Kidney Transplantation under Minimal Immunosuppression after Pretransplant Lymphoid Depletion with Thymoglobulin or Campath

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BACKGROUND: Multiple drug immunosuppression has allowed the near elimination of rejection, but without commensurate improvements in longterm graft survival and at the cost of quality of life. We have suggested that transplantation outcomes can be improved by modifying the timing and dosage of immunosuppression to facilitate natural mechanisms of alloengraftment and acquired tolerance.

STUDY DESIGN: Two therapeutic principles were applied for kidney transplantation: pretransplant recipient conditioning with antilymphoid antibody preparations (Thymoglobulin [Sangstat] or Campath [ILEX Pharmaceuticals]), and minimal posttransplant immunosuppression with tacrolimus monotherapy including "spaced weaning" of maintenance doses when possible. The results in Thymoglobulin- (n = 101) and Campath-pretreated renal transplantation recipients (n = 90) were compared with those in 152 conventionally immunosuppressed recipients in the immediately preceding era.

RESULTS: Spaced weaning was attempted in more than 90% of the kidney transplant recipients after pretreatment with both lymphoid-depleting agents, and is currently in effect in two-thirds of the survivors. Although there was a much higher rate of acute rejection in the Thymoglobulin-pretreated recipients than in either the Campath-pretreated or historic control recipients, patient and graft survival in both lymphoid depletion groups is at least equivalent to that of historic control patients. In the Thymoglobulin-conditioned patients for whom followups are now 24 to 40 months, chronic allograft nephropathy (CAN) progressed at the same rate as in historic control patients. Selected patients on weaning developed donor-specific nonreactivity.

CONCLUSIONS: After lymphoid depletion, kidney transplantation can be readily accomplished under minimal immunosuppression with less dependence on late maintenance immunosuppression and a better quality of life. Campath was the more effective agent for pretreatment. Guidelines for spaced weaning need additional refinement. (J Am Coll Surg 2005;200:505–515. © 2005 by the American College of Surgeons)

Kidney transplantation became a practical clinical service after it was shown that rejections developing under azathioprine were highly reversible with prednisone, and that the reversals often were succeeded by the emergence of variable donor-specific nonreactivity (ie, tolerance).[1] In 1966, antilymphoid globulin (ALG) was added to azathioprine and prednisone as a steroid-sparing adjunct.[2] A short course of antilymphoid globulin was begun preoperatively and continued for several posttransplant days or weeks.[2,3] The pretransplant portion of the course was subsequently deemphasized and omitted because of uncertainty about its value and because the time constraints of cadaveric transplantation made the pretreatment impractical. Instead, antilymphoid globulin usually was started on the day of, or day

after, transplantation (induction therapy), and in addition, prednisone or other agents were increasingly instituted at this time in multiple drug regimens designed to eliminate the threat of acute rejection.[4]

After elucidation of the mechanisms of alloengraftment,[5,6] it was apparent that this treatment policy could subvert the seminal mechanism of clonal exhaustion-deletion.[7] Consequently, we suggested modifications of immunosuppression in accordance with two principles. The first was lymphoid depletion before rather than after transplantation to reduce the anticipated donor-specific response into a more easily deletable range. The second was avoidance of so much posttransplant immunosuppression that the immune activation-dependent mechanism of clonal exhaustion-deletion would be interdicted. Pretransplant conditioning was done with a single infusion of rabbit antithymocyte globulin (rATG, Thymoglobulin [Genzyme]),[8,9] or alternatively, of alemtuzumab (Campath 1H [ILEX Pharmaceuticals]).[10-14] Minimalistic posttransplant immunosuppression was begun with relatively low doses of tacrolimus monotherapy with the intention of dose weaning after the first few months of highest immunologic risk. Our initial experience[15,16] and that reported here suggest that this approach to management can be carried out efficiently and safely.

METHODS

Institutional review process

Modifications in the timing and dosage of conventional immunosuppression were undertaken in July 2001. The primary purpose was to improve the quality-of-life outcomes and patient and graft survival across the full spectrum of all kinds of adult kidney recipients in our clinical practice. The modifications were submitted in this context to the University of Pittsburgh Institutional Review Board (IRB), which judged the changes to be within the boundaries of historically based standard treatment. The treatment protocols were reviewed by the Presbyterian University Hospital Committee on Innovative Practices and the Pharmacy and Therapeutic Practices Committee, with approval by both. All patients provided informed consent. In addition, separate informed consent was obtained with IRB approval for studies of immune variables not routinely assayed in our conventional practice. Safety and efficacy monitoring were assured by formal weekly reviews of all patients.

Patient selection

No adult kidney recipients were denied access to the reforms in management because of high risk factors. Recipients who received previous, simultaneous, or subsequent nonkidney solid organ allografts or bone marrow were removed from this analysis, as were kidney-only transplantations performed in the period of time when Thymoglobulin and Campath pretreatment were both used. The three study populations were compiled during the eras of March 2000 to July 2001 (historic controls, no pretreatment: n = 152); July 2001 to October 2002 (Thymoglobulin pretreatment: n = 101); and March 2003 to September 2003 (Campath pretreatment: n = 90) (Table 1).

