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ORIGINAL ARTICLE

Outcomes at 3 years of a prospective pilot study of Campath-1H and sirolimus immunosuppression for renal transplantation

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Summary

Campath-1H (alemtuzumab) induction was used for renal transplantation in combination with sirolimus as immunosuppression. We previously reported a high (28%) rate of early rejection with this regimen, and now report 3-year outcomes. Twenty-nine patients were recipients of either deceased donor or non-HLA (Human Leukocyte Antigen) identical living donor primary renal allografts. Clinical parameters including infection, malignancy, kidney function, and kidney histology were followed prospectively for 3 years. Three-year cumulative graft and patient survival were 96% and 100%, respectively. Twenty patients were maintained on steroid-free immunosuppressive regimens, and 15 patients were maintained on monotherapy for immunosuppression (12 on sirolimus). No serious infectious complications were observed and two patients developed basal cell skin cancer. The 3-year results of our initial pilot study demonstrate good graft (96%) and patient (100%) outcomes. Campath-1H induction has yielded a high proportion of patients maintained on immunosuppressive monotherapy (57%) without serious infectious- and no malignancy-related complications. The reported regimen yielded novel insights into both Campath-1H and sirolimus therapy in renal transplantation. Because of the higher incidence of early rejection, we recommend a modified strategy of immunosuppression including a brief course of a calcineurin inhibitor.

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Introduction

Lymphocyte depletion strategies attempt to provide improved graft survival, reduce maintenance immunosuppression requirements, and may be useful as a component of tolerance protocols. Campath-1H (alemtuzumab) depletes CD52+ cells from the periphery and lymph nodes of recipients with excellent efficiency and variable duration. The CD52 marker is expressed on T- , B- , NK cells, monocytes, and macrophages. The depletion of each of these subsets is variable, with T cells being the most completely and durably depleted. Cells are eliminated from the circulation via complement and antibody-mediated cell cytotoxicity after binding of the humanized anti-CD52 monoclonal antibody Campath-1H [1].

The short-term effects of Campath-1H therapy on early rejection rates and mechanisms of rejection have been reported by groups attempting the tolerance strategies, calcineurin/steroid avoidance, and monotherapy protocols. The summary of these results suggests that Campath-1H therapy alone will not induce a tolerant state, that monotherapy and drug minimization strategies will succeed in a, albeit slight, majority of patients, and

humoral rejection episodes may be more prevalent with some of these strategies [2–5].

The University of Wisconsin conducted a pilot trial of Campath-1H induction therapy for renal transplantation with enrollment concluding in 2002. Groups from the National Institutes of Health, University of Pittsburgh, and Cambridge University have reported on the other cohorts of patients under a variety of maintenance immunosuppressive protocols [3,4,6]. This 3-year follow-up report demonstrates good patient and graft survival of an initial group of 29 patients who received Campath-1H induction therapy for renal transplantation with relatively minimal maintenance immunosuppressive drug therapy. Our findings are comparable with those reported by Kaufman *et al.* [7,8].

Patients and methods

Patient selection

Twenty-nine patients were enrolled at the University of Wisconsin-Madison in an IRB-approved protocol after obtaining informed consent (Table 1). The maximum length of follow-up under the protocol was 3 years. FDA surveillance was also provided through an Investigational New Drug Application (IND) to Stuart J. Knechtle (S.J.K.) for off-label use of Campath-1H. Patients received primary renal transplants from deceased or living donors and had current panel reactive antibody (PRA) < 10%, peak PRA < 25%, and a negative National Institutes of Health (NIH) T-cell crossmatch to donors. Kidneys from 0-mismatch donors, extended criteria donors [United Network for Organ Sharing (UNOS) criteria], and donors after cardiac death were excluded. Kidney biopsies were performed at 6 and 12 months per protocol and as indicated clinically throughout later time points. Through years 2 and 3, blood specimens were analyzed monthly for creatinine, hemoglobin, platelets, and white blood cell (WBC) counts with differential. Flow cytometry provided data on lymphocyte subsets that were performed every 6 months.

Table 1. Patient demographics and HLA matching of 29 patients entered into study.

