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Clinical Significance of the Pharmacological Efficacy of Tacrolimus Estimated by the Lymphocyte Immunosuppressant Sensitivity Test (LIST) Before and After Renal Transplantation

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The lymphocyte immunosuppressant sensitivity test (LIST) with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay procedure can predict the pharmacological efficacy of immunosuppressive agents. A previous study reported the pharmacological efficacy of tacrolimus evaluated by LIST just before renal transplantation significantly correlated with the incidence of acute rejection episodes. However, the pharmacological efficacy of tacrolimus has not been estimated after renal transplantation. Therefore, the present study evaluated the pharmacological efficacy of tacrolimus by LIST using the MTT assay procedure before and 1, 3, and 12 months after transplantation in 17 renal transplant recipients that received tacrolimus-based immunosuppressive therapy. The tacrolimus pharmacological efficacies before and after the procedure were also compared with incidence of acute rejection and cytomegalovirus (CMV) infection episodes. The individual values of tacrolimus 50% inhibition of lymphocyte proliferation (IC_{50}) varied widely before transplantation, and the mean value of the IC_{50} was 126.4 ± 337.7 ng/ml. The patients were divided into two groups according to the tacrolimus IC_{50} values evaluated before transplantation. The rate of acute rejection episodes in the tacrolimus high-sensitivity group was significantly lower than that in the tacrolimus low-sensitivity group ($p = 0.005$). The tacrolimus IC_{50} deviation between patients expanded further at one and three months after surgery. However, the sensitivity deviation almost converged at 1 year after surgery. Moreover, the pharmacological efficacy of tacrolimus evaluated at 1, 3, and 12 months after transplantation did not significantly correlate with the incidence of acute rejection episodes. The pharmacological efficacies of tacrolimus evaluated at both before and after surgery were not significantly correlated with the episodes of CMV infection. These findings suggest that the pharmacological efficacy of tacrolimus evaluated with LIST before surgery is a useful biomarker for predicting the occurrence of acute allograft rejection in renal transplantation.

Key words: Tacrolimus; Renal transplantation; Lymphocyte immunosuppressant-sensitivity test (LIST); 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT); Peripheral-blood mononuclear cells (PBMCs)

INTRODUCTION

Tacrolimus is used as a major immunosuppressive agent to prevent acute rejection in renal transplantation. Renal transplant recipients are treated with a combination of tacrolimus, glucocorticoid, and mycophenolate mofetil (MMF), with or without basiliximab. Therapeutic drug monitoring (TDM) of tacrolimus is generally performed to examine the dose of the drug that should be used to optimize the immunosuppressive efficacy. However, estimation of the efficacy of immunosuppressive agents should include pharmacokinetics and pharmacodynamics based on the sensitivity of patient-derived cells to the immunosuppressive agents.

The lymphocyte immunosuppressant sensitivity test (LIST) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay procedure can evaluate the pharmacological efficacy of immunosuppressive drugs using peripheral blood mononuclear cells (PBMCs) of transplant recipients (7–10). A previous study evaluated the pharmacological efficacy of tacrolimus before surgery without basiliximab immunosuppressive treatment in renal transplant recipients (9). The tacrolimus concentration that yielded 50% inhibition of patient cell

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growth (IC_{50}) evaluated by LIST with the MTT assay procedure varied widely between patients before transplantation (9). Furthermore, the rate of rejection episodes was significantly correlated between the tacrolimus high- and low-sensitivity recipients (9). Therefore, the LIST can be used for prevention of acute rejection episodes in renal transplant recipients that receive a tacrolimus immunosuppressive regimen without basiliximab.

The cyclosporine IC_{50} values vary widely before transplantation (7,10). Furthermore, the pharmacological efficacy of cyclosporine and mycophenolic acid (MPA) have been estimated by LIST with the MTT assay procedure before and 1, 3, and 12 months after transplantation (8,10). The deviation of cyclosporine IC_{50} values between recipients further increases at 1 month after transplantation; however, the deviation tended to converge by 3–12 months after transplantation (10). The pharmacological efficacy of MPA assessed before transplantation shows small individual variations (8,13), whereas the variability greatly increased at 1 and 3 months posttransplantation. The individual IC_{50} variation among these subjects at 1 year after surgery is very similar to that observed prior to transplantation (8).

