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Peripheral Lymphocyte Response to Mycophenolic Acid In Vitro and Incidence of Cytomegalovirus Infection in Renal Transplantation

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The lymphocyte immunosuppressant sensitivity test (LIST) with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay procedure has been used to predict the pharmacological efficacy of immunosuppressive agents to prevent acute rejection episodes for renal transplant recipients. In this study, mycophenolic acid (MPA) pharmacological efficacies were evaluated by LIST at both prior to and just after renal transplantation. We compared the efficacies to the clinical outcome of these recipients. MPA's pharmacological efficacy was evaluated by LIST not only before the operation but also at 2, 4, and 6 weeks after transplantation in 16 renal transplant recipients. These recipients were divided into high- and low-sensitivity groups according to peripheral blood mononuclear cell (PBMC) sensitivity to MPA in vitro. The MPA sensitivities were compared to cytomegalovirus (CMV) infection and acute rejection episodes in these recipients under MPA immunosuppressive therapy. The rate of CMV infection episodes in the low-MPA pharmacological efficacy group categorized at 2 weeks after renal transplantation was 5/6 (83.3%), which was significantly higher than the rate of 1/10 (10.0%) ($p<0.01$) in the high-MPA sensitivity group. However, the MPA pharmacological efficacy evaluated both before and after transplantation had no relationship with the incidence of rejection episodes. These findings suggest that the MPA pharmacological efficacy evaluated by LIST at 2 weeks after operation is a useful biomarker for predicting the following occurrence of CMV infection episodes in renal transplant recipients.

Key words: Cytomegalovirus (CMV); Lymphocyte immunosuppressant sensitivity test (LIST); Mycophenolate mofetil (MMF); Mycophenolic acid (MPA); Peripheral blood mononuclear cell (PBMC); Renal transplantation

INTRODUCTION

Cytomegalovirus (CMV) infection is still a cause of morbidity among solid organ transplant recipients. The treatment of CMV infections in renal transplant recipients is mainly carried out by ganciclovir or valganciclovir. CMV infection incidence at 3 years after transplantation in a treatment group with high dosage of mycophenolate mofetil (MMF) (3.0 g/day) was reported to be higher than that in an azathioprine-treated group (16). Furthermore, renal transplant recipients with ganciclovir resistance have been reported (5). However, the prediction of the occurrence of CMV infection episodes in each patient is difficult after renal transplantation.

We previously reported the clinical significance of the pharmacological efficacy of mycophenolic acid (MPA), mizoribine, and azathiorine (6-mercaptopurine) evaluated by the lymphocyte immunosuppressant sensitivity test (LIST) with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay procedure (14). The MPA pharmacological efficacy to suppress mitogen-activated peripheral blood mononuclear cells (PBMCs) in renal transplant recipients and healthy subjects displayed the smallest degree of variation between the study subjects in comparison to that of the other purine synthesis inhibitors (14). MPA pharmacological efficacy has been evaluated by LIST not only prior to operation but also at 1, 3, and 12 months after transplantation (9). The variability of MPA efficacy greatly increased at 1 and 3 months posttransplantation, whereas the variation in IC_{50} values among the subjects

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after 1 year was almost similar to that observed prior to surgery (9). However, MPA pharmacological efficacy has not been evaluated by LIST at the time just after renal transplantation.

Thus, in this study, MPA pharmacological efficacy was evaluated prior to and at 2, 4, and 6 weeks after renal transplantation, and these MPA pharmacological efficacies were compared with the occurrence of CMV infection and rejection episodes in renal transplant recipients.

MATERIALS AND METHODS

Patients

The study was approved by the Ethics Review Board of the Medical Faculty of Niigata University, and all patients included in this study provided written informed consent. Heparinized venous blood (20 ml) was taken from the 16 renal transplant recipients (12 males and 4 females) before and at 2, 4, and 6 weeks after transplantation. The mean age of these recipients was 42.9 ± 14.0 years. The mean human leukocyte antigen (HLA)-AB mismatch number was 2.56 ± 0.73, and the mean HLA-DR mismatch number was 1.19 ± 0.54. 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. Serum creatinine was measured by automated enzyme immunoassay system (Kainos, Tokyo, Japan) before and at 2, 4, and 6 weeks after transplantation. All of the recipients underwent renal transplantation from February 2009 to May 2010 at Niigata University Medical and Dental Hospital, Niigata, Japan.