Age (years)	Mean 41, range 19–60
Sex (M:F)	19:10
Dialysis pretransplant	
Yes	16
No	13
Type of donor	
CAD	6
LRD	16
LURD	7
PRA level	
0	27
3%	1
8%	1
Cause of renal failure	
Diabetes mellitus	5
Polycystic kidney disease	5
Focal glomerulosclerosis	4
Hypertension	3
Glomerulonephritis	2
Congenital	2
IgA nephropathy	2
Hereditary	2
Reflux	2
Interstitial nephritis	1
Unknown	1
HLA mismatch	
0	0
1	1
2	5
3	12
4	1
5	6
6	4

CAD, deceased donor; LRD, living related donor; LURD, living unrelated donor; PRA, panel reactive antibody; HLA, human leukocyte antigen.

Immunosuppression

Patients received Campath-1H (Millennium Pharmaceuticals, Cambridge, MA, USA and Ilex, Inc., San Antonio, TX, USA) 20 mg i.v. at the time of transplant and on postoperative day 1. Methylprednisolone 500 mg i.v. was administered at the time of surgery, and sirolimus (Wyeth, Philadelphia, PA, USA) was started on day 1 at 2 mg b.i.d. to achieve target levels of 8–12 ng/ml. Patients 26–29 received additional induction therapy with Thymoglobulin (SangStat, Menlo Park, CA, USA) 1.5 mg/kg on day 1 and tapered steroids over 2 weeks. Patients had steroids tapered off by 2 weeks, at which point they were on sirolimus monotherapy. With episodes of cellular rejection, patients were treated with steroid boluses plus modification of therapy including discontinuation of sirolimus, and possible addition of tacrolimus, mycophenolate mofetil (MMF), and steroids. With episodes of humoral rejection, patients were treated with plasmapheresis, cytomegalovirus (CMV) immunoglobulin (CytoGam; MedImmune, Gaithersburg, MD, USA), rituximab (Rituxan; Genentech, San Francisco, CA, USA and Biogen Idec, Cambridge, MA, USA), Thymoglobulin, and modification of sirolimus maintenance therapy as above.

Pathology

Biopsies were performed in patients with evidence of renal allograft dysfunction, as indicated by a rise in serum

creatinine >20% over baseline, and at 6 and 12 months post-transplant. Sections were stained with hematoxylin and eosin and immunostaining for C4d was performed as previously described [5]. Rejection was diagnosed on biopsy according to Banff '97 criteria.

Postoperative monitoring

All patients were monitored for leukocyte subset recovery by flow cytometry at frequent intervals through the first 6 months and at yearly intervals thereafter. Serum creatinine was followed at monthly intervals after the first post-transplant year. Prophylaxis for CMV consisted of i.v. ganciclovir during the transplant hospitalization and oral acyclovir or ganciclovir for 3 months post-transplant. Trimethoprim-sulfamethoxazole was administered for 12 months as prophylaxis for *Pneumocystis carinii*. Patients were evaluated at regular intervals at the University of Wisconsin Hospital and Clinics.

Statistical methods

Estimates of graft survival, patient survival, and rejection rates at 3 years post-transplant were based on all patients that were followed for at least 3 years. The reported serial cell depletion percentages were based on the average changes in cell counts from the time of transplantation. The 3-year serum creatinine and urine protein levels were compared between a group of subjects who had experienced rejection prior to the 3-year period and a group of subjects who had not experienced rejection at that time using a two-sample *t*-test. *P*-values <0.05 were considered as significant.

Results

Three-year patient and graft survival are 100% and 96%, respectively, based on 28 of the original 29 patients transplanted; one patient had been lost to follow-up during the first year.

