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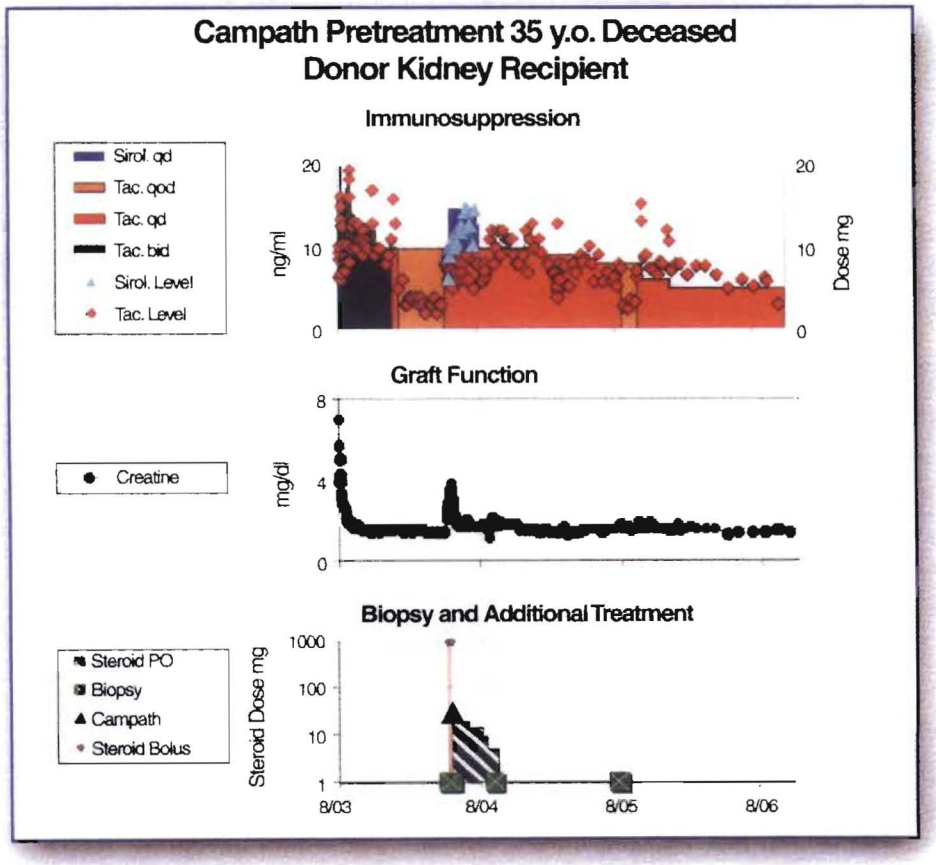

Campath Preconditioning for Renal Transplantation (pp. 1125-1132)

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Alemtuzumab Preconditioning With Tacrolimus Monotherapy—The Impact of Serial Monitoring for Donor-Specific Antibody

Ron Shapiro,[1,4] Adriana Zeevi,[2] Amit Basu,[1] Henkie P. Tan,[1] Liise K. Kayler,[3] Deanna M. Blisard,[1] Ngoc L. Thai,[1] Alin L. Girnita,[2] Parmjeet S. Randhawa,[2] Edward A. Gray,[1] Amadeo Marcos,[1] and Thomas E. Starzl[1]

Background. Antibody preconditioning with tacrolimus monotherapy has allowed many renal allograft recipients to be maintained on spaced weaning.

Methods. Of 279 renal allograft recipients transplanted between March 2003 and December 2004, 222 (80%) had spaced weaning (i.e., reduction of tacrolimus monotherapy dosing to every other day, three times a week, twice a week, or once a week) attempted. Routine monitoring for donor-specific antibody (DSA) was begun in September 2004. Mean follow-up is 34±6.5 months after transplantation and 26±8.1 months after the initiation of spaced weaning.

Results. One hundred and twenty-two (44%) patients remained on spaced weaning. One- and 2-year actual patient/graft survival was 99%/99%, and 97%/96%. Fifty-six (20%) patients experienced acute rejection after initiation of spaced weaning. One- and 2-year actual patient/graft survival was 100%/98%, and 94%/78%. Forty-two (15%) patients with stable renal function had spaced weaning stopped because of the development of DSA, which disappeared in 17 (40%). One- and 2-year actual patient and graft survival was 100% and 100%.