Immunosuppressive Therapy

These patients were primarily treated with maintenance immunosuppressive therapy after renal transplantation that included a combination of either tacrolimus (FK-506; Prograf cap., Astellas Co., Tokyo, Japan) or cyclosporine (Neoral cap., Novartis Pharma Co., Basel, Switzerland) with either 20 mg basiliximab (Simulect, Novartis Pharma Co.) on days 0 and 4 plus methylprednisolone (Medrol; Pfizer Co., Groton, CT, USA) and mycophenolate mofetil (MMF Cellcept 250 mg cap., Chugai Pharmaceutical Co., Tokyo, Japan). Two patients who received tacrolimus-based immunosuppressive therapy were not administered basiliximab. The starting doses for tacrolimus were 0.05 mg/kg/day intravenously or 0.2 mg/kg/day orally, and the starting doses for cyclosporine were 2–3 mg/kg/day intravenously or 8 mg/kg/day orally. The starting dose for methylprednisolone was 125 mg/day, and that for MMF was 1,000 or 2,000 mg b.i.d. The LIST was carried out for each patient to measure the response of PBMCs to the pharmacological efficacy of MPA in vitro. Blood calcineurin inhibitor concentration was measured by chemiluminescence immunoassay (CLIA; Abbott Laboratories, Abbott Park, IL, USA).

Diagnosis of CMV Infection and Acute Rejection Episode

CMV infection episode was diagnosed by detection of serum CMV IgG and IgM as CMV biomarkers (CMV antigenemia; Mitsubishi Chemical Medience Corporation, Tokyo, Japan). Furthermore, the rejection episode was proven by an ultrasound-guided kidney needle biopsy (Hitachi-Aloka Medical Ultrasonography System, Tokyo, Japan, and 16-gauge biopsy needle, C.R. Bard, Inc., Murray Hill, NJ, USA) of the graft. The graft biopsy is processed for routine light microscopy, immunofluorescence [mouse anti-human IgG, IgA, IgM, κ, λ, complement 1q (C1q), C3c, C4c, and fibrinogen with fluorescent-labeled anti-mouse Igs as second antibodies (Dako, Kyoto, Japan)], and electron microscopy and then diagnosed by a renal pathologist.

Reagents

MPA was obtained from Wako Chemical Co. (Osaka, Japan). Tetrazolium salt of MTT was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Ficoll-Paque was purchased from Amersham Pharmacia Biotech (Little Chalfont, Buckinghamshire, UK). Roswell Park Memorial Institute (RPMI) 1640 medium, fetal bovine serum, and Hanks' balanced salt solution 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 (20 ml) was taken from these recipients before and at 2, 4, and 6 weeks after transplantation. The blood sampling was carried out just before the administration of immunosuppressive drugs on a day when hemodialysis was not performed. The isolation and culture of PBMCs were carried out according to the methods described previously (3,7–14). 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 was taken and rinsed three times with Hanks' balanced salt solution. 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 (Becton Dickinson, Franklin Lakes, NJ, USA). Saline containing concanavalin A was added to each well to a final mitogen concentration of 5.0 μg/ml. Subsequently, an ethanol solution containing MPA was added to give a final drug concentration of 0.001, 0.01, 0.1, 1, 10, 100, 1,000, or 10,000 ng/ml. The same volume of each vehicle solution was added to the control wells (no concanavalin A or MPA treatment).

The plate was then 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 (7–14). The plates were centrifuged at $375 \times g$ for 5 min to precipitate the cells and the formazan produced by the growing cells. Aliquots of the supernatant were removed from each well, and dimethyl sulfoxide was added to dissolve the formazan crystals followed by shaking of the plate on a microshaker (Micromix5; Siemens, Munich, Germany) for 10 min. The absorbance was read with a microplate reader (Immuno-mini NJ-2300; Biotec, Tokyo, Japan) at 550 nm. Dose–response curves were plotted, and the IC_{50} values of the drug were calculated.

