtuzumab, are compared with nondepleting agents, such as anti-CD25 induction therapy. While the majority of experience in solid organ transplantation has been in kidney transplantation, there is more limited experience in liver, pancreas, islet, small bowel, and lung transplantation. We herein review some of the lessons learned from clinical experience to date in solid organ transplantation using alemtuzumab as an immunosuppressant.

Introduction

While initially used for the treatment of chronic lymphocytic leukemia, there is a growing body of literature describing the use of alemtuzumab (Campath-1H; Berlex, Montville, NJ, USA) as immunosuppressive agent for organ transplantation. The adaptation of this drug for transplantation is based on its profound lymphocyte-depleting effects. This review will briefly examine the history of the development of alemtuzumab, its mechanism of action, followed by a review of the available data describing the use of alemtuzumab in solid organ transplantation (Table 1) and possible future applications.

History

The first description of complement-fixing anti-CD52 antibodies was reported by Waldmann *et al.* in 1984 [1]. While the early isotypes (Campath-1M and Campath-1G) were found to be very efficient at lymphocyte depletion *in vitro* and were used for bone marrow depletion for cellular transplantation [1,2], they were noted to be quite immunogenic, which made *in vivo* clinical use more challenging. Early clinical trials in organ transplantation using these nonhumanized isotypes illustrated their potent lymphocyte-depleting effects. Unfortunately, their use was severely limited given the unacceptably high rates of viral complications. It should, however, be noted that these studies were performed in an era, where effective antiviral therapies were not yet available [3,4]. Subsequent observations noted that human IgG1 and IgG3 were most efficient at inducing complement-mediated cell lysis. Alemtuzumab is a humanized, rat IgG1κ monoclonal antibody directed against the CD52 cell surface antigen, which makes it more suitable for *in vivo* use. CD52 is a glycoprotein expressed on approximately 95% of peripheral blood lymphocytes, natural killer cells, monocytes, macrophages, and thymocytes; therefore, almost all

Table 1. Summary of Campath Studies.

Author (year)	Organ	Study design	*n*	Induction	Dose	Major endpoints	Results	Follow-up
Kirk (2003) [6]	Kidney	Prospective observational tolerance trial: induction without maintenance immunosuppression	7	Alemtuzumab	0.3 mg/kg × 3 OR 0.3 mg/kg × 4	Rejection Histology	100% Profound lymphocyte depletion. Monocyte predominant rejection.	1 year
Knechtle (2003) [10]	Kidney	Prospective observational: induction + rapamycin monotherapy	29	Alemtuzumab or alemtuzumab and Thymoglobulin	20 mg × 2 or 20 mg × 2 and 1.5 mg/kg	Pt survival Graft survival Rejection	100% 97% 28%	3–29 months
Ciancio (2004) [7]	Kidney	Prospective observational: alemtuzumab + maintenance minimization	44	Alemtuzumab	0.3 mg/kg × 2	Pt survival Graft survival Rejection Serious infection Steroid avoidance	100% 100% 9% 9% 86%	1–19 months
Knechtle (2004) [20]	Kidney	Retrospective review with historical control: alemtuzumab + low-dose steroids versus other induction + standard triple therapy	1241	Alemtuzumab versus CD25 antibody or Thymoglobulin or Other	30 mg × 2 versus variable vs. 1.5 mg/kg × >4 vs. Variable	Patient survival Graft survival Rejection Infection DGF	No difference Increased* Decreased* No difference No difference	1 year
Watson (2005) [12, 13, 16]	Kidney	Retrospective review with contemporaneous control: induction + cyclosporine monotherapy versus standard triple therapy	99	Alemtuzumab versus none/Thymoglobulin	20 mg × 2	Pt survival Graft survival Rejection	12% vs. 17% 21% vs. 26% 32% vs. 34%	5 years
Shapiro (2005) [11]	Kidney	Prospective observational with historical control: induction + maintenance minimization versus standard triple therapy	343	Alemtuzumab versus Thymoglobulin versus none	30 mg vs. 5 mg/kg vs. None	Patient survival Graft survival Graft function Rejection Spaced weaning	No difference No difference No difference Increased (Thymo)* 74% vs. 68% vs. None	12–39 months
Kaufman (2005) [21]	Kidney	Retrospective review with historical control: induction + steroid-free maintenance immunosuppression	278	Alemtuzumab versus Basiliximab	30 mg vs. NR	Patient survival Graft survival Rejection	96% vs. 99% 92% vs. 95% 15% vs. 14%	3 years
Ciancio (2005) [22]	Kidney	Randomized, prospective trial: comparison of induction agents with steroid-avoidance in alemtuzumab group	90	Alemtuzumab versus Thymoglobulin versus daclizumab	0.3 mg/kg × 2 vs. 0.1 mg/kg × 7 vs. 1 mg/kg × 5	Patient survival Graft survival Rejection Serious infection	100% vs. 92% vs. 88% 100% vs. 88% vs. 88% 18% vs. 18% vs. 19% 20% vs. 23% vs. 13%	1 year