The pharmacological efficacy of tacrolimus has not been estimated by LIST after renal transplantation, and therefore, the present study estimated the pharmacological efficacy of tacrolimus by LIST not only before transplantation but also 1, 3, and 12 months after transplantation in 17 renal transplant recipients. Furthermore, the study examined whether the pharmacological efficacy of tacrolimus by LIST evaluated before and after transplantation correlate with the occurrence of acute rejection and infection episodes.

MATERIALS AND METHODS

Patients

All patients provided written informed consent. Heparinized venous blood (20 ml) was taken from the 17 renal transplant recipients (13 males and 4 females) before and 1, 3, and 12 months after transplantation. The mean age of these recipients was 36.8 ± 15.7 years. The mean human leukocyte antigen (HLA)-AB mismatch number was 2.29 ± 1.05, and the mean HLA-DR mismatch number was 1.0 ± 0.71. The characteristics of these recipients are shown in Table 1. All of the transplant recipients received a renal allograft from living donors after blood-sampling for analysis of their PBMC response to immunosuppressive agents in vitro. All of the recipients underwent renal transplantation from October 2004 to December 2008 at Niigata University Medical and Dental Hospital. The study was approved by the ethics review board of the Medical Faculty of Niigata University.

These patients were primary treated with maintenance immunosuppressive therapy after renal transplantation that included a combination of tacrolimus (Prograf cap., Astellas Co., Japan), with either 20 mg basiliximab at day 0 and day 4 or without basiliximab, plus methylprednisolone and mycophenolate mofetil (MMF; Celcept 250 mg Cap., Chugai Co., Japan). The starting doses of these agents were 0.05 mg/kg/day intravenously or 0.2 mg/kg/day orally for tacrolimus, 125 mg/day for methylprednisolone, and 1,000 or 2,000 mg b.i.d. for MMF. The LIST was carried out for each patient to measure the response of PBMCs to the pharmacological efficacy of tacrolimus in vitro. Any patient that experienced an acute allograft rejection episode was treated with bolus methylprednisolone therapy (pulse therapy) or 15-deoxyspergualin.

Table 1. Characteristics of 17 Renal Transplant Recipients

Male (%)	13/17 (76.5%)
Female (%)	4/17 (23.5%)
Mean age (SD)	36.8 (15.7)
Mean HLA-AB mismatch number (SD)	2.29 (1.05)
Mean HLA-DR mismatch number (SD)	1.0 (0.71)

HLA, human leukocyte antigen.

Reagents

Tacrolimus was kindly provided by the Astellas Co. (Tokyo, Japan). Tetrazolium salt of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Ficoll-Paque was purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). RPMI 1640 medium, fetal bovine serum, and Hanks' balanced salt solution (HBSS) were obtained from Gibco Laboratories (Rockville, NY, USA). Concanavalin A was obtained from Seikagaku Kogyo Co. (Tokyo, Japan). All other reagents were of the highest grade available.

Isolation of PBMCs

Venous blood was taken from the recipients before and 1, 3, and 12 months after transplantation. Venous blood (20 ml) was taken just before the operation and the administration of immunosuppressive drugs on a day when hemodialysis was not performed. Venous blood was taken at 1, 3, and 12 months after transplantation. The blood was taken just before the administration of immunosuppressive agents. Isolation and culture of PBMCs were carried out according to the method described previously (3,5,7–14). Briefly, 5 ml of heparinized blood was loaded onto 4 ml of Ficoll-Paque and centrifuged at $900 \times g$ for 20 min at room temperature. The buffy coat containing lymphocytes was taken and rinsed three times with Hanks' balanced salt solution (HBSS). PBMCs, including lymphocytes, were suspended in RPMI 1640

medium containing 10% fetal bovine serum to a cell density of 1×10^6 cells/ml.