Statistical Analysis

The renal transplant recipients were divided into two subgroups according to their PBMC sensitivity to MPA estimated in vitro by LIST (9). The threshold of the MPA IC_{50} values to divide the patients into two groups was 287.3 ng/ml, which was calculated from the mean ± 2 SD of the values of renal transplant recipients we reported previously (9).

The patients who showed MPA IC_{50} values of <287.3 ng/ml were classified as an MPA high-sensitivity group, while the patients with MPA IC_{50} values of >287.3 ng/ml were classified as an MPA low-sensitivity group (9). The difference of reinfection of CMV antigenemia episodes or acute rejection episodes within 3 months after transplantation between any two recipient subgroups was estimated by the Fisher's exact probably test. Values of $p < 0.05$ were considered to indicate statistical significance. These data analyses were performed using the PASW statistics base 18.0 software package (SPSS Japan Inc., IBM, Tokyo, Japan) and EXCEL 2010 (Microsoft, Redmond, WA, USA).

RESULTS

The pharmacological efficacy of MPA was estimated by LIST before transplantation and at 2, 4, and 6 weeks after transplantation. Figure 1 shows typical dose–response curves for MPA against concanavalin A-stimulated proliferation of PBMCs for one recipient who experienced a CMV infection episode. Pre- and posttransplant IC_{50} values for MPA in 16 recipients are listed in Table 1 together with CMV infection and acute rejection episodes. The mean (±SD) MPA IC_{50} value before transplantation in these recipients was 161.5 ± 270.9 ng/ml, and the median (range) was 38.2 (0.25–1,000.0) ng/ml. The mean (±SD) MPA IC_{50} at 2 weeks after transplantation increased to 2,143.9 ± 3,651.4 ng/ml, and the median (range) was 55.5 (0.01–10,000.0) ng/ml. Thus, the deviation of the IC_{50} values between the subjects also increased at 2 weeks

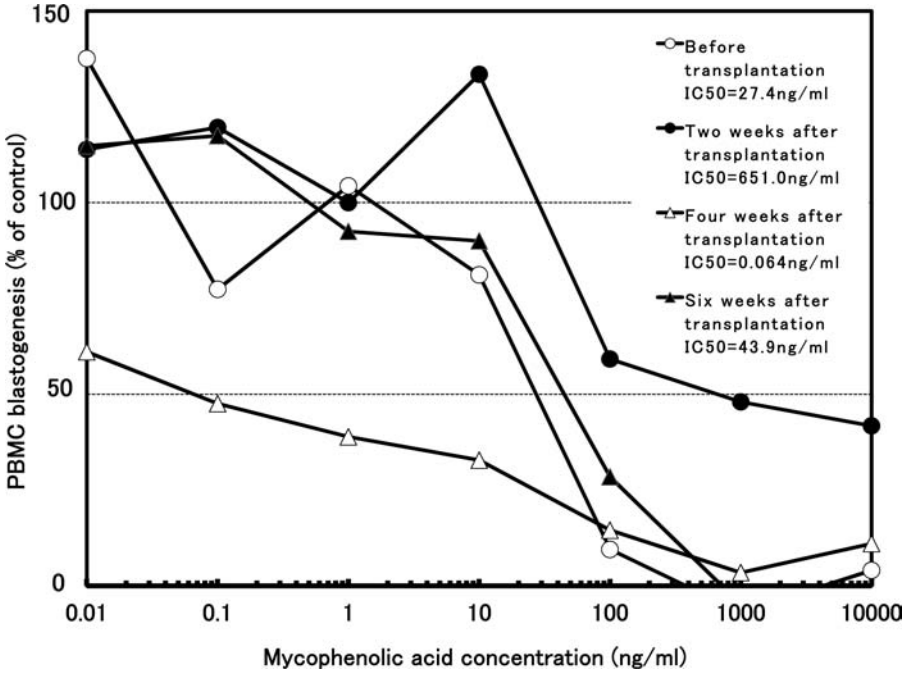

Figure 1. Dose–response curve of MPA on PBMCs at different time points from transplantation. Typical dose–response curves of the effect of mycophenolic acid (MPA) on concanavalin A-stimulated proliferation of peripheral blood mononuclear cells (PBMCs) before transplantation and at 2, 4, and 6 weeks after transplantation in one renal transplant recipient.