Differences in donor characteristics reflected nationwide efforts to expand the donor pool by using older deceased donors and more living donors. The large complement of nonrelated live donors (mostly spousal) is noteworthy. The mean histocompatibility match was worst and the mean ischemic time best in the Campath pretreatment series. Differences in recipient demographic factors were not statistically significant.

Immunosuppression
Historic controls

Patients treated between March 2000 and June 2001 received multidrug immunosuppression that included tacrolimus, a 5-day intravenous and oral prednisone taper (200 → 40 mg in 40 mg decrements) followed by oral prednisone (20 mg/d) and often a third agent (usually mycophenolate mofetil or sirolimus). The multiagent immunosuppression (particularly the steroid component) was weaned slowly throughout the first 6 to 12 posttransplant months.

Lymphoid depletion

Lymphoid depletion between July 2001 and October 2002 was done with an infusion of 5 mg/kg rabbit antithymocyte globulin (rATG, Thymoglobulin). The Thymoglobulin was administered over several hours before allograft reperfusion. The antibody infusion was accompanied by 1 or 2 g methylprednisolone to prevent cytokine release consequences. The same steroid doses were used when alemtuzumab (anti-CD52 mAb, Campath 1-H) infusion of 30 mg was substituted for Thymoglobulin as the conditioning agent.

Minimalistic immunosuppression

The lymphoid depleted patients were started on twice daily tacrolimus (Prograf) on postoperative day 1, with a

Table 1. Population Characteristics

Characteristic	Historic controls	Thymoglobulin pretreatment	Campath pretreatment
n	152	101	90
Accrual dates	3/00 to 7/01	7/01 to 10/02	3/03 to 9/03
Followup, (mo)	39 to 54	23 to 39	12 to 18
Recipient age, (y)	50.6 ± 14.9	51.1 ± 14.5	50.8 ± 16.6
Recipient gender (M/F), %	61/39	65/35	63/37
African-American recipients, %	14	14	18
Primary Tx/Re-Tx, %	78/22	85/15	87/13
Recipient PRA > 20%, %	26	18	19
Donor age, (y)	35.5 ± 18.0	**39.4 ± 15.4**	**41.7 ± 16.0**
Donor gender (M/F), %	57/43	**40/60**	47/53
African-American donors, %	10	9	8
Living/cadaveric donors, %	20/80	**46/54**	**39/61**
Sibling-sibling	12	14	6
Parent-offspring	1	6	4
Offspring-parent	3	7	5
Other related	3	5	4
Nonrelated (incl. spouse)	11	14	16
Cadaveric ischemia time, (h)	27.7 ± 8.69	26.4 ± 6.37	**20.8 ± 7.64**
ABDR mismatch	3.21 ± 1.61	3.12 ± 1.72	**3.64 ± 1.57**

Significant variations of the lymphoid depletion/minimum immunosuppression populations as compared with the historic controls are highlighted in bold with p ranging from 0.04 to less than 0.001. All significant differences in the populations are attributable to the cadaveric donor subgroup, because live donor characteristics were statistically similar.
ABDR, ; PRA, panel reactive antibodies; Tx, transplant; Re-Tx

target 12-hour trough level of 10 ng/mL. In a few cases, sirolimus (or cyclosporine) was substituted for tacrolimus because of nephro- or neurotoxicity. Renal function was monitored primarily with serum creatinine determinations. Suspected rejection was confirmed by biopsy and treated with one or more boluses of methylprednisolone, muronmonab-CD3 (OKT3), or alemtuzumab. Oral steroids or other secondary agents such as sirolimus were added only as necessary.

Spaced weaning

At some time after 3 to 4 months in lymphoid depleted patients who had been stable on tacrolimus monotherapy, the twice daily doses were consolidated to a single daily dose. For example, someone on 2 mg twice a day would be converted to a single 4-mg dose. One and a half or more months later, weaning to every-other-day dosing was begun (ie, 4 mg every other day). Subsequent weaning to three times weekly, twice weekly, and once weekly tacrolimus was instituted on an individualized basis.

Cellular immunologic monitoring

Peripheral blood mononuclear cells were obtained from recipients who had been followed up for 1 year or more.

The cells were separated by a standard gradient centrifugation method[17] and used fresh for in vitro functional assays.