Conclusion. Adult renal transplant recipients who are able to be maintained on spaced weaning have excellent outcomes. Patients with stable renal function who have reversal of weaning because of the development of DSA also have excellent outcomes. Routine monitoring for DSA may allow patients to avoid late rejection after spaced weaning.

Keywords: Antibodies, Kidney transplantation, Outcome.

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The potency of alemtuzumab as a preconditioning or an induction agent in renal transplant recipients has been demonstrated in a number of reports (*1–5*). A humanized anti-CD52 monoclonal antibody, alemtuzumab (Campath 1H; Berlex, Montville, NJ) rapidly depletes T and B lymphocytes, monocytes, and natural killer cells, and this depletion can last for many months. When it has been used in a regimen with minimal posttransplant immunosuppression using tacrolimus monotherapy, excellent short-term patient and graft survival rates and very low rates of early acute rejection have been seen in both adult and pediatric patients, with very low rates of viral complications and posttransplant diabetes mellitus (PTDM) (*6–8*). Spaced weaning, to every other day tacrolimus or less, has also been achieved in a variable percentage of patients, ranging from 40% to 60%. Although most of the patients undergoing spaced weaning have done well, an important and troubling minority have developed acute rejection. In an attempt to minimize or prevent the development of postweaning rejection, we began to monitor patients for the development of donor-specific antibody (DSA) (*9*). We used the new onset of DSA as a marker for impending rejection and abandoned spaced weaning when it occurred. In this report, we describe the outcomes of this work.

[1] Department of Surgery, The Thomas E. Starzl Transplantation Institute, Pittsburgh, Pennsylvania.
[2] Department of Pathology, The Thomas E. Starzl Transplantation Institute, Pittsburgh, Pennsylvania.
[3] Department of Transplant Surgery, University of Florida Medical Center, Gainesville, Florida.
[4] Address correspondence to: Ron Shapiro, M.D., Thomas E. Starzl Transplantation Institute, University of Pittsburgh Medical Center, Montefiore 7 South, 3459 Fifth Avenue, Pittsburgh, PA 15213.
E-mail: shapiror@upmc.edu
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PATIENTS AND METHODS

Recipient and Donor Demographics

Between March, 2003, and December, 2004, 279 adult kidney transplantations were performed in 278 recipients (Table 1) . The mean recipient age was 50.8±15.8 (SD) years (range, 18–82). Thirty-eight (14%) were undergoing retransplantation, and 39 (14%) were sensitized, with a panel-reactive antibody (PRA) levels more than 20%. The mean donor age was 39.6±15.1 years (range, 1–72). There were 121 (43%) living and 158 (57%) deceased donors. The mean cold ischemia time for the deceased donors was 22.5±7.1 hr. The average number of human leukocyte antigen (HLA) mismatches was 3.5±1.7.

We also analyzed outcomes in 152 patients transplanted between March, 2000, and July, 2001, before the beginning of the preconditioning era. These patients served as a reference group. The mean recipient age was 50.6±14.9 years (range, 18–86). Thirty-three (22%) were undergoing retransplantation, and 40 (26%) had a PRA levels more than 20%. The mean donor age was 35.5±18.0 years (range, 1–78). There were 30 (20%) living and 122 (80%) deceased donors. The mean cold ischemia time for the deceased donors was 27.7±8.7 hr. The average number of HLA mismatches was 3.2±1.6.