Table 1. Mycophenolic Acid Pharmacological Efficacy Estimated by the LIST Before and 2, 4, and 6 Weeks After Transplantation and Clinical Events in 16 Renal Transplant Recipients

					Clinical Events (Days After Operation)			
Recipients	Before Transplantation	2 Weeks After Transplantation	4 Weeks After Transplantation	6 Weeks After Transplantation	CMV Reinfection Episode at (Days)	Rejection Episode at (Days)	Main CNI	Conversion of CNI at (Days)
1	0.25	10,000.0	0.0025	1,259.6	16	–	CyA	–
2	56.7	6,043.1	0.01	3.2	20	–	FK+Bax	–
3	0.89	5,868.8	84.6	10,000.0	20,48	–	FK	–
4	426.1	1,459.8	447.2	10,000.0	34	–	CyA	–
5	27.4	651.0	0.064	43.9	34	–	CyA→FK	7
6	39.1	0.17	0.01	27.4	27	–	CyA	–
7	37.3	10,000.0	524.9	0.17	–	41	CyA	–
8	0.32	92.7	75.1	2.8	–	37	CyA	–
9	510.9	66.7	123.2	45.1	–	2	CyA	–
10	29.4	44.3	57.9	94.2	–	17	FK+Bax	–
11	192.2	31.8	139.7	27.9	–	–	CyA	–
12	31.5	25.4	74.3	35.3	–	–	CyA	–
13	17.7	18.4	60.1	25.3	–	47	CyA	–
14	49.5	0.80	87.1	21.5	–	–	CyA	–
15	165.3	0.01	60.3	45.2	–	–	FK	–
16	1000.0	0.01	35.4	5,973.6	–	–	CyA→FK	6
Mean	161.5	2,143.9	110.6	1,725.3				
SD	270.9	3,651.4	153.2	3,555.9				
Median	38.2	55.5	67.3	39.6				
Minimum	0.25	0.01	0.0025	0.17				
Maximum	1000.0	10,000.0	524.9	10,000.0				

LIST, lymphocyte immunosuppressant sensitivity test; CMV, cytomegalovirus; CNI, calcineurin inhibitor; CyA, cyclosporine; FK, tacrolimus (FK-506) without basiliximab immunosuppressive therapy; FK+Bax, tacrolimus with basiliximab immunosuppressive therapy.

postoperation. On the other hand, the mean IC_{50} (±SD) at 4 weeks after operation recovered to 110.6 ± 153.2 ng/ml, but the median (range) remained at 67.3 (0.0025–524.9) ng/ml. However, the mean IC_{50} (±SD) at 6 weeks after transplantation increased again to 1,725.3 ± 3,555.9 ng/ml, and the median (range) recovered to 39.6 (0.17–10,000.0) ng/ml. Primary CMV antigenemia (+) reinfection episodes occurred in 6 of 16 (37.5%) recipients at 16, 20, 20, 27, 34, and 34 days, respectively, after transplantation (Table 1). On the other hand, primary acute rejection episodes occurred at 2, 17, 37, 41, and 47 days after transplantation in five different recipients. The incidence of acute rejection episodes in these recipients was 5/16 (31.3%) (Table 1).

Two of the 16 patients were converted from their immunosuppressive therapy of cyclosporine to tacrolimus (12.5%) on days 6 and 7, respectively (Table 1). The mean serum creatinine concentrations (±SD) before and at 2, 4, and 6 weeks after transplantation in these 16 recipients were 10.5 ± 3.81, 1.65 ± 0.9, 1.51 ± 0.88, and 1.50 ± 0.73 mg/dl, respectively. The mean serum creatinine concentration did not significantly differ between the high- and low-MPA sensitivity groups (data not shown).