Leukocyte surface markers

Three- or four-color flow cytometry with appropriate flurochrome conjugated mAb combinations, and isotype-matched nonspecific mAbs (negative controls) were used to determine lymphocyte subsets. Data acquisition and analysis was performed on a Coulter EPICS XL flow cytometer (Beckman Coulter Corp). In conventionally gated cells for lymphocytes, 50,000 $CD45^+$ events were typically collected per sample and analyzed with EXP032 software (Applied Cytometry System).[17,18]

Mixed lymphocyte reaction

Conventional unidirectional mixed lymphocyte reaction (MLR) cultures were set up for 6 days using 1×10^5 irradiated stimulator cells (2000R). The degree of $[^3H]$ thymidine incorporation was assessed during the final 20 hours of incubation.

Cell-mediated lymphocytoxicity

The cytolytic activity of recipient lymphocytes toward donor and third party targets was assessed in cell-

—o— Historical Control —×— Thymoglobulin —■— Campath

Overall Patient Survival

Overall Graft Survival

Live Donor Patient Survival

Live Donor Graft Survival

Cadaveric Donor Patient Survival
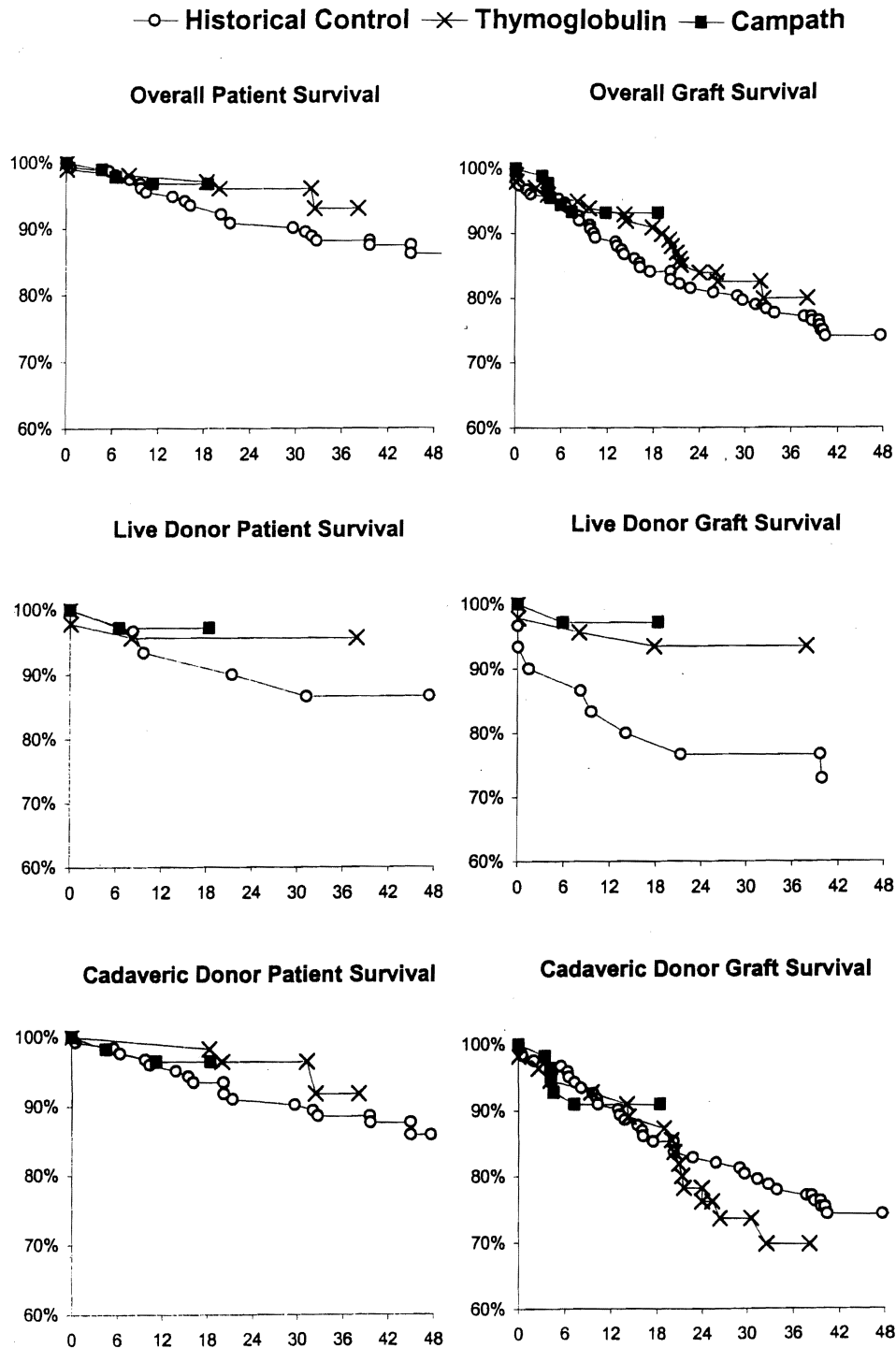
Cadaveric Donor Graft Survival

Figure 1. Patient and graft survival in historic control (oo), Thymoglobulin-pretreated (X), and Campath-pretreated (black squares) kidney recipients.