Vathsala (2005) [23]	Kidney	Randomized, prospective, multicenter trial: alemtuzumab + low-dose cyclosporine versus standard triple therapy	30	Alemtuzumab versus none	20 mg × 2 vs. none	Patient survival Graft survival Rejection Cyclosporine levels	95% vs. 100% 85% vs. 100% 25% vs. 20% Decreased*	6 months
Kirk (2005) [24]	Kidney	Prospective observational tolerance trial: induction without maintenance immunosuppression	5	Alemtuzumab and deoxyspergualin	0.3 mg/kg × 4 and 4 mg/kg loading + 2.5 mg/kg/day × 13	Rejection Histology	100% Profound lymphocyte depletion. Monocyte predominant rejection	2 years
Gruessner (2005) [25]	SPK/PTA	Prospective observational with historical control: induction + alemtuzumab/MMF maintenance therapy versus induction + standard triple therapy	341	Alemtuzumab and Thymoglobulin versus Thymoglobulin	30 mg × 4 AND 1.25 mg/kg × 1 vs. NR	Patient survival Graft survival Rejection Serious infection Creatinine clearance	No difference No difference No difference No difference Increased	6 months
Kaufman (2006) [26]	SPK	Retrospective review with historical control: induction + steroid-free, tacrolimus/sirolimus-based maintenance therapy	88	Alemtuzumab versus Thymoglobulin	30 mg vs. 1 mg/kg × 6	Patient survival Kidney graft survival Pancreas graft survival Rejection Viral infections	91% vs. 92% 91% vs. 86% 92% vs. 97% 8% vs. 5% 10% vs. 40%*	22–67 months
Marcos (2004) [30]	Liver	Prospective observational with contemporaneous control: induction + tacrolimus maintenance versus tacrolimus + steroids/MMF	160	Alemtuzumab versus none	30 mg vs. none	Patient survival (HCV^-) Graft survival (HCV^-) Patient survival (HCV^+) Graft survival (HCV^+)	97% vs. 86% 90% vs. 79% 71% vs. 65% 70% vs. 54%	14–22 months
Tryphonopoulos (2005) [28,29]	Liver	Prospective, observational with contemporaneous control: induction + tacrolimus maintenance versus tacrolimus + steroids	127	Alemtuzumab versus none	0.3 mg/kg × 4 vs. none	Patient survival Graft survival Rejection	No difference No difference 51% vs. 65%*	1 year
Garcia (2004) [33]	Intestine/MV	Retrospective review: comparison of small bowel allograft biopsy results by induction agent	87	Alemtuzumab versus daclizumab	NR versus NR	Overall rejection rate Rejection severity (<6 weeks) Rejection severity (>6 weeks)	19% vs. 33% Lower* No difference	250 days
Kato (2005) [32]	Intestine/MV	Retrospective review with historical control: induction + variable maintenance therapy	108	Alemtuzumab versus daclizumab (late) versus daclizumab (early) versus none OR OKT3 OR cyclophosphamide	NR versus 2 mg/kg × 8 then 1 mg/kg × 6 versus same versus NR	Patient survival Rejection	41% vs. 83% vs. 54% vs. 44%* Variable by transplant type	0.5–108.5 months

Table 1 (contd)