In the case of recipients with CMV reinfection without rejection ($n = 6$), the MPA IC_{50} values estimated by LIST before and at 2, 4, and 6 weeks after transplantation are shown in Figure 2. Furthermore, recipients without CMV infection and with acute rejection episode ($n = 5$) have MPA IC_{50} values estimated by LIST before and at 2, 4, and 6 weeks after transplantation and are shown in Figure 3. Similarly, in patients without CMV infection or a rejection episode ($n = 5$), the MPA IC_{50} values estimated by LIST before and at 2, 4, and 6 weeks after transplantation are shown in Figure 4.

The clinical significance of the MPA pharmacological efficacy was compared between the two patient subgroups divided according to their PBMC sensitivity to MPA at 2 weeks after transplantation by the cutoff IC_{50} value of 287.3 ng/ml. The baseline characteristics of these two groups are shown in Table 2. The incidence of CMV antigenemia (+) reinfection episodes in the MPA high-sensitivity group classified at 2 weeks after transplantation was 1/10 (10.0%), whereas the incidence in the low-MPA sensitivity recipients was 5/6 (83.3%). Thus, the incidence was higher in the low-MPA sensitivity group, and the difference in the incidence rate of the CMV antigenemia (+) reinfection episodes was statistically significant according to Fisher's exact probability test between the MPA high- and low-sensitivity recipients at 2 weeks after operation ($p < 0.01$) (Table 2). The incidence of acute rejection episodes in the MPA high-sensitivity group classified at 2 weeks after transplantation was 4/10 (40.0%), whereas the incidence of acute rejection episodes in the low-MPA sensitivity recipients was 1/6 (16.7%) (Table 2). The difference in the incidence rate of acute rejection episodes was not statistically significant between the high- and low-MPA sensitivity

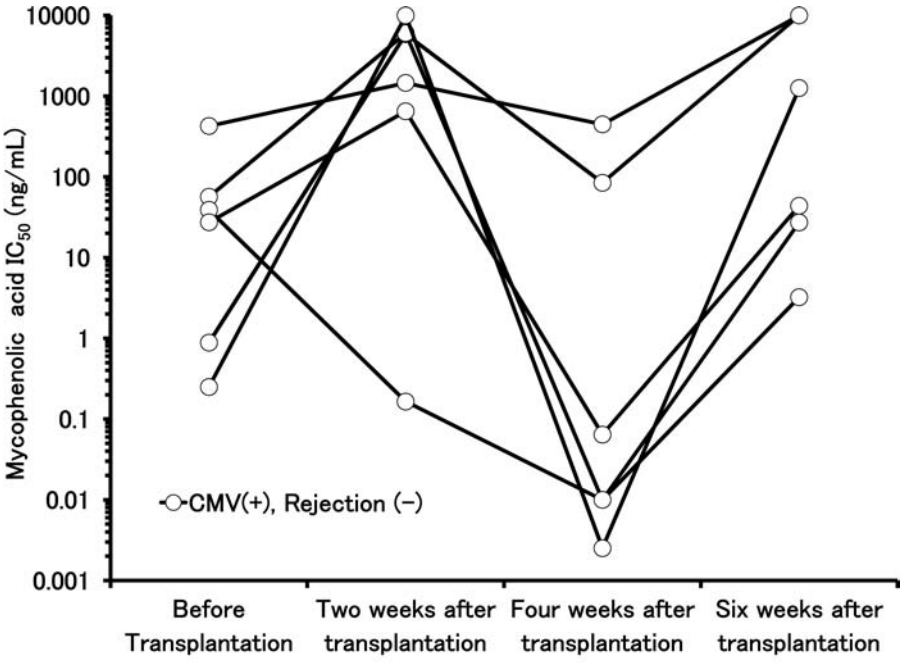

Figure 2. MPA IC_{50} variation in CMV reinfection recipients. Variation of MPA IC_{50} values in six recipients who experienced cytomegalovirus (CMV) reinfection episodes but no rejection episode before transplantation and at 2, 4, and 6 weeks after transplantation.