Table 2. Mean Creatinine (mg/dL) at Successive Posttransplant Times of Allografts that Still Function

n	1 wk	1 mo	3 mo	6 mo	1 y	2 y	3 y	Current
Historic controls (n = 113)								
Mean	4.45	2.03	1.75	1.68	1.56	1.48	1.53	1.64
SD	4.13	1.47	0.89	0.59	0.57	0.56	0.79	1.02
Thymoglobulin (n = 83)								
Mean	3.90	2.24	1.84	1.60	1.58	1.89*	—	2.08*
SD	4.32	1.14	1.08	0.62	0.55	0.95	—	1.23
Campath (n = 84)								
Mean	1.95	1.74	1.54	1.47	1.59	—	—	1.69
SD	1.60	0.71	0.58	0.54	0.75	—	—	0.83
p Value	<0.001	0.025	0.073	0.051	0.957	<0.001	—	—

*Removal of 5% (n = 4) of the outlying results for the Thymoglobulin group results in 2-y and current creatinines of 1.74 ± 0.07 and 1.86 ± 0.86, respectively.

mediated lymphocytotoxicity. In these assays, effector lymphocytes were incubated with Cr-labeled donor phytohemagglutinin-induced target cells at various E:T ratios, ranging from 10:1 to 30:1.

Limiting dilution assay

Cytolytic T lymphocyte precursor frequency was analyzed as previously described by Kaminski and colleagues[19] using recipient cells as responders and donor cells as stimulators, with the addition on days 4 and 6 of recombinant interleukin-2 to fresh medium to give a final concentration of 10 U/mL. On day 10, a cytotoxic assay was carried out for each culture using ^{51}Cr- labeled phytohemagglutinin-induced blast cells from the original stimulator as the targets. Supernatants harvested from each well were measured for ^{51}Cr along with appropriate positive and negative controls; cytolytic T lymphocyte precursor frequency was considered as minimum or background with frequencies of less than 1 in 300,000.[20,21]

Antibody monitoring

Lymphocytotoxic crossmatches were negative in all cases. The pre- and posttransplant sera of 92 recipients were screened by ELISA for the presence of IgG anti-HLA class I and class II alloantibodies according to the manufacturer's instructions (One Lambda Inc). The resulting optical densities were analyzed by LATTM software for Windows (One Lambda Inc).[22,23]

Pathologic studies

Posttransplant biopsies were not taken on protocol but rather because of clinical evidence (or suspicion) of rejection. But a few biopsies in the historic control patients were obtained in pursuit of unrelated research projects,

and some of the lymphoid depleted patients had baseline biopsies before beginning spaced weaning.

Tissues were handled according to hospital procedures.[15] Biopsy findings were categorized by the standardized Banff system[24] and CAN was scored with a scale of 0 to 3 for each of four kinds of abnormality:
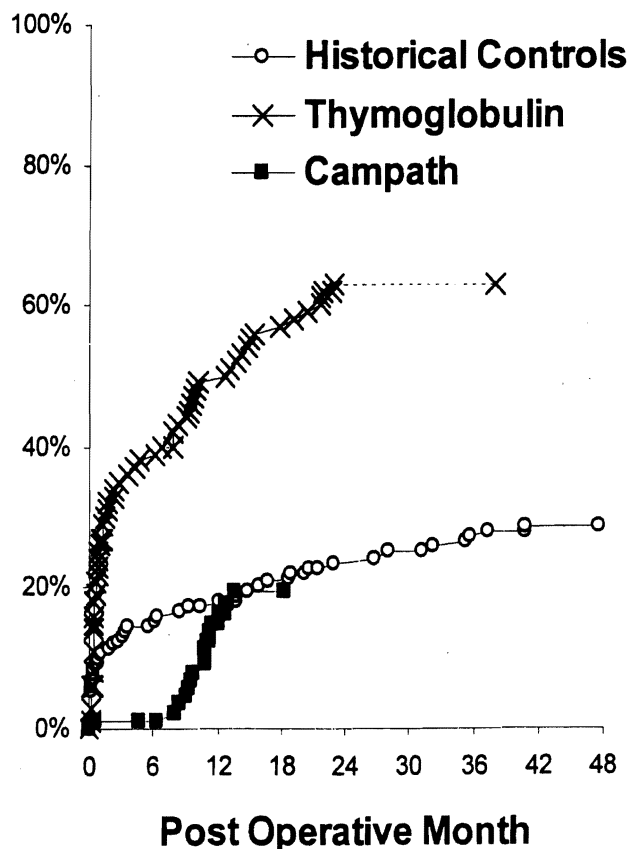