Author (year)	Organ	Study design	*n*	Induction	Dose	Major endpoints	Results	Follow-up
Reams (1999) [34]	Lung	Case report: treatment of refractory rejection	1	N/A	3 mg/10 mg/30 mg/ 30 mg	Recurrent rejection	None	8 months
McCurry (2005) [35]	Lung/HL	Retrospective review with historical control: induction plus tacrolimus monotherapy	76	Alemtuzumab versus Thymoglobulin versus daclizumab	30 mg vs. 4–7 mg/kg vs. 1 mg/kg × 5	Pt survival FEV1 Rejection	90% vs. 97% vs. 89% 80% vs. 70% vs. NR 20% vs. 68% vs. 65%	6 months

DGF, delayed graft function; HCV, hepatitis C virus; HL, heart–lung; MMF, mycophenolate mofetil; MV, multivisceral; NR, not reported; PTA, pancreas transplant alone; SPK, simultaneous pancreas–kidney transplant.
*P-value < 0.05.

mononuclear cells are affected [5]. There does not appear to be any effect on plasma cells and similar to other induction agents, alemtuzumab appears to spare memory type cells [6]. CD52 is not present on granulocytes, platelets, erythrocytes, or hematopoietic stem cells. After binding to its target, alemtuzumab causes cell death through several mechanisms including complement-mediated cytolysis, antibody-mediated cytotoxicity, and apoptosis. While the plasma elimination half-life of alemtuzumab is approximately 12 days, its clinical effects are far more persistent. Lymphocyte depletion of >99% can be seen after a single dose with varying rates of cellular recovery depending upon the subpopulation of interest [7]. Additionally, alemtuzumab has significant depletional effects on lymph node lymphocytes although lymph node depletion takes 3–5 days compared with <1 h seen in peripheral lymphocytes [6]. To date, there has been no data published on the effects on splenic or thymic lymphocytes. The profound T-cell depletion is comparable with that seen using immunotoxin although cell recovery may be more protracted after alemtuzumab induction [8]. Monocyte and B-cell recovery can be seen at 3 and 12 months, respectively, while T-cell levels recover to only 50% of baseline at 36 months [9,10]. Although T-cell repopulation has been reported to take at least 3 months with rabbit antilymphocyte globulin (Thymoglobulin; Genzyme Inc., Cambridge, MA, USA) [11], a direct comparison of the kinetics cellular recovery with alemtuzumab has not been described to date. Alemtuzumab was approved by the Food and Drug Administration for the treatment of lymphoid malignancies in 1999 and has been increasingly studied off-label for use in organ transplantation.

Renal transplantation

Thus far, the most extensive experience with the use of alemtuzumab in solid organ transplantation has been in renal transplantation. The first reports of renal transplant recipients treated with alemtuzumab induction and cyclosporine maintenance therapy without azathioprine or steroids were described by Calne *et al.* [12,13]. They speculated that profound lymphocyte depletion could potentially induce the immune system into a tolerogenic state when encountering a newly transplanted graft, or at least develop a state of 'prope (almost) tolerance' with minimal requirements for further immunosuppressive therapy. Indeed, we and others have demonstrated that depletion of T cells with immunotoxin in the nonhuman primate model can induce a state of donor-specific tolerance [14,15]. In the initial series, 31 patients were given alemtuzumab induction therapy followed by one-half of the usual dose of cyclosporine and no other agents.

Short-term follow-up demonstrated an approximately 20% rejection rate with most patients maintaining graft function at 15–28 months on low-dose cyclosporine monotherapy. They recently reported their 5-year follow-up of this recipient group and noted no significant difference in rejection episodes, graft function or graft and patient survival [16]. Importantly, there were no differences in *de novo* malignancy or infection. They did, however, report more episodes of late rejection in the alemtuzumab treated group. The cause is speculative, but may be secondary to lower cyclosporine levels, which did not protect renal grafts from recovering lymphocytes or from other mechanisms of immunity which were not inhibited by maintenance immunosuppressive agents: mycophenolate and steroids. Interestingly, two episodes of autoimmune disease were noted in alemtuzumab-treated patients. Coles *et al.* [17] reported a 33% incidence of autoimmune thyroid disease in patients treated with five doses of alemtuzumab for multiple sclerosis. This phenomenon has not been described in other transplant studies and may therefore be specifically related to patients with multiple sclerosis or simply underreported. With regard to the induction of tolerance, these patients were no more tolerant to renal grafts than controls at 5 years. They did, however, demonstrate that alemtuzumab induction therapy could enable a steroid-free immunosuppressive regimen with low-dose cyclosporine with graft function equivalent to standard triple therapy with no untoward effects.