PBMC Culture and Evaluation of Drug Potency

The cell suspension was placed into each well of a 96-well flat-bottomed microplate. Saline-containing concanavalin A was added to each well to a final mitogen concentration of 5.0 μg/ml. Subsequently, an ethanol solution containing tacrolimus was added to give a final drug concentration of 0.0001, 0.001, 0.01, 0.1, 1, 10, 100, or 1,000 ng/ml. The same volume of each vehicle solution was added to control wells. The plate was incubated for 4 days in an atmosphere containing 5% CO_2 at 37°C.

MTT Assay

After 4 days of culture, 10 μl of 5 mg/ml MTT solution dissolved in saline was added to each well, and then the cultures were reincubated under 5% CO_2 at 37°C for 4–5 h (7–13). The plates were centrifuged at $375\times g$ for 5 min to precipitate the cells and formazan produced by the growing cells. Aliquots of the supernatant were removed from each well, and dimethyl sulfoxide was added followed by shaking of the plate on a microshaker for 10 min to dissolve the formazan crystals. The absorbance was read with a microplate reader at 550 nm. Dose–response curves were plotted, and the IC_{50} of the drug was calculated.

Statistical Analysis

The IC_{50} raw data were log-transformed before the statistical analysis because they showed a skewed distribution. The variance of tacrolimus IC_{50}s between only one group (1 month and 12 months after transplantation) was assessed using the Friedman test. Values of $p<0.05$ were considered to indicate statistical significance. The time-course variations of tacrolimus IC_{50}s at before and 1, 3, and 12 months after transplantation were tested by Friedman's repeated measures analysis of variance by ranks. The rate of acute rejection (except accelerated acute rejection episode) and cytomegalovirus (CMV) infection episode in patients treated by tacrolimus without basiliximab immunosuppressive therapy was compared between the tacrolimus high and low sensitivity groups by Fisher's exact probability tests. These data analyses were performed using the PASW statistics base 18.0 software package (SPSS Japan, Inc., an IBM company) and EXCEL 2007 (Microsoft).

RESULTS

The present study compared the pharmacological efficacy of tacrolimus before and 1, 3, and 12 months after transplantation. Figure 1 shows typical dose–response curves for tacrolimus against concanavalin A-stimulated blastogenesis of PBMCs of one recipient before and after

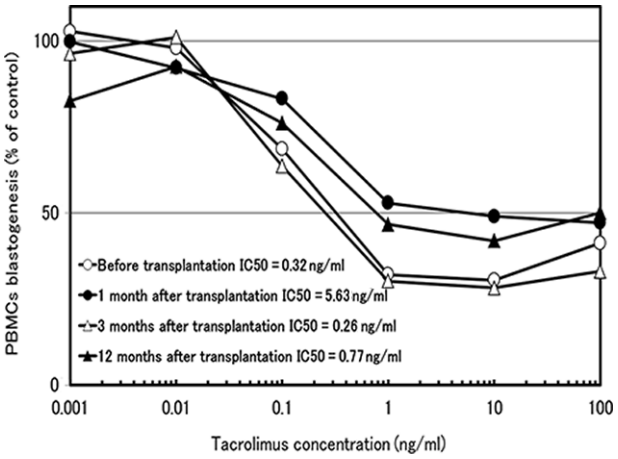

Figure 1. Typical dose–response curves of the effect of tacrolimus on concanavalin A-stimulated blastogenesis of peripheral blood mononuclear cells (PBMCs) before and 1, 3, and 12 months after transplantation in one renal transplant recipient.