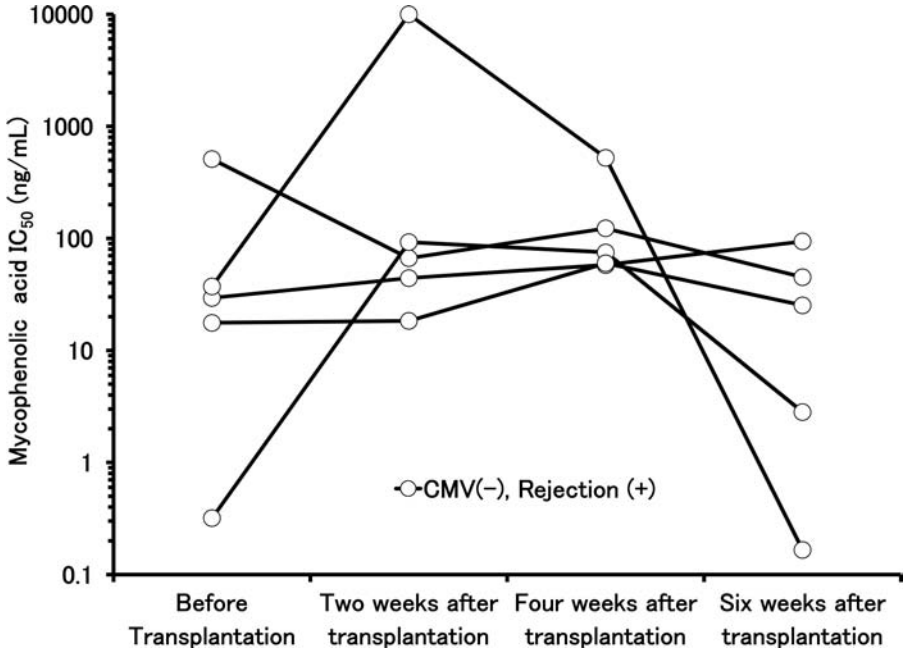

Figure 3. MPA IC_{50} variation in recipients who experienced rejection. Variation of MPA IC_{50} values in five recipients who experienced rejection episodes but no CMV infection episode before transplantation and at 2, 4, and 6 weeks after transplantation.

groups. Furthermore, no statistically significant difference in other indices was observed between the high- and low-MPA sensitivity groups (Table 2).

Moreover, we also divided these 16 recipients into two subgroups based on the MPA IC_{50} values evaluated before and at 4 or 6 weeks after transplantation. The incidence rate of CMV infection or rejection episodes were similar between the two recipient subgroups, and thus no statistically significant difference in the incidence rate was observed between the two groups.