TABLE 1. Recipient and donor demographics

	Campath	Reference
Recipient demographics		
Time frame	March 2003 to Dec 2004	March 2000 to July 2001
N	279	152
Age (yrs)	50.8±15.8	50.6±14.9
Range	18–82	18–86
Retransplantation	38 (14%)	33 (22%)
PRA >20%	39 (14%)	40 (26%)
Donor demographics		
Age (yrs)	39.6±15.1	35.5±18.0
Range	1–72	1–78
Living	121 (43%)	30 (20%)
Deceased	158 (57%)	122 (80%)
Cold ischemia time (hrs)	22.5±7.1	27.7±8.7
HLA mismatch	3.5±1.7	3.2±1.6

Immunosuppression

In the preconditioning patients, alemtuzumab 30 mg was administered intravenously over 2 hrs intraoperatively, after induction of anesthesia. Premedication was with methylprednisolone 1 g intravenously (IV), diphenhydramine 50 mg IV, acetaminophen 650 mg orally, and famotidine 20 mg IV; a second dose of methylprednisolone 1 g IV was administered during the arterial anastomosis (steroids were administered to minimize cytokine release symptoms). Oral tacrolimus 3 mg twice daily was started on postoperative day 1, with a target 12-hr trough level of 10 ng/mL (using the Abbott whole blood IMX assay) for at least the first 3.5 to 4 months after transplantation. At that point, patients were consolidated to once daily tacrolimus, that is, a patient on 3 mg twice daily would be converted to 5 or 6 mg once daily. Two to four months later, patients who were stable on once daily tacrolimus would be converted to every other day dosing (i.e., from 5 mg once a day to 5 mg every other day). By 1 year after transplantation, stable patients could be weaned to three times weekly tacrolimus. Less commonly, weaning to twice weekly or once weekly tacrolimus was carried out; this was generally limited to HLA-identical living related donor recipients.

Beginning in September, 2004, routine monitoring for DSA was initiated, beginning 1 and 3 months after transplantation, 1 and 3 months after any major change (i.e., consolidation or spaced weaning), and every 3 months chronically. A combination of enzyme-linked immunosorbent assay (ELISA) and Luminex methods was used for the detection and specificity analysis of donor-specific anti-HLA antibodies (DSA). We used commercial LATM, LAT1288, LAT1HD, and LAT240 ELISA kits (One Lambda, Canoga Park, CA), in accordance with the manufacturer's instructions, to identify IgG anti-HLA class I- and class II-specific antibodies independently. Briefly, diluents, control serum, or patients' samples were added to plates coated with purified class I or class II HLA antigens. The plates were incubated for 60 min, followed by the addition of enzyme-conjugated anti-IgG secondary antibody. After 40 min, substrate was added, the trays were incubated for 10 to 15 min, and the reactions were stopped. Anti-HLA IgG antibodies were measured indirectly, by a second enzyme-linked colorimetric reaction, and assays were read at 630 nm using an ELISA reader (ELX 800NB, Bio-Tek Instruments, Inc., Winooski, VT, and One Lambda computer software). The positive cutoff was calculated as 10% of average positive IgG control (*10*).

The Luminex system (One Lambda) is a multiplexed microsphere-based suspension array platform capable of analyzing and reporting up to 100 different reactions in a single reaction vessel (*11*). The serum is first incubated with LABScreen beads. Any anti-HLA antibodies present in the serum bind to the antigens and then are labeled with R-phycoerythrin-conjugated goat anti-human IgG. The Labscan 100 flow analyzer detects the fluorescent emission of R-phycoerythrin from each bead, allowing real-time data acquisition. We used LSM12 beads for the detection of class I and class II anti-HLA antibody, LS1PRA and LS2PRA for specificity analysis, and LS1A/LS2A for single-antigen analysis. We applied the manufacturer's positive cutoff for LABScreen, the normalized background ratio, which is considered positive when greater than 15%.

Patients with class I and class II ELISA screens less than 10% were generally assumed not to have DSA; in patients with ELISA less than 10%, further analysis was performed to look for DSA. Although it is possible that patients with ELISA screens less than 10% might have had low level DSA by more sensitive testing (i.e., Luminex), this testing was initially not available to our laboratory at the time, and began to be used only in the last 16 months. In patients on spaced weaning who developed a de novo DSA, weaning was abandoned, and patients were taken back to once daily tacrolimus. If the DSA did not disappear over the next few months, mycophenolate mofetil 250 to 500 mg twice daily was added to the immunosuppressive regimen. In patients on once daily tacrolimus, the development of DSA would preclude any attempt at spaced weaning, and would generally lead to an increase in immunosuppression to twice daily tacrolimus, with or without the addition of mycophenolate mofetil.