transplantation. Pretransplant and posttransplant IC_{50} values for tacrolimus in recipients are listed in Table 2 together with clinical events, including acute rejection episodes, cytomegalovirus (CMV) infection, and conversion from tacrolimus to cyclosporine. The log-transformed IC_{50}s of all recipients were plotted as shown in Figure 2. The mean tacrolimus IC_{50} before transplantation was 126.4 ± 337.7 ng/ml (median, 0.63 ng/ml) and ranged from 0.0075 to 1042 ng/ml. The mean tacrolimus IC_{50} was 235.7 ± 437 ng/ml (median, 0.16 ng/ml) 1 month after transplantation and the range was 0.0001–1,000 ng/ml. On the other hand, the mean IC_{50} at 3 months after transplantation was 65.0 ± 241.6 ng/ml (median, 0.64 ng/ml), and the range was 0.00091–1,000 ng/ml. The mean IC_{50} 12 months after transplantation was 118.1 ± 331.9) ng/ml (median 0.55 ng/ml), and the range was 0.06–1,000 ng/ml.

A statistical analysis using the Friedman test showed significant differences in the variance of tacrolimus log transfomed-IC_{50}s between the values at 1 month and 12 months after transplantation ($p=0.01$). In brief, the efficacy of tacrolimus showed large deviations before and at 1 month after transplantation, but the variation of the efficacy decreased sharply at 12 months after the operation (Fig. 2). The IC_{50} values in the recipients with no rejection episodes also showed large deviations after transplantation (Fig. 3).

There were three cases of primary acute rejection episodes in 17 recipients 3/17 (17.6%) during the first 6 months after transplantation. These primary episodes occurred at 7, 9, and 28 days after transplantation. The pharmacological efficacies tacrolimus 1 month after transplantation were shifted to high sensitivity in these recipients who experienced an acute rejection episode in

Table 2. Tacrolimus Pharmacological Efficacy Estimated by the LIST Before and 1, 3, and 12 Months After Transplantation and Clinical Events in 17 Renal Transplant Recipients

Recipients (Rejection Episodes and Primary Used CNI)	Tacrolimus IC_{50} (ng/ml)				Clinical Events (Days After Operation)		
	Before Transplantation	1 Month After Transplantation	3 Months After Transplantation	12 Months After Transplantation	Rejection Episode at: (Days)	CMV Infection Episode at: (Days)	Conversion of CNI at: (Days)
Rejection (–) Tac	0.0075	0.00010	0.50	0.50	–	–	–
Rejection (–) Tac	0.046	0.10	0.095	0.23	–	–	–
Rejection (–) Tac	0.14	1000	0.78	0.61	–	14	–
Rejection (–) Tac	0.48	0.042	0.58	1.08	–	20	–
Rejection (–) Tac	0.63	0.07	0.93	0.85	–	34	–
Rejection (–) Tac	0.65	1000	0.25	1000	–	19	–
Rejection (–) Tac	0.67	0.00028	5.01	0.47	–	–	–
Rejection (–) Tac	0.79	0.16	0.61	1.15	–	–	77
Rejection (–) Tac	0.90	1000	6.93	0.73	–	113	–
Rejection (+) Tac	1.04	0.37	0.00091	0.36	28,88	–	–
Rejection (+) Tac	100	0.34	72.40	1000	7	66	–
Rejection (+) Tac	1042	0.0013	9.99	0.44	9	–	–
AAR (+) Tac	0.37	0.0061	1000	0.55	2,17	12	–
AAR (+) Tac	0.63	1000	0.001	0.11	2	–	–
AAR (+) Tac	1000	0.65	5.78	0.27	3,9	–	–
Rejection (–) Bax	0.18	0.060	0.64	0.06	–	–	–
Rejection (–) Bax	0.32	5.63	0.26	0.77	–	–	–
Mean	126.4	235.7	65.0	118.1			
SD	337.7	437.0	241.6	331.9			
Median	0.63	0.16	0.64	0.55			
Minimum	0.0075	0.0001	0.00091	0.06			
Maximum	1042.0	1000.0	1000.0	1000.0			

LIST, lymphocyte immunosuppressant sensitivity test; CMV, cytomegalovirus; CNI, calcineurin inhibitor; AAR, accelerated acute rejection; Tac, tacrolimus without basiliximab immunosuppressive therapy; Bax, tacrolimus with basiliximab immunosuppressive therapy.