Lymphocyte depletion and reconstitution

Campath-1H provided for near-complete depletion of peripherally detected T- and B lymphocytes. Patients with episodes of rejection also received therapy that could include Thymoglobulin and rituximab. CD3-positive T cells demonstrated 99.4% peripheral depletion at 2 weeks post-transplant, 77% peripheral depletion at 1 year, 58% at 2 years, and 52% at 3 years (Fig. 1a). CD4-positive T cells were peripherally depleted by 99.7% 2 weeks post-transplant, 85% depletion at 1 year, 69% depletion at 2 years, and maintained 63% peripheral depletion at 3 years (Fig. 1b). CD20-positive B cells were peripherally depleted by 99.2% 2 weeks post-transplant, but demonstrated only 54% peripheral depletion at 6 months with reconstitution to baseline values by 2 years (Fig. 1c). WBC were minimally affected by Campath-1H therapy with only one patient demonstrating lymphopenia (WBC of 1.6) at 2 weeks post-transplant, and an average WBC of 4.8 among all patients at this same time point.

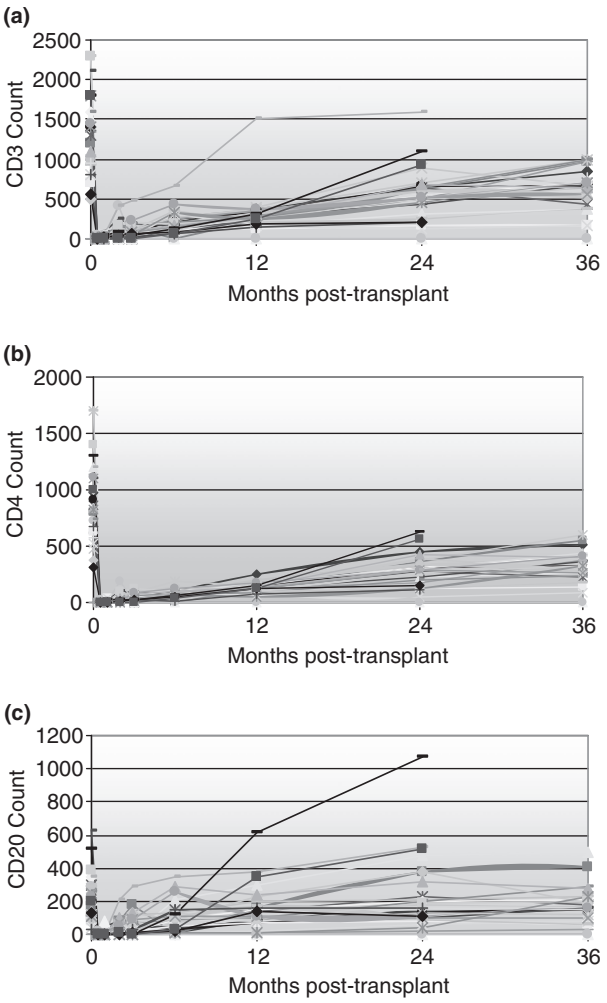

Figure 1 Depletion and reconstitution of lymphocyte subsets after Campath induction therapy. (a) Peripheral blood CD3 counts demonstrated 99.4% depletion and were reconstituted to nearly 50% baseline at 3 years. (b) Peripheral blood CD4 counts demonstrated 99.7% depletion and reconstitution to 37% baseline at 3 years. (c) Peripheral blood CD20 counts demonstrated 99.2% depletion with nearly 50% reconstitution at 6 months.

Rejection episodes

Thirteen patients (46%) had experienced an episode of cellular or humoral rejection 3 years post-transplant.

Table 2. Rejection episodes and treatment. Thirteen of 28 patients had episodes of rejection in 3-year study period, seven patients had components of humoral rejection, and one patient lost their graft secondary to rejection. The timing, biopsy results, and treatment therapy are described.