Rejection was biopsy proven more than 95% of the time. Acute rejection was treated initially with steroids and an adjustment of the tacrolimus dosage. Steroid resistant rejection was treated with antibody therapy, either additional alemtuzumab, thymoglobulin, or OKT3, and the addition of mycophenolate mofetil or sirolimus. Antibody-mediated rejection was treated with plasmapheresis/IVIg, and an adjustment in the tacrolimus dosage, with or without the addition of mycophenolate mofetil or sirolimus. In patients on spaced weaning with acute rejection, the tacrolimus dosage was converted back to once daily, in addition to the other therapies just described.

In the reference group, immunosuppression was generally with tacrolimus, mycophenolate mofetil, and steroids, without antibody induction. Occasional patients received sirolimus instead of mycophenolate mofetil as part of a pilot trial. The diagnosis and treatment of acute rejection was carried out as described above.

Statistics

This study was conducted as a retrospective medical records review, and data were managed under the auspices of our institutional review board-authorized honest brokering system. Categorical data were expressed as frequency and per-

centages. Nonparametric variables were expressed as mean value and standard deviation.

Institutional Oversight

This immunosuppressive regimen, which was used as the standard of care for our patients, was approved by the Innovative Clinical Practice Committee and the Pharmacy and Therapeutics Committee of the University of Pittsburgh Medical Center (*12*). Data analysis was performed under an institutional review board-approved protocol. Institutional support was always present throughout this time period.

RESULTS

The mean follow-up was 34±6 months in the alemtuzumab preconditioning patients, and 67±5 months in the reference group.

Patient and Graft Survival

Overall 1- and 2-year patient and graft survival rates in the preconditioning group were 97% and 94%, and 94% and 87%, respectively (Fig. 1a). Corresponding survival rates in the reference group were 95% and 91%, and 89% and 81%, respectively. In general, outcomes in the preconditioning patients were at least as good, and perhaps slightly better than in the reference group. Subgroup analyses of graft survival in the preconditioning group were performed in living versus deceased donor cases, primary versus retransplantation, African Americans versus non-African Americans, recipients with

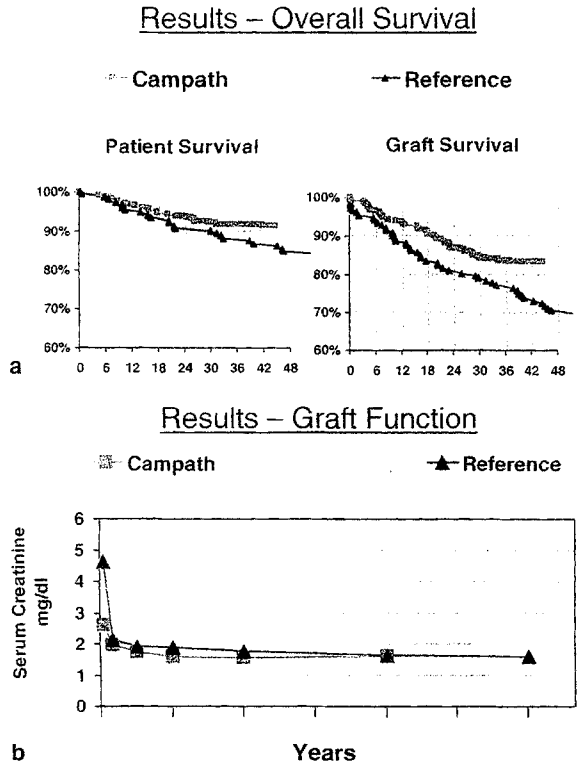

FIGURE 1. (a) Patient and graft survival in the alemtuzumab and reference groups. (b) Serum creatinine over time in the alemtuzumab and reference groups.