Figure 2. Incidence and time to first acute rejection in historic control (oo), Thymoglobulin-pretreated (X), and Campath-pretreated (black squares) recipients.
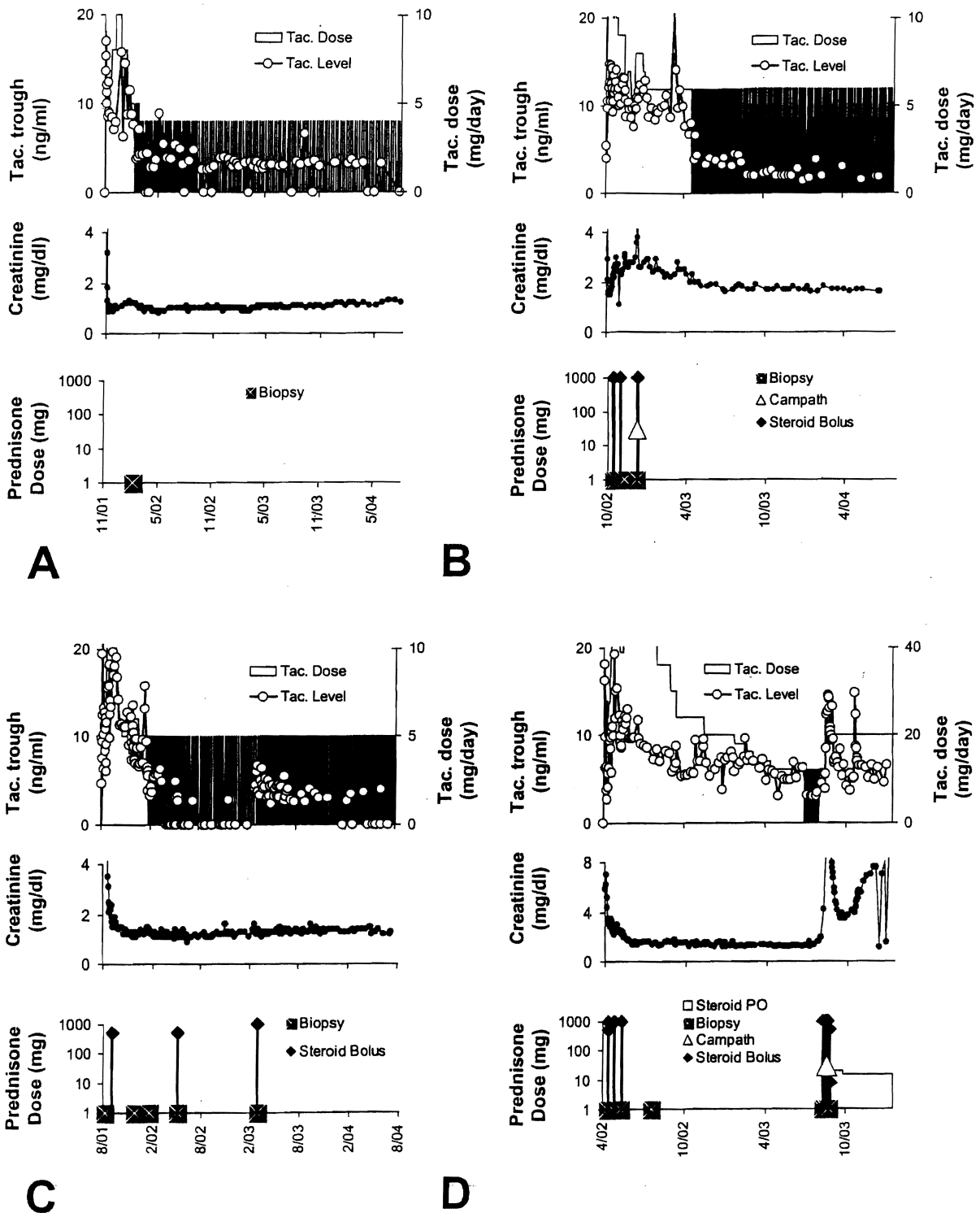
Figure 3. Variable preweaning courses and weaning outcomes in Thymoglobulin-pretreated kidney recipients. Solid shade = daily tacrolimus (Tac) dosing. Spaced weaning represented by spikes that indicate frequency and dose. (A) Uncomplicated weaning from daily to once weekly doses of tacrolimus between 4 and 10 posttransplant mo in a cadaver kidney recipient who has been rejection free for more than 3 y. (B) Spaced weaning begun after 7 mo after a difficult rejection at 2½ mo. Tacrolimus doses have been three times per week for the past year in this recipient of a kidney from a live unrelated donor. (C) Reweaning in a patient who developed a mild rejection after 9 mo on one dose of tacrolimus per week. The eventual (and current) dose is three times per week. (D) Irreversible rejection of a cadaver kidney 4 wk after an attempt to reduce tacrolimus doses to every other day. The patient's original disease was lupus nephritis.

glomerulopathy (cg), interstitial fibrosis (ci), tubular atrophy (ct), and chronic vasculopathy (cv).