Ciancio *et al.* [7] at the University of Miami described a similar experience with alemtuzumab induction therapy for renal transplantation. Their group of 44 patients undergoing primary renal transplant received alemtuzumab induction followed by a steroid-free protocol including low-dose tacrolimus and low-dose mycophenolate. Preliminary short-term data suggested similar rates of acute rejection as well as graft and patient survival compared with earlier protocols. Importantly, this once again demonstrated the ability to implement a regimen with lower exposure to immunosuppressive agents without deleterious effects.

Based upon the encouraging early data demonstrating good outcomes of renal transplants using depletional therapy with alemtuzumab and low-dose calcineurin inhibitors, our group sought to evaluate the efficacy of alemtuzumab induction therapy in conjunction with rapamycin (sirolimus, Rapamune; Wyeth Inc, Madison, NJ, USA) monotherapy [10]. It was hypothesized that this immunosuppressive protocol could be a means to completely avoid the toxicity associated with calcineurin inhibitors and possibly improve long-term graft function. Results of a pilot study noted an unacceptably high rate of acute humoral rejection (17%) when compared with the estimated rates of 10% using traditional, triple immunosuppressive therapy [18,19]. This phenomenon was particularly observed in younger (<45 years) recipients. While similar rates of transplant and graft survival were noted compared with patients undergoing standard immunosuppressive therapy, the combination of alemtuzumab induction with rapamycin monotherapy appears to lack protection against humoral rejection. The suggestion is that calcineurin inhibitors may be a valuable adjunctive therapy in combination with alemtuzumab as this trend was not noted in earlier experiences utilizing low-dose calcineurin inhibitors [13]. It is important to note that this study once again implied that alemtuzumab induction therapy may allow lower doses of other immunosuppressive agents without compromise of long-term graft and patient survival.

It becomes clear that alemtuzumab may be an important induction agent in organ transplantation, but the optimal combination of immunosuppressive therapy remains to be determined. At the University of Wisconsin, a subsequent immunosuppressive protocol was then adopted consisting of two doses of alemtuzumab at the time of renal transplant in combination with low-dose steroids (10 mg methyprednisolone per day), mycophenolate mofetil (1000 mg b.i.d.: MMF, CellCept; Roche, Nutley, NJ, USA), and either tacrolimus (Prograf; Fujisawa, Deerfield, IL, USA) or cyclosporine (Neoral; Novartis, East Hanover, NJ, USA) [20]. Patients enrolled in this protocol were compared with historical controls that received induction therapy with anti-CD25 antibody, Thymoglobulin or OKT3 (Muromonab-CD3; Ortho Biotech, Bridgewater, NJ, USA) followed by maintenance immunosuppression with a calcineurin inhibitor, mycophenolate mofetil, and steroids. It was noted that the alemtuzumab-treated group experienced less acute rejection, particularly in those patients experiencing delayed graft function (DGF). It appears that recipients are 'protected' from rejection during the DGF period while calcineurin inhibitors are typically with-held to prevent further renal insult. Interestingly, a significantly greater number of patients in the alemtuzumab treated group were retransplants which are generally considered to be higher risk patients compared with those receiving a primary transplant. While overall actuarial renal allograft survival was greater in the alemtuzumab group, the follow-up period was only approximately 1 year. It was also noted that there was no increased risk of infection or *de novo* malignancy during the follow-up period.