Patient	Date	Indication	Biopsy findings	Treatment of rejection
1	POD 1010	Increased creatinine	Acute renal allograft rejection (Banff 1B)	Steroids, MMF
7*	POD 6	Increased creatinine	Acute humoral rejection	Re-biopsy
	POD 8	Increased creatinine	Thrombotic microangiopathy versus acute humoral rejection	Rituximab, plasmapheresis, Thymoglobulin × 1 day, steroids
	POD 112	Increased creatinine	Moderate acute rejection (Banff 2A), acute and chronic glomerulopathy	Steroids, increased MMF
8*	POD 8	Increased creatinine	Mild acute rejection (Banff 1A), acute tubular injury	Steroids, MMF
	POD 372	Month 12 protocol	Positive staining for anti-C4d antibody	CMV IgG
13	POD 21	Increased creatinine	Mild acute tubular injury	
	POD 24	Increased creatinine	Moderate acute tubular injury	Steroids, MMF
	POD 182	Month 6 protocol	Minimal acute rejection (Banff suspicious)	No treatment
14*	POD 29	Proteinuria (3G)	Negative for acute cellular rejection	
	POD 34	Increased creatinine	Acute tubular injury	Steroids, start MMF
	POD 147	Increased creatinine	Mild acute rejection (Banff 1A), peritubular capillary C4d deposition	Steroids, start tacrolimus, plasmapheresis
	POD 183	Month 6 protocol	Mild acute rejection (Banff 1A), C4d positive	Steroids, Thymoglobulin, CMV IgG, plasmapheresis
	POD 365	Month 12 protocol	Mild acute rejection (Banff 1A)	No treatment
	POD 456	Increased creatinine	Cellular rejection (Banff 1A), positive C4d stain	Steroids
15*	POD 11	Increased creatinine	Acute antibody-mediated rejection	Steroids, rituximab, plasmapheresis, i.v. MMF
	POD 35	Nephrectomy	Severe cellular and antibody mediated rejection	
19	POD 565	Increased creatinine	Acute cellular rejection (Banff 1A), grade 1 chronic allograft nephropathy	Steroids
20	POD 275	Increased creatinine	Borderline, mild chronic transplant glomerulopathy	Steroids, tacrolimus
23*	POD 17	Increased creatinine	Acute rejection (Banff 1A)	MMF, steroids, CMV IgG, plasmapheresis
	POD 30	Increased creatinine	Positive C4d staining consistent with humoral rejection	Steroids, rituximab, Thymoglobulin, CMV IgG, plasmapheresis
	POD 184	Month 6 protocol	Mild acute cellular rejection (Banff 1A)	Steroids, CMV IgG
	POD 359	Month 12 protocol	Acute cellular rejection (Banff 1B)	Steroids, CMV IgG × 1 dose, tacrolimus
24	POD 118	Increased creatinine	Acute renal allograft rejection (Banff 1B)	Steroid taper
	POD 169	Month 6 protocol	Acute renal allograft rejection (Banff 1A)	Steroids 250 mg i.v.
27	POD 31	Increased creatinine	Acute cellular rejection (Banff 1B), negative C4d staining	Steroids, Thymoglobulin, plasmapheresis, MMF, tacrolimus
	POD 629	Increased creatinine	Acute cellular rejection (Banff 1B), negative C4d staining	Steroids
28*	POD 26	Increased creatinine	Acute cellular rejection (Banff 2A), positive C4d staining	Steroids, rituximab, Thymoglobulin, plasmapheresis, MMF, tacrolimus
29*	POD 39	Increased creatinine	Positive C4d staining	Steroids, MMF, tacrolimus

POD, postoperative day; MMF, mycophenolate mofetil; CMV, cytomegalovirus.
*Humoral rejection component to rejection.

Seven patients experienced humoral rejection demonstrated by C4d positivity on immunohistochemistry or evidence of thrombotic microangiopathy on routine hematoxylin and eosin histologic analysis. There was a median time of 1 month to the first humoral rejection episode. These patients were treated with regimens that included combinations of CMV immunoglobulin, plasmapheresis, Thymoglobulin, rituximab, steroid boluses, MMF, and tacrolimus (Table 2). The one kidney that was lost to rejection experienced an early (day 11) episode of humoral rejection, and was removed on postoperative day 35 after failing to respond to therapy of rituximab, plasmapheresis, and MMF. Final pathology demonstrated widespread cortical necrosis and hemorrhage secondary to severe cellular and probable antibody-mediated rejection.

Twelve of the 13 patients with rejection episodes experienced cellular rejection with a median time of 3.8 months to first rejection episode. Episodes were trea-

ted with steroid boluses, addition of MMF, and initiating calcineurin-inhibitor therapy. No isolated cellular rejection episode resulted in graft loss.