Data management and statistical analysis

An honest brokering system approved by the University of Pittsburgh IRB was used for data management. Data were extracted, related, reviewed, augmented (where required) for accuracy and completeness, and deidentified for statistical analysis. Differences in means and standard deviations calculated from participant characteristics by treatment group were evaluated for the statistical significance using t-tests and ANOVAs for continuous comparisons and chi-square tests for categorical comparisons. Kaplan-Meier survival curves were generated and evaluated for significance using a log-rank test. Kaplan-Meier comparisons were adjusted for significant population differences to ascertain their effect. A p value <0.05 two sided was considered significant.

RESULTS

Survival and graft function

Survival

Survival of live donor grafts (Fig. 1) was better in both the Thymoglobulin- and Campath-pretreated patients than in the live donor historic controls ($p \leq 0.037$). Otherwise, patient and graft survival were not significantly different in either of the lymphoid depletion populations versus the historic controls overall ($p = 0.12$ to 0.59) or in the subgroups of the respective cadaveric or liver donor recipients ($p = 0.25$ to 0.61). These findings did not change when survival was adjusted for population differences.

Graft function

Campath-pretreated patients had the best early mean serum creatinine, but there was no difference in the three populations at 1 year (see Table 2). Mean serum creatinine at 2 years and currently in Thymoglobulin-pretreated patients was higher than in historic controls, mostly because of four outliers (see footnote to Table 2).

Rate of acute rejection

The three groups showed a markedly different incidence and time to acute rejection (Fig. 2). In the Thymoglobulin-pretreated patients, the onset of rejection was earlier ($p < 0.001$) and the incidence was higher than in either the Campath or historic control recipients.

The incidence of rejection during the first 6 months after Campath pretreatment was 1%. Rejections that occurred after 6 months were frequently associated in both the Thymoglobulin- and Campath-pretreatment groups with attempts to space wean (see below).

Weaning

After Thymoglobulin pretreatment

Spaced weaning was attempted in 91 (90.1%) of the 101 recipients after a mean of 5.9 ± 1.4 months. Clinical courses were highly variable before and after weaning. The weaning process was uncomplicated in the majority of cases in which it was attempted (Fig. 3A and B). But in 45% of the patients in whom spaced weaning was started, daily therapy was resumed because of acute rejection. If the rejection promptly responded to 1 or 2 boluses of prednisone, less aggressive spaced weaning subsequently was resumed (Fig. 3C).

Weaning either could not be successfully done or was never attempted in about one-third of the recipients. In some of these patients and in others in whom spaced weaned was never tried, grafts were lost to nonreversible rejection. In the patient depicted in Figure 3D, the original renal disease was lupus nephritis.

With followups of 24 to 39 months, 68% of the 83 patients with currently surviving grafts are on spaced doses of maintenance immunosuppression. Another 25% are on daily monotherapy. Only 7% are receiving more than one immunosuppressant (Table 3).

After Campath pretreatment

Spaced weaning was attempted in 83 (91.2%) of the 90 patients after a mean of 6.3 ± 1.2 months. The decision to go forward with weaning was more straightforward than it was in the Thymoglobulin-conditioned patients because of the nearly complete absence of preweaning rejection. In addition, the incidence of postweaning rejection (20%) has been lower (Fig. 2).

With followups of 12 to 18 months, 62 (74%) of the 84 Campath-pretreated patients are on spaced weaning, 14% are on daily monotherapy, and only 12% are receiving more than a single agent (Table 3).

Morbidity

In the historic controls, the incidence of symptomatic infections by cytomegalovirus and by BK virus was 4.5% and 3.2%, respectively. The incidence of posttransplant lymphoproliferative disease was 2%, and 9.7% had new

Table 3. Current Immunosuppression for Recipients with Surviving Grafts

Immunosuppression	Historic controls (n = 113)		Thymoglobulin (n = 83)		Campath (n = 84)	
	n	%	n	%	n	%
Daily monotherapy	46	41	21	25	12	14
Multidrug therapy	67	59	6	7	10	12
Spaced dose weaning	—		56	68	62	74
Once a week	—		12		—	
Twice a week	—		18		6	
Three times a week	—		17		40	
Every other day	—		9		16	

onset diabetes. These complications were not seen in the lymphoid depleted patients, except for a 2% incidence of BK virus infection in the Thymoglobulin series and a 2.2% incidence of new onset diabetes in the Campath patients.

Pathology
Acute rejection
Acute cellular rejection was diagnosed and graded for severity according to the Banff 1997 schema of renal allograft pathology.[24] Patients were biopsied only when there was a rise in creatinine. Several of these specimens showed intimal arteritis, glomerulitis, and focal or diffuse C4d deposition. The incidence of subclinical rejection and its effect on graft morphology in the nonbiopsied patients cannot be addressed because no protocol biopsies were performed. It is notable that only one rejection episode occurred during the preweaning period after a Campath infusion.