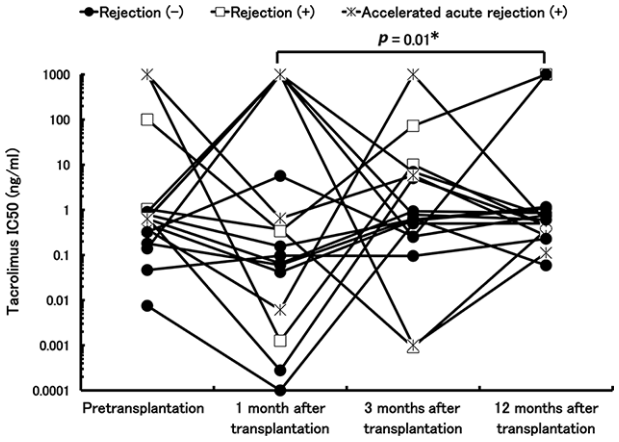

Figure 2. Variation of tacrolimus IC_{50}s evaluated by lymphocyte immunosuppressant sensitivity test (LIST) before and 1, 3, and 12 months after transplantation in 17 renal transplant recipients. The Friedman test was applied to compare the IC_{50} values. The variance of tacrolimus IC_{50}s was only significant between 1 month and 12 months after transplantation (p=0.01 by Friedman test).

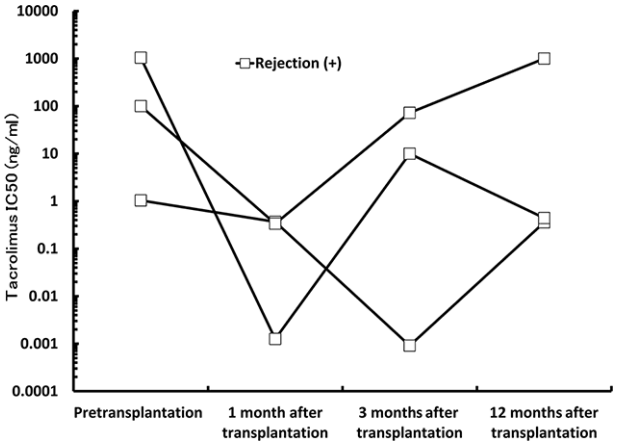

Figure 4. Variation of tacrolimus IC_{50}s evaluated by LIST before and 1, 3, and 12 months after transplantation in the recipients who experienced episodes of acute rejection.

comparison to those before surgery (Fig. 4). There were three cases of accelerated acute rejection that were not due to immunological mechanisms, (3 of 17; 17.6%). These primary episodes occurred at 2, 2, and 3 days after transplantation (Fig. 5).

The IC_{50} values before and after renal transplantation in the recipients who experienced primary acute rejection or accelerated acute rejection also deviated between the recipients (Figs. 4 and 5). Recurrence of acute rejection episodes did not occur in these recipients during the 12 months after transplantation. The mean of the serum creatinine concentration at 1, 3, and 12 months after transplantation in all of the recipients was 1.3±0.3, 1.3±0.3, and 1.2±0.3 mg/dl, respectively. Graft function tended to stabilize at 3 and 12 months after transplantation. CMV antigenemia test positive results occurred in 7/17 (41.2%) cases, at 12, 14, 19, 20, 34, 66, and 113 days after transplantation. The IC_{50} values in the recipients experienced CMV infection episodes were also deviated largely after transplantation (Fig. 6).

One of the 17 patients was converted from tacrolimus to cyclosporine (5.9%) at day 77 (Table 2).

Finally, the clinical significance of the pharmacological efficacy of tacrolimus evaluated before and after transplantation was examined. Twelve patients without an accelerated acute rejection episode and experienced tacrolimus with basiliximab immunosuppressive therapy were divided into two groups according to their PBMC sensitivity to tacrolimus before transplantation by the cutoff IC_{50} value of 1.0 ng/ml, established in a previous study (9).