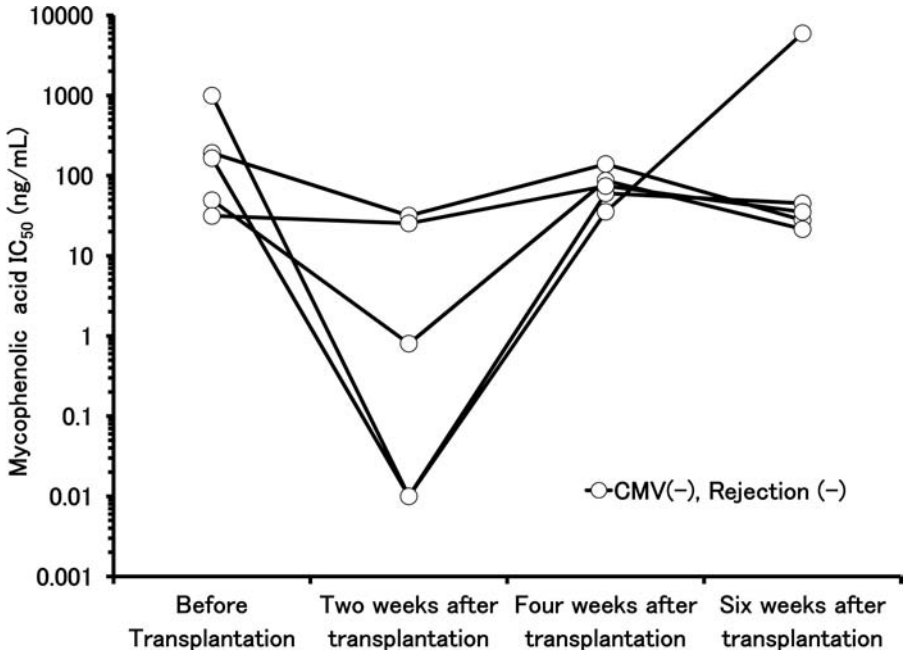

Figure 4. MPA IC_{50} variation in recipients who experienced neither rejection nor CMV infection. Variation of MPA IC_{50} values in five recipients who did not experience rejection and CMV infection episodes before transplantation and at 2, 4, and 6 weeks after transplantation.

Table 2. Comparison of Baseline Characteristics and Incidence of Cytomegalovirus Infection or Rejection Episodes Between the High- and Low-Mycophenolic Acid Sensitivity Groups Classified With a Mycophenolic Acid IC_{50} Value (287.3 ng/ml) at 2 Weeks After Transplantation

Indices	Mycophenolic Acid High-Sensitivity Group (IC_{50} < 287.3 ng/ml) (n = 10)	Mycophenolic Acid Low-Sensitivity Group (IC_{50} > 287.3 ng/ml) (n = 6)	
Male/female	2/8	2/4	NS
Mean age (SD)	36.6 (15.4)	53.5 (165.4)	NS
Mean HLA-AB mismatch number (SD)	2.3 (0.67)	3.0 (0.63)	NS
Mean HLA-DR mismatch number (SD)	1.3 (0.48)	1.0 (0.63)	NS
Cyclosporine-based immunosuppressive therapy	8/10 (50%)	4/6 (66.7%)	NS
Tacrolimus-based immunosuppressive therapy	2/10 (20%)	2/6 (33.3%)	NS
Incidence of CMV reinfection episode	1/10 (10.0%)	5/6 (83.3%)	*p < 0.01
Incidence of rejection episode	4/10 (40%)	1/6 (16.7%)	NS

HLA, human leukocyte antigen.
*Significant by Fisher's exact probably test.

DISCUSSION

This study evaluated the MPA pharmacological efficacies by LIST with MTT assay procedure in 16 renal transplant recipients before transplantation and at 2, 4, and 6 weeks after transplantation, and the efficacies were related to CMV infection or rejection episodes after transplantation. The rate of CMV reinfection episodes was significantly different between the high- and low-MPA sensitivity groups classified at 2 weeks after renal transplantation ($p < 0.01$). However, the rate of CMV infection was not significantly different between the two groups, before and at 4 or 6 weeks after transplantation. The rate of acute rejection episodes was not significantly different between the high- and low-MPA sensitivity groups classified before and at 2, 4, and 6 weeks after operation. We also monitored blood concentration of calcineurin inhibitor in these renal transplant recipients. There were no significant differences in the blood concentration of calcineurin inhibitor between the high- and low-MPA sensitivity groups. However, we did not measure MPA blood concentration in these recipients.

In our previous study, the MPA pharmacological efficacy before transplantation showed small individual variations (9,14), whereas the variability greatly increased at 1 and 3 months posttransplantation. However, the individual IC_{50} value variation among these subjects at 1 year postoperation was closely similar to that observed before transplantation (9). In the present study, the median and the range of MPA IC_{50} values evaluated before transplantation were similar to those reported in our previous studies (9,14), whereas the value largely deviated at 2 weeks after transplantation (Table 2). Thus, the data suggested that the high-dose immunosuppressive therapy in the early phase of renal transplantation causes a change in PBMC sensitivity to MPA. Furthermore, the data also suggested that the decreased response of PBMCs to the suppressive efficacy of MPA in the early phase of transplantation was related to an increase in the patient's sensitivity to CMV infection. While the mechanism underlying this remains to be elucidated, no significant difference in the total dose of calcineurin inhibitors, MMF, or steroids, as well as PBMC stimulation index by T-cell mitogen, was observed between the high- and low-MPA sensitivity groups (data not shown).