TABLE 2. Campath subgroup survival

	%	1-yr graft survival %	2-yr graft survival %
Donor type			
Living	43	98	90
Deceased	57	90	86
Graft type			
Primary	86	95	89
Nonprimary	14	87	82
Recipient race			
African American	13	83	81
Non-African American	87	95	89
Preoperative PRA			
≥20%	14	89	82
<20%	86	94	88
HLA mismatches			
0	10	96	96
1	3	100	88
2	13	94	86
3	18	100	90
4	22	90	80
5	24	94	92
6	10	93	82
Acute rejection—reference			
Overall	28		
Steroid-resistant	3		
Preweaning rejection—Campath			
Overall	8		
Steroid-resistant	2		
Delayed graft function			
Campath	12		
Reference	31		

Complications	Campath (%)	Reference (%)
CMV disease	0	5.3
BK virus	0.7	2.6
PTLD	0.4	2.6
PTDM	1.2	11.8

PRA, panel-reactive antibody; HLA, human leukocyte antigen; CMV, cytomegalovirus; PTLD, posttransplant lymphoproliferative disorders; PTDM, posttransplant diabetes mellitus.

PRA levels more than or equal to 20% or less than 20%, and 0 to 6 antigen mismatch cases, and are shown in Table 2.

Renal Function

Renal function over time was similar between the preconditioning and the reference groups (Fig. 1b). The mean serum creatinine 2 years after transplantation was 1.5 mg/dL in both groups of patients.

Acute Rejection

The incidences of acute rejection and of steroid resistant rejection before spaced weaning in the preconditioning

group were 8% and 2%, respectively, and in the reference group were 28% and 3% (Table 2).

Infectious Complications, Posttransplant Diabetes Mellitus, and Delayed Graft Function

The incidences of cytomegalovirus, BK virus, posttransplant lymphoproliferative disorders, and PTDM in the preconditioning and reference groups, as shown in Table 2, were generally lower in the preconditioning group. Delayed graft function (DGF) was also observed less commonly in the preconditioning group.

Spaced Weaning

Spaced weaning was not attempted in 57 (20%) patients. These were generally patients with poor graft function or who had experienced difficult early rejection episodes. Spaced weaning was attempted in 222 (80%) patients an average of 8.0±3.0 months after transplantation. Spaced weaning was able to be continued in 122 (44% of the overall population of 279 patients, 55% of the 222 patients in whom spaced weaning was attempted) patients. Weaning was interrupted in 56 (20%/25%) patients because of acute rejection, and in 42 (15%/19%) patients because of the development of DSA. One additional patient had interruption of spaced weaning because of an elevated Cylex level of 508, and another had interruption of spaced weaning because he was thought to have developed DSA, although he had not done so. One- and 2-year patient and graft survival rates in the four groups are shown in Table 3. The worst outcomes were in patients who were never weaned. The patients who were weaned and who remained on spaced weaning had excellent outcomes, as did those who had weaning interrupted because of the development of DSA. In patients who had weaning interrupted because of acute rejection, patient survival was reasonable (94% at 2 years), but graft survival was down to 78% at 2 years. A subgroup analysis of spaced weaning is shown in Table 3. Eighty percent of patients receiving 0 antigen mismatch kidneys were able to undergo spaced weaning. Individual cases of successful spaced weaning, weaning interrupted by acute rejection, and weaning interrupted by the development of DSA are shown Figure 2 (a–c).

Donor-Specific Anti-Human Leukocyte Antigen Alloantibodies

Donor-specific anti-HLA alloantibodies (DSA) were detected in 42 (15%) patients by ELISA or Luminex (Table 4).

TABLE 3. Monitoring for donor-specific antibody (DSA)

	N (%)	Patient/graft 1 yr	Survival % 2 yr	HLA mismatch
Never weaned	57 (20)	86/70	79/63	3.6±1.4
Remained on spaced dosing	122 (44)	99/99	97/96	3.2±1.9
Ended spaced dosing at rejection	56 (20)	100/98	94/78	3.6±1.4
Ended spaced dosing at DSA	42 (15)	100/100	100/100	4.1±1.5

Campath subgroup weaning	Spaced dosing %	Ended spaced dosing %	
		At rejection	At DSA
Donor type			
Living	62	24	15
Deceased	48	27	23
Graft type			
Primary	55	25	19
Nonprimary	52	28	17
Patient race			
African American	48	24	12
Non-African American	55	25	20
Preoperative PRA			
≥20%	40	29	36
<20%	57	25	16
HLA mismatches			
0			
1	80	7	4
2	29	12	12
3	65	23	12
4	48	31	12
5	50	23	16
6	52	17	20
6	45	17	28

PRA, panel-reactive antibody; HLA, human leukocyte antigen.