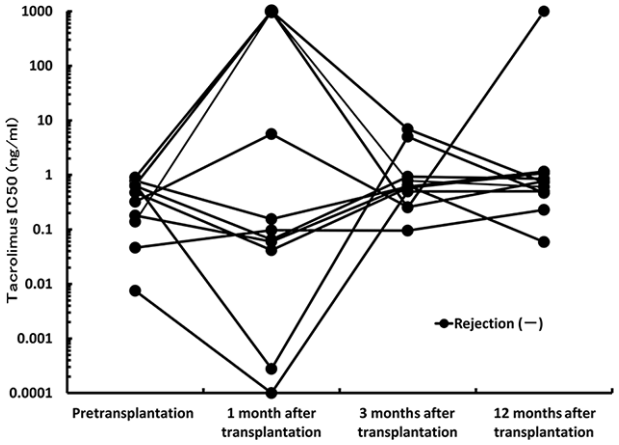

Figure 3. Variation of tacrolimus IC_{50}s evaluated by LIST before and 1, 3, and 12 months after transplantation in recipients with no rejection episodes.

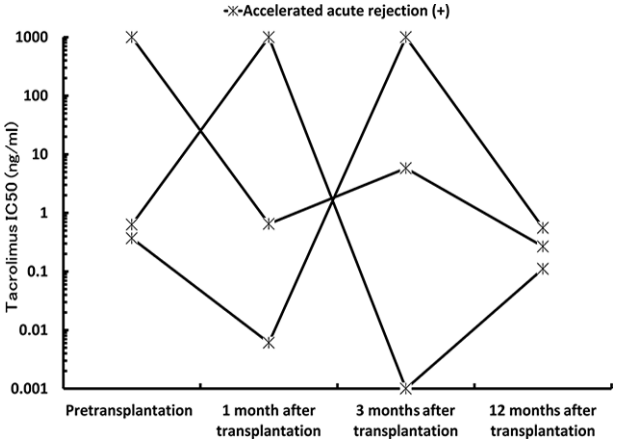

Figure 5. Variation of tacrolimus IC_{50}s evaluated by LIST before and 1, 3, and 12 months after transplantation in the recipients who experienced accelerated acute rejection episodes.

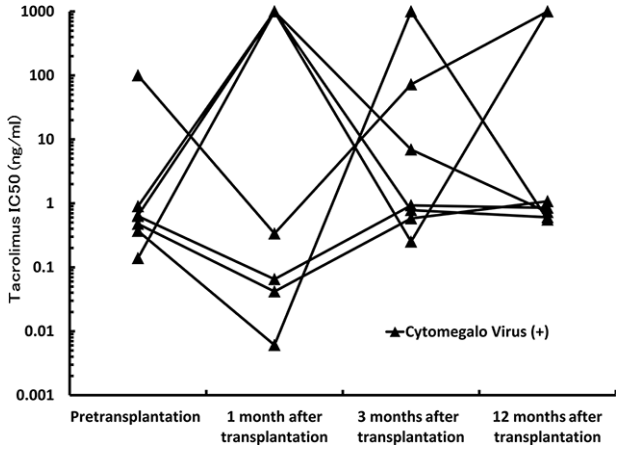

Figure 6. Variation of tacrolimus IC_{50}s evaluated by LIST before and 1, 3, and 12 months after transplantation in the recipients who experienced cytomegalovirus (CMV) infection episodes.