Immunosuppressive therapy was composed either of tacrolimus or cyclosporine, MMF, methylprednisolone, and basiliximab for renal transplant recipients. Among those, calcineurin inhibitors are the main immunosuppressive agents for the prevention of acute rejection episodes in renal transplant recipients. In our previous reports, tacrolimus pharmacological efficacy was evaluated by LIST just before transplantation in renal transplant recipients treated with tacrolimus without basiliximab immunosuppressive therapy after surgery (8). The rate of acute rejection episodes in the tacrolimus high-sensitivity group was significantly lower than that in the low-sensitivity group (8,10). Therefore, the pharmacological efficacy by the LIST with MTT assay procedure was generally evaluated just prior to operation. However, the rate of CMV infection episodes was not significantly different between the high- and low-tacrolimus sensitivity groups classified before operation (8,10). The pharmacological efficacies of calcineurin inhibitors were also previously estimated by LIST with MTT assay procedure in renal transplant recipients before and at 1, 3, and 12 months after surgery (10–12). 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 (12). Furthermore, the tacrolimus IC_{50} values varied widely before transplantation. 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 (10). The cyclosporine and tacrolimus pharmacological efficacies evaluated by the LIST using the MTT assay procedure before and at 1 and 12 months after renal transplantation were given a significant correlation in these transplant recipients. However, no statistically significant relationship was observed between the pharmacological efficacies of the two calcineurin inhibitors at 3 months after transplantation (11). Thus, the pharmacological efficacies of these immunosuppressive agents were unstable in renal transplant recipients at a time shortly after transplantation.

In the immunosuppressive therapy of renal transplantation, MMF is a relatively new immunosuppressive agent belonging to a class of purine synthesis inhibitors that has shown promise mainly in renal transplantation. In particular, a lower rate of treatment failure resulting from biopsy-proven allograft rejection has been reported in renal transplantation with MMF in comparison with azathioprine (15). Therefore, MMF is currently used instead of azathioprine as an immunosuppressive therapy in renal transplantation. MMF is a prodrug of MPA, and the bioavailability of MPA was improved by esterification to be MMF (4). MPA selectively inhibits the enzyme inosine monophosphate dehydrogenase (IMPDH) in the de novo pathway of purine synthesis. There are two major pathways of purine synthesis; cell types and tissues can be classified according to their dependence on the de novo and salvage pathways of purine synthesis. Most tissues, including brain tissues, are able to use both of these pathways and are thus in an intermediate category. However, lymphocytes show extreme dependence on de novo synthesis. MMF is a potent, noncompetitive, reversible inhibitor of the enzyme IMPDH, and therefore this drug selectively suppresses the proliferation of both T and B lymphocytes (1). Raggi et al. reported that the IMPDH activity of lymphocytes was significantly different between recipients who experienced acute rejection episodes and those without rejection episodes in renal transplantation. These two groups also showed significantly different IMPDH activities at 1 and 2 weeks after renal transplantation (6). Furthermore, Glander et al. reported that individual IMPDH activity shows a wide individual variation in healthy volunteers (2). Moreover, the administration of MMF rapidly decreased IMPDH activity in both the recipients of primary renal transplantation and the recipients with stable renal function maintained with long-term MMF therapy (2). Therefore, in addition to the present findings, the measurement of IMPDH activity would also be an effective clinical biomarker in renal transplant recipients.

In this study, the rate of CMV infection episodes was significantly higher in the low-MPA sensitivity groups classified by LIST at 2 weeks posttransplantation. Therefore, we conclude that the MPA pharmacological efficacy evaluated by LIST using the patient's PBMCs at 2 weeks after transplantation could be a useful biomarker for predicting the occurrence of CMV infection episodes in renal transplant recipients.

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