Two recently published studies sought to directly compare the effects of induction agent on outcome when compared with historical controls [11,21]. Shapiro *et al.* at the University of Pittsburgh compared alemtuzumab induction therapy with Thymoglobulin. Both groups received steroid-free maintenance immunosuppressive

therapy and low-dose tacrolimus [11]. These groups were then compared with historical controls receiving standard maintenance therapy with tacrolimus, steroids and mycophenolate, or sirolimus. Additionally, depleted patients underwent a 'spaced weaning' protocol to further decrease the exposure to calcineurin inhibitors beginning 3–4 months post-transplant. While there were more episodes of acute cellular rejection in the Thymoglobulin induction group, the patient and graft survival rate was equivalent to the other two cohorts at 24–40 months. Similar to the University of Wisconsin results, the average time to rejection in the alemtuzumab depleted group was later than comparison groups. They did note that late rejection episodes (>6 months) in both depletional groups were increased, likely secondary to the aggressive calcineurin inhibitor weaning protocols. Despite this fact, they were still able to further reduce tacrolimus doses in 74% of alemtuzumab-treated patients at 12–18 months. While all three regimens had good short-term outcomes, all patients receiving depletional therapy were able to be maintained on low-dose tacrolimus monotherapy, and alemtuzumab-depleted patients had overall decreased rates of rejection. Kaufmann *et al.* [21] examined the long-term outcomes of kidney recipients in a nonrandomized, retrospective study comparing the use of alemtuzumab versus anti-CD25 for induction. Both groups received low-dose tacrolimus and mycophenolate for maintenance therapy. They noted no difference in long-term patient or graft survival at a minimum of 30 months follow-up. Additionally, they observed a decreased rate of early (<3 months) rejection episodes in the alemtuzumab group, similar to other reports. However, by 1 year, there was no difference between the two groups.

To date, there are only two published, randomized trials involving the use of alemtuzumab induction in renal transplantation. At the University of Miami, 90 patients were randomized to received induction with either Thymoglobulin, alemtuzumab, or anti-CD25 antibody [22]. Additionally, they also examined the effect of differing maintenance regimens whereby the Thymoglobulin and anti-CD25-treated groups received triple therapy with tacrolimus, mycophenolate and steroids while the alemtuzumab treated group received lower-dose tacrolimus and mycophenolate in a steroid-free regimen. At a median follow up of 15 months, they demonstrated no differences in graft function, rejection episodes, infection or patient, and graft survival. They were, however, able to achieve these results with less overall exposure to maintenance immunosuppression in the alemtuzumab group. In an Asian randomized, prospective, multi-center trial, 30 patients were randomized to receive either triple therapy with cyclosporine, azathioprine and steroids, or alemtuzumab induction with low-dose cyclosporine monotherapy alone [23]. In the induction group, cyclosporine trough levels were significantly lower than the triple therapy group. At 6 months post-transplant, there were no differences in rates of rejection or patient and graft survival. However, of the remaining 17 patient in the induction group with functioning grafts, 15 remained steroid-free. While these studies are encouraging regarding the use of alemtuzumab with minimization of maintenance immunosuppressive therapy, larger randomized studies with longer follow-up are needed to determine the optimal drug regimens and dosing protocols to maximize long-term outcomes.

When reviewing the rejection episodes seen under alemtuzumab induction, some interesting histological findings are noted. Calne *et al.* [13] originally observed an acellular, 'vascular' type rejection in a single patient while Kirk *et al.* [6] noted a marked macrophage infiltration in patients with early rejection who received alemtuzumab induction without maintenance immunosuppressive therapy. Indeed, several authors have now reported that a number of these rejection episodes demonstrate positive staining for C4d, indicative of acute humoral rejection [7,10,11,24]. It is unclear if the rates of $C4d^+$ rejection are increased with alemtuzumab induction as this technique has only been employed for a relatively short period of time. Ciancio *et al.* observed that the incidence of $C4d^+$ rejection was no different between patients undergoing induction with alemtuzumab or with other agents [22]. While it may be that depletion with alemtuzumab causes dysregulation of B-cell function with a resultant increased rate of acute humoral rejection, this hypothesis clearly requires further testing. It is possible that we are only observing this phenomenon now because we have the appropriate tools.