The rate of acute rejection episodes in the tacrolimus high sensitivity group (tacrolimus $IC_{50} < 1.0$ ng/ml) was 0/9 case (0%). In contrast, the rate in the low sensitivity group (tacrolimus IC_{50} value > 1.0 ng/ml) was 3/3 (100%). Therefore, the incidence of acute rejection episodes was significantly higher in the tacrolimus low sensitivity group ($p = 0.005$). However, the incidence of acute rejection episodes was not significantly different between tacrolimus high- and low-sensitivity groups that were classified based on the PBMC sensitivity to tacrolimus at 1, 3, and 12 months posttransplantation. Furthermore, the CMV infection rate was not significantly different between the tacrolimus high- and low-sensitivity groups classified based on the tacrolimus IC_{50} values before and 1, 3, and 12 months after transplantation. Table 3 shows the incidences of rejection episode and CMV infection in relation to PBMC sensitivity to tacrolimus before surgery in these 12 renal transplant recipients.

DISCUSSION

This study evaluated the pharmacological efficacy of tacrolimus by LIST with MTT assay procedure in 17 renal transplant recipients before and 1, 3, and 12 months after transplantation. The tacrolimus IC_{50} values varied widely before transplantation, which was consistent with previous data (7,9). The deviation of tacrolimus IC_{50} values increased further at 1 and 3 months after transplantation in the current series; however, the deviation tended to converge at 12 months after transplantation. A previous study evaluated the tacrolimus pharmacological efficacy by LIST just before transplantation in renal transplant recipients treated with tacrolimus without basiliximab immunosuppressive therapy after surgery (9). The rate of acute rejection episodes in the tacrolimus high-sensitivity group is significantly lower than that in low-sensitivity group (9). The present results showed that the rate of acute rejection episode in the recipients with low tacrolimus sensitivity (tacrolimus $IC_{50} > 1.0$ ng/ml) evaluated before transplantation was also significantly high, which was consistent with that of the previous study (9). However, there was no significant difference in the rate of accelerated acute rejection episodes that were not associated with immunological mechanisms between the recipients with high and low tacrolimus IC_{50} values estimated before surgery.

However, the rate of acute rejection episode was not significantly different in the tacrolimus high- and low-sensitivity groups estimated at 1, 3, and 12 months posttransplantation. Furthermore, CMV infection showed no difference between the tacrolimus high- and low-sensitivity groups estimated before, 1, 3, and 12 months after transplantation. These observations suggest that the pharmacological efficacy tacrolimus estimated by LIST before transplantation is the most useful biomarker for prediction of acute rejection in renal transplantation.

The pharmacological efficacy of cyclosporine was previously estimated by LIST with MTT assay procedure in renal transplant recipients before and 1, 3, and 12 months after surgery (10). The cyclosporine IC_{50} values vary widely before transplantation. The deviation of cyclosporine IC_{50} values between patients increased further at 1 month after transplantation. However, the deviation tended to converge by 3–12 months after

Table 3. Number of Cases With Allograft Rejection Episode and CMV Infection in Relation to PBMC Sensitivity to Tacrolimus Before Operation in the 12 Renal Transplant Recipients*

	High-Sensitivity Group ($IC_{50} < 1.0$ ng/ml)	Low-Sensitivity Group ($IC_{50} > 1.0$ ng/ml)	
Clinical Event	$n = 9$	$n = 3$	p
Rejection episode	0/9 (0%)	3/3 (100%)	0.005*
CMV infection episode	5/9 (55.7%)	1/3 (33.3%)	0.52

CMV, cytomegalovirus; PBMC, peripheral blood mononuclear cells.
*These recipients who experienced an accelerated acute rejection episode and the recipients under tacrolimus with basiliximab immunosuppressive therapy were excluded.

transplantation. In contrast to the present study, the pharmacological efficacy cyclosporine before transplantation did not correlate with the rate of the acute rejection episodes. These recipients received cyclosporine-based immunosuppressive therapy combined with basiliximab and other immunosuppressive agents. Therefore, basiliximab may also prevent acute rejection in recipients with low sensitivity due to cyclosporine (10) due to a strong potentiating effect of basiliximab with cyclosporine by blocking IL-2 receptor on T cells. Furthermore, the MPA efficacy was previously estimated using LIST before and 1, 3, and 12 months after transplantation in renal transplant recipients (8). These recipients received MMF, cyclosporine, basiliximab, and methylprednisolone as immunosuppressive therapy. The pharmacological efficacy of MPA before transplantation showed small individual variations (8,13), whereas the variability greatly increased at 1 and 3 months posttransplantation. However, the individual IC_{50} variation among these subjects at 1 year after operation was closely similar to that observed before transplantation (8).