Chronic allograft nephropathy
The numbers of recipients having a baseline biopsy within the first 30 days and later biopsies in the historic control, Thymoglobulin- and Campath-pretreated groups were 58 (38%), 52 (51%), and only 8 (9%), respectively. These frequencies largely reflected the relative incidence of early renal dysfunction from primary graft dysfunction or acute rejection. Although these and subsequent samples were in no sense protocol biopsies, enough histopathology was available to tentatively assess the posttransplant progression of CAN.

Only a minority of the baseline (control) biopsy samples were completely free of findings that contribute to the 12-point maximum CAN score. The baseline CAN scores averaged from 1.3 to 2.0 to 2.6 in the three successively compiled series. The acceptance of kidneys with an increasing incidence and severity of preexisting

donor disease reflected nationwide efforts to expand the organ pool, ie, by relaxing the criteria for organ acceptance.

During the first year posttransplantation, CAN progressed from baseline by 2.5 points in recipients pretreated with Thymoglobulin, and by 3.0 points in the historic control patients. The 1-year increase was only 1.4 points in the recipients pretreated with Campath, but this was based on a very small number of biopsies.

In years 2 and 3 posttransplantation, average CAN progressed an additional 1.7 and 0.34 points, respectively, for the Thymoglobulin patients and 1.5 and 1.2 points, respectively, in the historic controls. In the absence of protocol biopsies, an important caveat to the third year value is that very few samples were available at this time point: 13 in the reference group and 7 in the Thymoglobulin group.

Cellular immunologic monitoring
Pretreatment with 5 mg/kg Thymoglobulin, as previously reported,[14,25] resulted in profound depletion of T cells (CD3$^+$) by postoperative day 1. T cell counts remained below baseline for 3 months, with a gradual return toward baseline values. The CD8 population recovered more promptly than the CD4 population, resulting in an inverted CD4/CD8 ratio that persisted up to 6 months posttransplant. B cells were not depleted. The T cell depletion was considerably greater and more sustained with Campath, and in addition, B cells were notably depleted.

Approximately 1 year after transplantation, more detailed studies were obtained in eight of the Thymoglobulin-pretreated patients. Seven of the eight were on spaced weaning, with doses of one per week (n = 2), two per week (n = 1), and three per week (n = 4). The eighth patient was on multiple drugs. The mean CD4/CD8 ratio, which had been 2.3 before lymphoid

depletion, remained less than 1.0 in five of the eight patients between 1.38 and 1.5 in the other three.

At 1 year, all seven of the patients on spaced weaning had donor-specific hyporeactivity demonstrated with MLR; the patient on multiple drugs had global hyporesponsiveness. In six of the seven hyporesponsive patients, kidney function was excellent at the time of testing. The kidney of the seventh patient was failing at the time of testing (serum creatinine 3.7 mg/dL). Biopsy of this allograft revealed recurrent membranous glomerulonephritis similar to that which had destroyed the native kidney, but with no histopathologic evidence of rejection.

In additional studies of four of the seven patients with MLR evidence of donor-specific hyporeactivity, the absence of donor cell killing was confirmed by cell-mediated lymphotoxicity assay, the essential absence with limiting dilution assay of cytolytic T lymphocyte precursor, or both findings. But one of these patients, who was on one dose per week at the time, subsequently developed a mild rejection that was treated with a single dose of prednisone and temporary reinstitution of daily tacrolimus. This patient is presently on three doses of tacrolimus per week.

Antibody monitoring

Serial samples were available from 92 of the 101 Thymoglobulin-pretreated recipients, all of whom had negative conventional crossmatches with their donors. In 59 (64%) of these patients, nonspecific anti-HLA antibodies were never detectable before or after transplantation. In 14 of the other 33, anticlass I antibodies predated transplantation (n = 12) or developed afterward (n = 2). These disappeared in 9 who had adequate subsequent samples, and they were known to persist in only 2 of the others. Similarly, the isolated finding of anticlass II antibodies before or after transplantation was frequently temporary or it waxed and waned. There were only three examples of a class switch or addition.

Combined anticlass I and class II antibodies predated transplantation in five patients and developed de novo after 2, 4, and 12 months in the three others. Of interest, four of the five patients with both kinds of preformed antibodies received zero mismatched kidneys. In two cases, the antibodies disappeared after transplantation.

Except for a trend to poorer results in patients with anticlass II antibodies (ie, graft losses or death at 8, 21, 24, and 28 months), the preexistence or de novo devel-opment of the anti-HLA antibodies did not appear to be associated with clinical outcomes. C4D staining on biopsy samples was not routinely carried out in these patients. So in these patients, a meaningful correlation of the serum antibody and tissue C4D complement deposition was not possible.

DISCUSSION

The efficacy of the multiple drug protocols of immunosuppression in wide use for kidney transplantation has been judged primarily by how well these regimens prevent acute rejection. The extent to which this objective can be accomplished was exemplified by our historic control group. Despite the low incidence of early rejection, however, there was a steady erosion of patient and graft survival that usually was related in some way to chronic rejection, organ-specific drug toxicity, immune depression per se, or combinations of these factors.