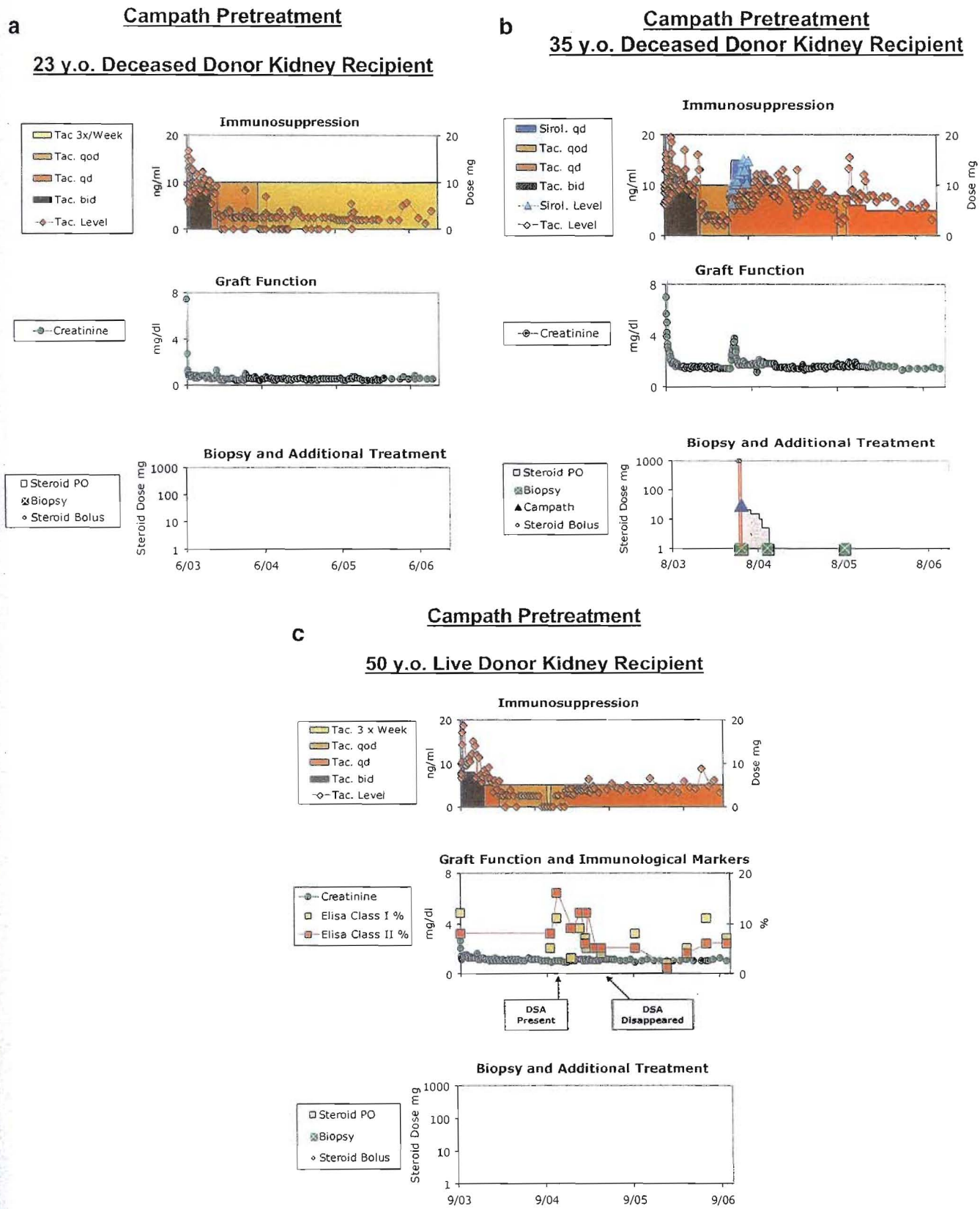

FIGURE 2. (a) Uncomplicated patient course. (b) Spaced weaning interrupted by rejection. (c) Spaced weaning interrupted by the development of DSA.