Important economic implications were noted from the University of Wisconsin and the Northwestern experiences. At the University of Wisconsin, there were overall decreased financial expenditures for patients receiving alemtuzumab therapy, particularly those experiencing DGF [20]. Prior to the institution of our alemtuzumab induction protocol, patients induced with either anti-CD25 antibody or Thymoglobulin who experienced DGF typically underwent routine biopsy on post-transplant day 7 to assess for underlying rejection. Those with histological evidence of rejection then underwent multiple infusions of high-dose steroids and antilymphocyte therapy. After switching to alemtuzumab induction, we noted a decrease in early rejection episodes from 40% from 45% with earlier protocols to 12% in patients with DGF. Given the markedly decreased early rejection rates in this subgroup, the practice of routine allograft biopsies has been abandoned and a decreased need for further antibody

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infusions was noted. The Northwestern group raised an important point about the economics of early versus late rejection episodes [21]. The need for biopsy and rejection therapy within the first 30–90 days post-transplant must be financially covered by the negotiated case rate for the initial transplant episode and therefore may have a significant negative financial impact on the transplant center. This fact must be considered as long as the timing of the rejection episode does not have a major impact on patient or graft well-being.

Solitary pancreas, simultaneous pancreas–kidney, and islet transplantation

While, there are no randomized, controlled trials evaluating the use of alemtuzumab in solitary pancreas, simultaneous pancreas–kidney or islet transplant patients, there are several retrospective reviews currently published. Gruessner *et al.* [25] at the University of Minnesota reported a novel approach to pancreas transplantation by incorporating aggressive lymphocyte depletion with four doses of alemtuzumab along with a single dose of Thymoglobulin to deplete $CD52^{-}$ immunocompetent lymphocytes. Additionally, alemtuzumab was used for maintenance therapy when absolute lymphocyte counts rose above $200/mm^3$ as well as for the treatment of rejection, along with mycophenolate dosing to keep absolute neutrophil counts below $1500/mm^3$. The purpose of this prospective, nonrandomized study was to attempt to reduce the exposure to calcineurin inhibitors and steroids and to avoid their adverse side effects. While, at 6 months follow-up, there was no difference seen in pancreatic graft survival or graft loss, there did appear to be a trend toward decreased pancreas graft survival and increased graft loss in the highest risk pancreas transplant alone group, when compared with historical controls. Also, noted was an increase in the rate of reversible rejection episodes in the simultaneous pancreas–kidney transplant group, several of whom required conversion to calcineurin inhibitors. While a combination such as this may not be suitable for patients receiving isolated pancreas grafts, it is encouraging to note that at 6 months, there was a trend toward increased glomerular filtration rates and decreased serum creatinine levels in all patients avoiding calcineurin inhibitors, thereby validating the soundness of concept. We are eagerly awaiting long-term follow-up of this and an ongoing randomized trial.

Most recently Kaufman *et al.* [26] published a study reporting the use of alemtuzumab along with a steroid-free protocol in simultaneous pancreas–kidney patients. In their nonrandomized, retrospective study, they compared Thymoglobulin with alemtuzumab induction, followed by a maintenance protocol consisting of mycophenolate, tacrolimus, and sirolimus. They observed no difference in 1 year actual or 3-year actuarial patient, pancreas graft or renal graft survival or function. Additionally, there was no difference in the 12-month rejection rate. While the overall rate of cytomegalovirus (CMV) infection appears to be lower using a steroid free protocol when compared with rates reported in the literature [27], a significantly higher incidence of viral complications (CMV, BK nephropathy, herpes, and parvovirus) was noted in the Thymoglobulin-treated group. There was a trend toward an increased rate of CMV infection in particular in the Thymoglobulin group, specifically in high-risk patients. This is likely because of the longer follow-up interval in this group as many of the infections appeared to occur at greater than 2-year post-transplant. As with renal transplantation, a significant cost benefit was realized in the alemtuzumab group (with a course of Thymoglobulin induction costing >400% more than alemtuzumab) with no apparent adverse effect on overall outcome.

To date, there are no published studies on the use of alemtuzumab in pancreatic islet cell transplant recipients. Early data presented by the Edmonton group did suggest that lymphocyte depletion with alemtuzumab may yield results equivalent to patients undergoing the original Edmonton protocol (Shapiro *et al.* [11], IPITA presentation 2003). At the University of Wisconsin, a very small experience has been accumulated thus far with alemtuzumab induction for islet transplantation with excellent results (personal communication); however, long-term outcomes await further study.