After the first post-transplant year, four patients had episodes of isolated cellular rejection, one patient had a humoral rejection episode, and one patient had a rejection episode with elements of both humoral and cellular rejection. Four of these six patients had previous rejection episodes during the first post-transplant year; only two of these patients experienced their first rejection episode after the first year and both of these were cellular rejection episodes.

Renal function

Twenty-seven of 28 patients had functioning renal allografts 3 years after transplantation. The median serum creatinine for all recipients at 3 years was 1.5 mg/dl (Fig. 2). The highest value of 5.0 mg/dl was observed in a patient without any rejection episodes, but with progressive renal failure associated with congestive heart failure, myocardial infarction and coronary bypass grafting requiring cardiopulmonary bypass. Patients who experienced a least one rejection episode (13 patients) demonstrated mean serum creatinine of 1.7 mg/dl at 3 years; this was not significantly different when compared with a median serum creatinine of 1.4 mg/dl among the 15 patients without any rejection episodes ($P = 0.4$), and was within 10% of prerejection values.

Spot urine protein was quantified in 19 patients at 3 years, with a median value of 290 mg/l (range 77–1656 mg/l). There was no significant difference ($P = 0.4$) between the median values of patients with rejection episodes (257 mg/l) and patients without rejection episodes (302 mg/l).

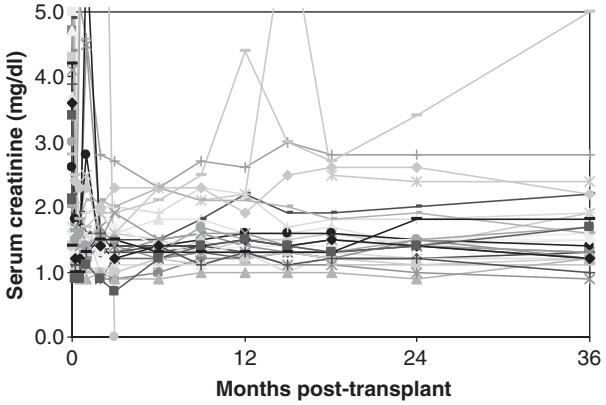

Figure 2 Serum creatinine values post-transplant. Median creatinine at 3 years was 1.5 mg/dl; one patient with renal failure associated with congestive heart failure after cardiac surgery had a serum creatinine of 5.0 mg/dl at 3 years.

Protocol biopsies

Protocol biopsies were performed in patients at 6 and 12 months post-transplant (Table 3). Patients without rejection episodes had nonspecific changes with only focal or minimal fibrosis at 6 and 12 months. Tubular atrophy and chronic glomerulopathy were not evident in these patients, most of whom remained on sirolimus monotherapy. Patients with rejection episodes had mild fibrosis and evidence of tubular atrophy or injury (5 of 8). These patients had immunosuppressive regimens that often included calcineurin inhibitors and/or steroids.

Immunosuppressive regimens at 3 years

Fifteen of 27 patients (57%) with functioning renal allografts at 3 years were on a single immunosuppressive drug. Thirteen of these patients were on the original sirolimus monotherapy that had been started immediately post-transplant. The mean level of sirolimus was 7 ng/ml. Two additional patients were on tacrolimus and prednisone monotherapy.

Eighteen patients (67%) were on steroid-free pharmacologic regimens at 3 years. Fourteen of these patients were on monotherapy, as mentioned above. Four additional patients were on a combination of MMF with either sirolimus or tacrolimus.

Eighteen patients were on immunosuppressive regimens that included sirolimus. Five patients had sirolimus discontinued secondary to an early rejection episode, three patients had sirolimus discontinued because of a wound healing complication, and one patient had sirolimus discontinued secondary to pneumonitis.

Infectious, malignant, and other complications

Over the 3-year study period, the most common infectious complications were urinary tract infections in 10 patients, pneumonia in five patients, herpes simplex virus I in five patients, herpes zoster in three patients, wound infections in three patients, and intra-abdominal abscess in two patients. None of the infectious complications resulted in graft loss or patient death. There were no CMV infections.

Only two patients developed malignancies in 3 years of follow-up, and both of these were limited to basal cell skin cancers that were completely excised without recurrence or sequelae. No cases of post-transplant lymphoproliferative disease were identified. No oral ulcers were seen, and the cholesterol profile of the patients has been published separately [9].