TABLE 4. Distribution of donor-specific anti-HLA alloantibody (DSA) after weaning cessation

Case	Anti-HLA class I DSA	Anti-HLA class II DSA	DSA disappeared
1		DQ4	Yes
2		DR17, DQ2	Yes
3	A1		Yes
4	B35	DR4, DR17, DR52	Yes
5		DR7, DR53	Yes
6	A1	DR17	Yes
7		DR15	Yes
8	B8		Yes
9	B27		Yes
10		DR13	Yes
11	B61		Yes
12		DR17	Yes
13	B7		Yes
14		DQ4	Yes
15	B53		Yes
16	B65		Yes
17	B8	DQ2	Yes
18		DR4, DQ7	No
19	B44	DR17, DQ2	No
20	A26, B7		No
21	B7, Cw2		No
22		DR17, DQA1*5	No
23		DR11, DR52, DQA1*3, DQA1*5	No
24		DR13, DQ5, DQ7, DQA1*5	No
25	A26		No
26		DR11, DQ7	No
27	A2, A26, B49	DR15, DR51	No
28		DR4, DR53	No
29	A68, B44, Cw5	DR4, DQ6, DQA1*3	No
30		DR7, DR53	No
31		DQ7	No
32		DQ3	No
33		DR7, DR53	No
34	A*6801		No
35		DR53, DQ2, DQ7, DQA1*5	No
36		DR8, DQ7	No
37		DQ6	No
38		DR52, DQ7, DQA1*5	No
39	Cw3	DR15, DR17, DQA1*0501	No
40*	B49	DQ6	No
41*		DR17, DQ2	No
42*	A24, B7		No

HLA, human leukocyte antigen.

Patients with an ELISA screen PRA lower than 10% were assumed not to have DSA; in the past 16 months, this was selectively confirmed by Luminex testing. In these patients we did not detect DSA before transplantation, and the T- and B-cell crossmatches were negative. After transplantation and

TABLE 5. Comparative ELISA PRAs in patients in whom DSA did or did not disappear

	DSA disappeared		
	No	Yes	*P*
DSA	38	33	NS
Class I DSA	33	36	NS
Class II DSA	29	17	0.08
HLA-A DSA	31	14	0.10
HLA-B DSA	32	40	NS
HLA-DRB1 DSA	35	33	NS
HLA-DRB3,4,5 DSA	30	20	0.12
HLA-DQB1 DSA	30	14	0.11

ELISA, enzyme-linked immunosorbent assay; PRA, panel-reactive antibody; DSA, donor-specific antibody; HLA, human leukocyte antigen.

initiation of spaced weaning, 12 patients developed anti-class I DSA, 22 patients developed anti-class II DSA, and 8 patients developed both anti-class I and anti-class II DSA. After weaning was abandoned in response to the development of DSA circulating DSA disappeared in 17 (40%) patients, and persisted in the other 25 (Table 4). The DSA specificity distribution was as follows: 9 anti-HLA A, 14 anti-HLA B, 3 anti-HLA C, 24 anti-HLA DRB1, 8 anti-HLA DQA1, 20 anti-HLA DQB1, and 10 anti-HLA DRB3,4,5. Interestingly, some DSA specificities disappeared more than others. The descending order of DSA disappearance was:

- Anti-HLA-B DSA disappeared in 9 of 14 cases (64%);
- Anti-HLA-DRB1 DSA disappeared in 10 of 24 cases (42%);
- Anti-HLA-A DSA disappeared in 3 of 9 cases (33%);
- Anti-HLA-DRB3,4,5 DSA and anti-HLA-DQB1 DSA disappeared in 22% (2 of 9) and 21% (4 of 19) of cases respectively;
- Anti-HLA-DQA1 DSA and anti-HLA-C DSA did not disappear in any case (0 of 8 and 0 of 3, respectively).

The anti-HLA antibody strength was expressed as a ratio of the patients' ELISA to the positive control. There was a trend toward a lower ELISA PRA in patients whose DSA disappeared for anti-HLA A, DRB3,4,5, and DQB 1 (Table 5).