Liver, intestine, and multivisceral transplantation

While induction therapy is not typically used in the setting of liver transplantation, the prospect of minimization of maintenance immunosuppressive agents makes this approach more attractive. In 2004, Tzakis *et al.* [28] at the University of Miami published their experience with alemtuzumab induction in liver transplant recipients. In their nonrandomized, prospective study, they compared a protocol of alemtuzumab induction followed by low-dose tacrolimus with standard therapy consisting of tacrolimus and steroids without induction. It is important to note that patients with hepatitis C virus or recipients of multi-organ transplants were excluded. Their data suggested no difference in patient or graft survival at 1 year. While there were significantly fewer early (<2 months) rejection episodes with alemtuzumab, the trend did not achieve statistical significance at 1 year. They were, however, able to surmize that results equivalent to standard therapy could be achieved with significantly less steroid use and significantly lower tacrolimus doses and levels. The lower

maintenance tacrolimus levels translated into lower serum creatinine levels in the induction group. In a more recent update of their data including a total of 77 alemtuzumab-treated patients, the Miami group was able to observe a statistically significant decrease in acute rejection episodes at 1 year [29]. Once again, there was a longer interval to the first rejection episode in the treated group. They did report an interesting observation that patients undergoing induction therapy had a significantly greater amount of intraoperative blood product use. While this may be a spurious occurrence as there are no other reports of bleeding diathesis in organ transplant patients receiving alemtuzumab, it warrants further investigation.

A similar study utilizing alemtuzumab induction with low-dose tacrolimus therapy was conducted at the University of Pittsburg [30]. In their study, patients were compared with those who received no lymphocyte depletion followed by standard maintenance therapy. Similar to observations by the Miami group, there were no differences in rejection or patient and graft survival at 1 year. It is important to note that in this study, patients with hepatitis C were not excluded from participation and these patients did significantly worse than hepatitis C-negative patients both in the induction and noninduction groups. However, in the depletional group, viral replication was frequently associated with alemtuzumab infusion. They noted a marked increase in viral load in the 2 months following depletional therapy. It therefore stands to reason that lymphocyte depletion may be permissive for unchecked viral replication and may lead to an earlier and more aggressive recurrence of disease.

Acute allograft rejection is one of the most serious complications of intestinal and multivisceral transplantation and severe rejection has been correlated with a particularly poor prognosis [31]. This fact generated significant interest in the role of lymphocyte depletion for intestinal transplantation. A recent retrospective analysis by Kato *et al.* [32] evaluated outcomes of 124 pediatric intestinal transplants depending upon the type of induction agent received (none, OKT3 or cyclophosphamide, daclizumab, or alemtuzumab). Preliminary results suggested a decrease in the incidence of acute allograft rejection with alemtuzumab induction, particularly in the first 2 months after transplant [33]. Unfortunately, this did not translate into improved survival rates. Conversely, longer-term follow-up demonstrated a significantly higher rate of death because of nonrejection causes such as infectious complications. Based on these data, alemtuzumab appears to be less well tolerated in children undergoing intestinal transplantation when compared with other induction agents. The role of alemtuzumab induction in pediatric intestinal transplantation has yet to be defined.

Thoracic organ transplantation

There is very little data published regarding the use of alemtuzumab in thoracic organ transplantation. The first single case report was described by Reams *et al.* in 1999 [34]. They reported a patient who underwent lung transplantation with persistent cellular rejection despite treatment with high-dose steroids, Thymoglobulin, and i.v. IgG. Reversal of the refractory rejection was only seen after treatment with a 4-day course of alemtuzumab. After 6 months alemtuzumab therapy, the patient remained free from rejection with preservation of graft function, no evidence of rejection by biopsy and persistent lymphocyte depletion at 8 months post-therapy demonstrating a possible role for alemtuzumab in the treatment of lung allograft rejection.