Seven patients developed hernias over the 3-year study period. Three of these patients had hernias for which

Table 3. Protocol biopsies. Patients had protocol biopsies performed at 6 and 12 months. Most biopsies from patients without rejection demonstrated non-specific changes with minimal fibrosis.

Subject	Baseline biopsy	6-month biopsy	12-month biopsy	Rejection
1		Minimal fibrosis	Mild fibrosis	POD 1010
2		Acute rejection (suspicious)	Mild fibrosis, tubular atrophy	
3	Mild acute renal tubular injury, mild interstitial fibrosis	NS, minimal fibrosis	Minimal fibrosis & mild arterial focal sclerosis	
4		NS	Mild renal tubular atrophy and striped fibrosis	
5		Mild fibrosis	Minimal fibrosis	
6	NS	Acute rejection (Banff 1A), mild interstitial fibrosis	NS	
7				POD 6
8	Renal cortex within normal limits	Focal mild tubular atrophy and interstitial fibrosis	Positive C4d	POD 8
9		Mild acute & chronic tubulointerstitial nephritis		
10	NS, focal fibrosis in interstitium	Minimal arteriolonephrosclerosis	Minimal interstitial fibrosis & tubular atrophy	
11		NS		
12	Minimal fibrosis			
13	NS, mild tubular injury	Acute rejection (Banff suspicious), minimal fibrosis	Mild tubular atrophy & fibrosis & mild arterial intimal fibrosis	POD 24
14	NS	Acute rejection (Banff 1A), positive C4d	Acute rejection (Banff 1A), chronic glomerulopathy, mild fibrosis	POD 34
15				POD 11
16		Renal cortex normal	NS	
17	Renal parenchyma within normal limits	NS	Renal cortex normal	
18	Mild hyaline arteriolo/arterionephro-sclerosis, mild acute tubular injury	Mild arteriolosclerosis & interstitial fibrosis	Minimal interstitial fibrosis	
19		Minimal arteriolosclerosis	Minimal interstitial fibrosis	POD 565
20		NS		POD 275
21		NS, trace focal fibrosis	Minimal interstitial fibrosis	
22		Minimal fibrosis	Renal cortex normal limits	
23	NS, mild acute tubular injury	Acute cellular rejection (Banff 1A), mild fibrosis	Acute cellular rejection (Banff 1B)	POD 17
24	Unremarkable renal cortex	Acute rejection (Banff 1A)	Minimal acute tubular injury	POD 118
25	NS, minimal sclerosis of renal arteries	Renal cortex normal	NS	
26	Mild acute tubular injury		Mild focal fibrosis	
27	Mild acute tubular injury, minimal interstitial fibrosis			POD 31
28	Renal cortex within normal limits	Minimal fibrosis		POD 26
29	Moderate acute tubular injury	Mild fibrosis	Suspicious acute rejection	POD 39

POD, postoperative day.

sirolimus was discontinued in order to address complicated wound-healing issues.

Discussion

Three-year results of an initial group of 28 patients receiving Campath-1H induction therapy followed by sirolimus therapy have demonstrated excellent graft and patient survival of 96% and 100%, respectively. The renal grafts also demonstrated excellent 3-year function as demonstrated by an average serum creatinine of 1.5 mg/dl and low urine protein levels. At 3 years, the majority of patients were on monotherapy (57%), calcineurin-free regimens (78%), and steroid-free regimens (67%). Only three patients were on standard triple immunosuppression at 3 years.