DISCUSSION

The paradigm shift of preconditioning with minimal posttransplant immunosuppression was undertaken because of a concern that modern multiagent immunosuppressive regimens preclude any donor-recipient immune system interaction, and thus compromise long-term graft survival (*7, 13, 14*). Registry data have confirmed these observations and have suggested that, despite falling acute rejection rates renal allograft half life has not improved over the past decade (*15*). Short-term outcomes in an unselected patient population with a regimen of alemtuzumab preconditioning and tacrolimus monotherapy after kidney transplantation have been quite reasonable, with 1- and 2-year patient and graft survival rates of 97% and 94%, and 94% and 87%, respectively, and have compared favorably with outcomes seen in conventionally immunosuppressed reference group. Rates

early acute rejection, viral complications, and DGF have all been low, and again have compared favorably with those seen in the reference group. Spaced weaning has been attempted in most of the preconditioning patients, and those patients who were able to be maintained on spaced weaning had excellent outcomes. Patients who had weaning abandoned because of acute rejection had reasonable patient survival but compromised graft survival at 2 years. Screening for the development of DSA, and interruption of weaning when DSA was noted was associated with good outcomes. We found that the persistence of donor-specific HLA antibody depends on the specificity itself. The fastest to disappear were anti-class I HLA-A and HLA-B antibodies, as well as anti-class II DRB1 antibodies. However, anti-HLA DQ alpha and beta, anti-DRB3,4,5, and anti-HLA C antibody specificities were significantly more persistent. A similar pattern was described in the Johns Hopkins desensitization protocol, after plasma exchange/IVIg (*16*). In three cases (40, 41, and 42, Table 4), some of the DSA disappeared, whereas other DSA, mostly anti-HLA DQ, persisted. Antibody strength might also be important, as was reported by Terasaki's group (*17*). In the present study, we also noted a tendency for lower antibody strength in some patients whose DSA disappeared. It is also noteworthy that all living-related grafts were in the subgroup where DSA disappeared (cases 7, 10, 16, Table 4). Furthermore, a single allelic difference was sufficient for humoral allosensitization (Table 4, case 34); the recipient typing was HLA-A*6802, and the donor typing was HLA-A*6801; all the other HLA-A, -B, -Cw, -DRB1, -DQA1, -DQB1, and -DRB3,4,5 loci were matched at the allelic level.

In 40% of patients, DSA disappeared after patients were put back on daily tacrolimus. This observation, that DSA can disappear when immunosuppression is intensified, is relatively novel and important. Others have shown that conversion from cyclosporine/azathioprine to tacrolimus/mycophenolate mofetil-based immunosuppression can reduce DSA (*18*). In our patients, increasing the dosage of tacrolimus from every other day or three times a week to daily dosing was often sufficient to reduce DSA. This observation offers the possibility of allowing maintained stable renal function and graft survival in patients who do not tolerate spaced weaning, and can continue to allow most patients to benefit from an immunosuppressive regimen that allows for minimal posttransplant immunosuppression.

Unfortunately, it is possible to develop postweaning rejection in the absence of DSA. Additional immunologic monitoring tools will have to be developed to allow safer immunosuppression minimization. We are currently studying the Cylex assay (*19*) as a routine clinical test, and are exploring other potentially useful immunologic markers.

There are a number of important limitations of this analysis. First, in most of the patients who developed postweaning rejection, the incidence of DSA was not known, because routine analysis for DSA was begun late in the series. Second, this was not a randomized trial, and so these observations lack a control group. The alemtuzumab and reference group recipient and donor demographics had some similarities, but were not identical. The reference groups cannot be compared with the Campath group statistically, because these were two different groups transplanted at two different time points, and assignment was not randomized. Third, routine staining for C4d was not performed by our pathologists during this time period.

To conclude, an immunosuppressive regimen with alemtuzumab preconditioning and tacrolimus monotherapy in unselected adult renal transplant recipients seems to be associated with excellent short-term patient and graft survival, low rates of early acute rejection, viral complications, PTDM, and DGF, and excellent renal function. Spaced weaning is possible in the most of the patients, with generally good outcomes. Monitoring for DSA may serve as a marker for impending rejection after weaning, and interruption of weaning in patients with stable renal function who develop DSA can lead to its disappearance, with maintained stable renal function and excellent graft survival.

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