The first series of patients reported utilizing alemtuzumab for induction therapy prior to lung transplantation was reported by McCurry *et al.* at the University of Pittsburgh [35]. In a retrospective analysis, patients who received alemtuzumab induction were compared with those who received Thymoglobulin induction followed by tacrolimus monotherapy or 'near monotherapy'. These two groups were then compared with historical controls receiving daclizumab with standard triple therapy. In this small study, they noted significantly fewer acute rejection episodes in the alemtuzumab group compared with the other two groups. It was also encouraging to note that the early rejections in the alemtuzumab group appeared to be less severe and there was a trend toward decreased rates of CMV infection. Overall, there were no differences in patient or graft survival at 6 months, pulmonary function tests, or infectious complications. It is unclear how lymphoid repopulation will manifest itself in the long term, particularly in light of minimization of calcineurin inhibitors. Additionally, it is unknown if it will be possible to continue with low-dose tacrolimus monotherapy without an unacceptably high risk of late rejection episodes. There is clearly a need for long-term randomized trials in this area of transplantation.

Conclusions and future directions

Profound lymphocyte depletion with alemtuzumab is a reasonable induction strategy in renal transplantation with short- and long-term graft and patient survival equivalent to that seen with other induction agents. The optimal dosing and ideal combinations of maintenance immunosuppressive strategies utilizing alemtuzumab have yet to be determined and are the subjects of ongoing studies. Based on the available data, it is difficult if not impossible to tease out whether there is a real difference in potency between lymphocyte depleting agents or if this

is simply related to a dose effect (Table 1). To date, there have not been any side-by-side comparisons examining the relative potencies of various lymphocyte depleting agents and equivalent dosing regimens have not been defined. One common theme noted with alemtuzumab depletion is a longer-time interval to the first rejection episode. It is possible that this is due to the fact that most studies implemented maintenance minimization strategies along with induction that were unable to inhibit recovering lymphocytes. Perhaps, we should change our paradigm of late minimization of immunosuppression or incorporate additional maintenance immunosuppressive therapy for higher-risk patients to prevent late rejection episodes.

Although alemtuzumab appears to be safe in the long term, it should be noted that there are only a few studies in transplant recipients with follow-up approaching 5 years and beyond. Although depletional therapy may be associated with an increased risk of infection, there are no reports of increased rates of malignancy to date. At the University of Wisconsin, we now have patients who are more than 7 years post-transplant with no obvious increase in adverse events.

There may be a possible niche for alemtuzumab as an induction agent in high-risk kidneys or as an adjunct when DGF is present. This stems from data that perceived a possible protective effect of alemtuzumab in DGF kidney transplantation with a decreased rate of early acute rejection and possible decreased need for surveillance biopsies during the period of recovery while unprotected with calcineurin inhibitors. It is encouraging that long-term follow-up of patients receiving alemtuzumab does not seem to demonstrate an increased risk of infection or malignancy, despite the profound and durable lymphocyte depletion seen.

We have previously proposed a potential role for alemtuzumab in children [36]. Children represent a particular challenge in transplantation, given the need for extremely long-term graft function and the potential compliance issues that arise, particularly in the teen years. Alemtuzumab represents an attractive option as emerging data may suggest a decrease in infectious complications and the possible need for a reduced level of maintenance immunosuppression.

The use of alemtuzumab as a primary treatment for allograft rejection remains a topic that has not been intensively studied. A single report from the University of Pittsburgh observed acceptable outcomes of the treatment of steroid resistant rejection or Banff 1B or greater rejection in renal transplant recipients [37]. While there are case reports demonstrating effectiveness in the treatment of rejection in several organ transplant types, there have been no randomized trials designed to specifically evaluate this modality of therapy. There may be a particular niche for alemtuzumab therapy in the treatment of acute cellular rejection; however, that will require further study in a randomized-controlled trial.

With regard to tolerance induction, alemtuzumab alone has not demonstrated the ability to induce stable tolerance in solid organ transplantation [6] even when combined with other agents such as deoxyspergualin [24]. It is indeed possible that in combination with other agents or by employing depletion strategies at other time points before or after transplantation, we may come closer to the goal of graft tolerance. This is currently being investigated at our center in 10 patients as a pilot trial supported by the Immune Tolerance Network.

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