With our revised strategy, improvement in the quality of recipient life replaced avoidance of acute rejection as the highest priority. Because the immune activation that can proceed to organ rejection also is the mandatory first step of the seminal tolerance mechanism of clonal exhaustion-deletion,[5-7,26] the greater than 50% rate of early and delayed acute cellular rejection in Thymoglobulin-pretreated patients (Fig. 2) was not necessarily viewed with alarm. Nevertheless, the frequent need for urgent intensification of immunosuppression mandated unusually close physician surveillance. Despite vigilant supervision, there were examples of nonreversible acute rejection that proceeded to graft loss. These cases included, but were not limited to, patients who were on spaced weaning.

The obvious question raised by these observations concerned the risk-to-benefit ratio of the tolerogenic strategy overall, with particular reference to the spaced weaning in the Thymoglobulin series. With followups of 2 to more than 3 years, patient survival, graft survival, and graft function of the Thymoglobulin-pretreated recipients are equivalent overall to the results in the historic controls. In the subgroup of recipients of live donor kidneys, survival parameters are superior to those in the historic controls. Although these results are encouraging, the effect of the high rate of rejection on longterm prognosis in the Thymoglobulin-pretreated recipients cannot yet be definitively evaluated.

Protocol biopsies for histopathologic studies were not

systematically obtained in either the historic control or Thymoglobulin-pretreated patients. Instead, most of the biopsies were done to confirm the clinically diagnosed rejections that were most common in the Thymoglobulin cohort. In cases in which multiple biopsies were done, the severity and progression of CAN were similar in the Thymoglobulin and historic control recipients. The lesions under both kinds of immunosuppression were particularly prevalent in kidneys from cadaveric donors. This was not surprising because it is well known that preexisting donor disease and ischemic injury may contribute to CAN[27-29] and aggravate drug nephrotoxicity.[30] It also is well established that any degree of acute rejection (even of the subclinical variety) may contribute to the arteriopathy, fibrosis, tubular atrophy, and glomerular loss that were the criteria for our semiquantitative histopathologic scale of CAN.[30,31]

Concerns about acute rejection were largely eliminated after Campath was substituted for Thymoglobulin as pretreatment. With Campath, the incidence of rejection before spaced weaning was 1%, and even afterward, the cumulative total has been only 20%. In addition, the trend of better patient and graft survival relative to historic controls out to 1 to 2 years, are much the same as in the Thymoglobulin-pretreated cohort. The superior performance of Campath may reflect, in part, the benefit of lessons previously accrued with the Thymoglobulin experience. For example, because of rejections associated with too rapid weaning in the Thymoglobulin series, spaced dosing beyond every other day or three times a week is no longer attempted until at least 1 year unless there are specific indications (eg, drug nephrotoxicity or neurotoxicity).

It should be emphasized that it is not yet known whether the exceptionally complete elimination of acute early rejection with Campath will carry a delayed price. In addition to its long biologic effect (6 to 12 months), unbound Campath remains in the circulation for 1 to 2 weeks after infusion (information from ILEX Inc), and theoretically could erode the mechanism of clonal exhaustion-deletion in the same way as multidrug posttransplant therapy.[7] If so, clinically silent CAN could develop and not be detected, particularly because the rate of biopsy sampling has been very low with the efficient avoidance of acute rejection.·

The most striking benefit of the tolerogenic immunosuppression in both the Thymoglobulin- and Campath-pretreatment series was the improvement in recipient quality of life. This was reflected in a very low incidence of infection and of de novo malignancies, and freedom from new onset insulin-dependent diabetes. The gains were clearly associated with reduced exposure to chronic daily immunosuppression. In both the Thymoglobulin and Campath series, only about 10% of the recipients who still bear functioning grafts are on more than a single immunosuppressive agent. The vast majority of the others are on spaced dose schedules of the monotherapy, including 12 patients in the Thymoglobulin cohort who are on one dose per week. Based on observations in adults, the Thymoglobulin-based strategy was adopted in April 2003 for all pediatric kidney recipients at our center, and more recently, Thymoglobulin was replaced with Campath.

In conclusion, recipient pretreatment by lymphoid depletion combined with minimalistic posttransplant immunosuppression is an acceptable way to manage kidney transplant recipients. Campath currently appears to be the most effective means of pretreatment. But the strategy, which is designed to permit natural mechanisms of tolerogenesis, is neither drug nor organ specific. It is most easily applied for live donor organ transplantation, but can be readily used for transplantation of cadaveric organs. Additional improvements should be possible, including development of better guidelines for the optimal timing and extent of drug weaning.

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Analysis and interpretation of data: Gray, Bass, Ness
Drafting of manuscript: Shapiro
Critical revision: Starzl
Statistical expertise: Bass, Ness
Supervision: Starzl, Fung, Marcos

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