The characteristics of rejection episodes were unique in that of the 13 of 27 patients who had rejection events, over half had components of humoral rejection. These events occurred early, within 1 month, and all episodes were successfully treated except for the one and only graft loss. The modified immunosuppressive regimen of

Campath-1H plus Thymoglobulin induction in four patients followed by sirolimus monotherapy did not successfully prevent the higher incidence of humoral rejection episodes. The possibility exists that the extremely small non depleted peripheral and nodal T cell populations provided B-cell help without the ability to mount a cellular rejection episode. Recent data suggests that the phenotype of these small residual T-cell populations may be that of a memory and/or regulatory phenotype, and that homeostatic proliferation of these cells accounts for a significant proportion of early repopulating T cells [10,11]. Additional studies by Kirk *et al.*[3] have also pointed to a macrophage/monocyte population that infiltrates rejecting grafts after Campath-1H induction without maintenance immunosuppression. Plasma cells are also known not to be depleted by Campath-1H, and other depleted B-cell populations reconstitute within months when compared with the prolonged T-cell depletion. Campath-1H trials that have utilized calcineurin inhibitors post-transplant have not seen these same elevated rates of humoral rejection [4,6,12,13]. These facts suggest that the current strategies of Campath-1H induction may require additional T-cell suppression in the first months post-transplant to prevent a residual humoral rejection that manifests in some patients. We have previously reported that flow cytometric crossmatches were done retrospectively on the patients with humoral rejection by using pretransplant serum. No patient had a positive crossmatch by flow to account for a subsequent antibody-mediated rejection [14].

Protocol biopsies within the first year revealed minimal changes in patients maintained on sirolimus monotherapy, while patients who experienced rejection episodes and had modified immunosuppressive protocols demonstrated mild fibrosis and tubular changes. The improved histologic appearance of these protocols may be secondary factors of both calcineurin avoidance and absence of rejection-mediated injury.

The patients in this study demonstrate the prolonged period of T-cell depletion after Campath-1H induction. The peripheral depletion of both CD3+ and CD4+ T-cell populations persists for years as reported by others [15]. Three-year postinduction CD3+ cells were only at 48% baseline values of 666 cells/μl and CD4+ cells were only at 37% baseline values of 320 cells/μl. This is in significant contrast to the peripheral populations of CD20+ B cells that were nearly 50% reconstituted to baseline values by 6 months. The incidence of lymphopenia was likewise not significant in this study as only one patient was lymphopenic at 2 weeks with a WBC of 1.6. The durable depletion of CD3+ and CD4+ T cells at 3 years may contribute to the high success of calcineurin- and/or steroid-free regimens.

The association of a lymphocyte depletion induction strategy with infectious and malignant complications was not significant at 3 years in this small group of patients. No mortality was observed in this study group. These patients did demonstrate higher than expected complications related to wound healing issues (i.e. frequent hernias), and this is presumably associated with the sirolimus-based post-transplant immunosuppressive regimen. This has been well described with other nondepleting induction therapies and may have no association with Campath-1H therapy [16,17]. Nonetheless, two-thirds of patients remained on sirolimus at 3 years with excellent graft survival, no evidence of chronic allograft nephropathy, and few rejection episodes after the first post-transplant year. In fact, only two patients had a first rejection episode after the first post-transplant year. The long-term results with this immunosuppressive regimen that was still largely based on sirolimus at 3 years may be as much secondary to the prolonged lymphocyte depletion as sirolimus therapy.

The uncontrolled study design and small low-risk patient population limit the conclusions of this study that can be applied to drug minimization, drug avoidance, or tolerogenic strategies. This study was not designed with any plan to evaluate whether lymphocyte depletion following sirolimus therapy may allow for the development of tolerance. However, the incidence of cellular and humoral rejection episodes post-transplant suggests that Campath-1H/sirolimus is ineffective at producing a tolerogenic response in the early post-transplant period. Residual lymphocyte and other cell populations have a significant ability to mount either cellular or humoral rejection episodes; however, these were all successfully treated without functional or immunological consequences detected at 3 years, with one exception of early graft loss. Patients at 3 years demonstrated excellent graft survival and function with most patients on either monotherapy, steroid-, or calcineurin-free immunosuppressive regimens. In subsequent clinical trials at our center, we have altered the immunosuppressive regimen described herein due to the high incidence of early rejection. A calcineurin-inhibitor is recommended for at least short-term use in order to prevent early rejection. Use of combined calcineurin inhibitor and sirolimus long-term may pose an increased risk of nephrotoxicity in contrast to sirolimus alone which may allow gradual increase in glomerular filtration rate and less histologic injury than calcineurin inhibitors [18].

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