The blood was obtained for LIST from recipients at the time of rejection episodes after the pulse therapy, and the tacrolimus sensitivity of $CD4^+$ and $CD8^+$ T cells in these recipients may have been affected by the immunosuppressive therapy at 1 and 3 months transplantation. Two recipients exhibited extremely low tacrolimus sensitivity as estimated by LIST at 12 months after renal transplantation. However, these two recipients showed stabilized renal function at 12 months after transplantation. Moreover, the pharmacological efficacy of tacrolimus in these two recipients strikingly fluctuated between before and after transplantation. Antirejection therapy possibly caused the shift in the tacrolimus pharmacological efficacy estimated with LIST in these recipients at 12 months after transplantation.

Most of the current recipients received tacrolimus without basiliximab as immunosuppressive therapy for prevention of acute rejection, while two recipients were treated by tacrolimus with basiliximab and other immunosuppressive agents. The pharmacological efficacy of tacrolimus after transplantation in these recipients was similar to that before transplantation. Therefore, basiliximab might prevent acute rejection episode and stabilize the pharmacological efficacy of tacrolimus on PBMCs after renal transplantation.

Blood samples were taken from the renal transplant recipients just before administration of immunosuppressive agents after transplantation, and therefore, the blood samples used for LIST contained tacrolimus. However, PBMCs were washed three times by HBSS, which was sufficient to wash out tacrolimus from the PBMC fraction (8,10).

All 17 renal transplant recipients were making satisfactory progress at 12 months after transplantation: They tion or acute rejection episodes at 12 months after transplantation. Individual variations in the pharmacological efficacy of tacrolimus decreased after a long-term immunosuppressive therapy in patients with stabilized allograft renal function without infection. No recipients presented acute rejection episodes or CMV and other infections at 12 months after renal transplantation.

Concanavalin A was used as a mitogen to induce blastogenesis in the patient's PBMCs (3,5,7–14). Concanavalin A can activate mitosis both CD4- and CD8-positive T cells. Therefore, the present result must reflect the sensitivity of both subpopulations of T lymphocytes. Flechner et al. reported that the population of CD4- and CD8-positive T lymphocytes fluctuates in cyclosporine or tacrolimus-based immunosuppressive therapy (1). Furthermore, the percentages of CD4- and CD8-positive T cells and the CD4/CD8 ratio are significantly different between day 0 and 3 or 6 months posttransplantation in recipients treated with either cyclosporine- or tacrolimus-based immunosuppressive therapy (6). In addition, he number of IL-2-expressing CD4 and CD8 T cells decreases significantly from days 0 to 3 or at 6 months posttransplantation. Therefore, the peripheral lymphocyte function of renal transplant recipients might be changed by chronic immunosuppressive therapy with calcineurin inhibitors (6). Furthermore soluble CD4 and CD8 levels are significantly higher in recipients with acute allograft rejection than those in recipients with stable allograft function (2). Similarly, the mean CD4 and CD8 levels in transplant recipients at the diagnosis of an acute rejection episode are significantly higher than those in the control group (4). Therefore, immunosuppressive therapy and acute rejection episodes are thought to affect CD4- and CD8-positive T cells in renal transplant recipients (2,4).

In conclusion, the current findings suggest that the pharmacological efficacy of tacrolimus evaluated by LIST before surgery is a useful biomarker for acute allograft rejection in renal transplantation. The deviation in the pharmacological efficacy of tacrolimus in recipients observed before operation expanded further at 1 and 3 months after transplantation, and the levels almost converged at 1 year after transplantation. However, the pharmacological efficacy of tacrolimus evaluated after surgery did not correlate with either of the incidence of acute rejection or CMV infection.

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