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Benefits of a loading dose of tacrolimus on graft survival of kidney transplants in nonhuman primates

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A B S T R A C T

We examined the benefit of a loading dose of tacrolimus on the production of donor-specific antibodies (DSA), occurrence of acute rejection (AR) episodes, graft survival, and histological evidence of antibody-mediated rejection (ABMR) after kidney transplantation in nonhuman primates. Eight cynomolgus monkeys were assigned to two groups ($n = 4$ each): a maintenance dose-group, orally administered 1 mg/kg/day tacrolimus from the day of transplantation; and a loading-dose group, orally administered 2 mg/kg/day tacrolimus for 21 days after transplantation followed by 1 mg/kg/day. The monkeys were observed for up to 178 days after transplantation. Plasma creatinine was monitored over time. Recipients with increased plasma creatinine levels of > 2 mg/dL received anti-acute rejection therapy. In the maintenance dose-group, DSA production, frequent AR episodes, and histological evidence of ABMR were observed in all recipients. Three of four recipients did not survive until the end of the observation period. In the loading-dose group, two recipients showed DSA production, frequent AR episodes and histological evidence of ABMR, while the remaining two had no DSA, AR episodes, or ABMR. Our findings indicate that a loading dose of tacrolimus may prevent DSA production, occurrence of AR events and ABMR, and prolong graft survival following kidney transplantation in monkeys.

1. Introduction

While the use of calcineurin inhibitors (CNIs) for the prevention of acute rejection (AR) has markedly improved kidney transplantation since the 1980s [1,2], both CNI toxicity [3–5] and chronic rejection (CR) remain important medical problems. Exploratory studies have attempted to identify replacement regimens to abrogate CNI-induced toxicity [6–10], but no agents to date have proven sufficiently efficacious to replace CNIs. Recently, patients have been prescribed low doses of CNIs to avoid their adverse effects [11]. While CR is the leading cause of late graft loss, details of its mechanism remain unclear. Recent reports have demonstrated that an increase in plasma creatinine (pCr) level within the first year of transplantation is an important predictive factor of CR [12,13].

Antibody-mediated rejection (ABMR), which is induced by the production of donor-specific antibodies (DSAs) [14–18], has drawn considerable attention in the past decade as a potential mechanism underlying late renal graft failure. Almost half of failed grafts are reportedly due to ABMR [19]. Given that expression of DSA adversely affects graft survival [20], antibody removal strategies such as plasmapheresis, intravenous immunoglobulin, and B cell depletion with rituximab have been employed as potential therapeutic interventions. However, such interventions have not reduced graft loss [21,22]. These findings suggest that DSA production itself in the early post-kidney transplantation period might be a critical event for ABMR. Further, recent reports indicate that DSA mediates and promotes both AR and CR [23]. Therefore, it might be prudent to focus on preventing DSA production to improve long-term outcomes.

Tacrolimus, a calcineurin inhibitor (CNI), prevents DSA production by strongly inhibiting T cell activation [24], suggesting that treatment with an optimal dose of tacrolimus could prevent CR. However, it may be difficult to reconcile the inhibition of DSA production and avoidance of CNI toxicities such as nephrotoxicity [25]. Therefore, it may be particularly valuable to devise a dosing regimen to augment the therapeutic benefits of tacrolimus.

We studied the effect of two dosage regimens, a tacrolimus maintenance-dose regimen and a tacrolimus loading-dose regimen, on DSA production, the incidence of AR episodes, graft survival and ABMR following kidney transplantation in cynomolgus monkeys.

Abbreviations: ABMR, antibody-mediated rejection; AR, acute rejection; CNIs, calcineurin inhibitors; CR, chronic rejection; pCr, plasma creatinine; DSA, donor-specific antibodies; GFR, glomerular filtration rate; IF/TA, interstitial fibrosis/tubular atrophy; MFI, mean fluorescence intensity; MMF, mycophenolate mofetil; PTC, peritubular capillaries

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2. Materials and methods

2.1. Animals

Eight male cynomolgus monkeys (*Macaca fascicularis*) weighing 3.8–5 kg, free of simian immunodeficiency virus, simian retrovirus, salmonella bacteria, dysentery bacteria, and B virus were obtained from Hamri Co., Ltd. (Ibaraki, Japan). All monkeys were housed in individual cages and allowed free access to water, and given food twice a day. All animals were adapted to their rearing environment for at least 1 week before use.

All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Astellas Pharma Inc. The Tsukuba Research Center of Astellas Pharma Inc. has been accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

2.2. Kidney transplantation

Each animal was used as both the donor and recipient. Donor and recipient pairs were selected according to ABO blood type compatibility. Each pair was negative for allo-antibodies using flow cytometry, and had a stimulation index (SI) > 2.5, where SI was calculated by dividing the proliferative response (3H-thymidine incorporation) in stimulated recipient cells by the background proliferative response in the one-way mixed lymphocyte reaction (Table 1). Kidney transplantation was performed using standard microsurgical techniques as previously reported [26,27]. Briefly, all donor and recipient monkeys were anesthetized using an intramuscular injection of 8 mg/head tiletamine and 10 mg/head zolazepam (USP, Rockville, MD, USA) for induction, and inhaled isoflurane (Pfizer Inc., NY, USA) for maintenance.

The left kidney of the donor was mobilized and excised, along with long segments of the renal vessels and ureter. The kidney allograft was perfused with Euro-Collins solution (4 °C) and stored in cold Euro-Collins solution while the recipient was prepared. After removal of the left kidney, the donor kidney was transplanted into the original position in the abdomen via end-to-side anastomoses of the renal artery to the aorta and the renal vein to the vena cava. End-to-end anastomosis of the donor and recipient ureters was performed using suture methods. Immediately after grafting, native nephrectomies were performed. After transplantation, 0.02 mg buprenorphine (Otsuka Pharmaceutical, Tokyo, Japan) as an analgesic was administered subcutaneously twice a day for 4 days. On the day of the operation, 500 mg cefazolin (Astellas Pharma Inc., Tokyo Japan) as an antibiotic prophylaxis was administered intramuscularly. In addition, 250 mg cefazolin was administered subcutaneously twice a day for 7 days following transplantation. Moreover, 1 mg metoclopramide hydrochloride (Astellas Pharma Inc.) and 5 mg famotidine (Astellas Pharma Inc.) as gastroprokinetic agents were administered subcutaneously twice a day for 3 days.

The general condition of animals was observed every day from the day of kidney transplantation. Body weight of each recipient was measured on the day of kidney transplantation and once a week thereafter. The function of the transplanted kidneys was monitored by measuring pCr levels twice a week. The day of the transplant operation was defined as day 0. Kidney allograft survival was defined as the interval in days between transplantation and the following: (i) the day before death; (ii) the day the animal became moribund; or (iii) survival to the end of the observation period of 178 days, described as > 178 days.

Recipients that showed anuresis received an ultrasonic renal examination. When ultrasonography indicated a ureter obstruction at the sutured site, a stent was placed in the ureter.

Table 1
Stimulation index (SI) in the mixed lymphocyte reaction assay.

Group	Animal ID	Stimulation index
Tacrolimus maintenance-dose	M-327	4.5
	M-328	6.1
	M-329	3.6
	M-330	3.7
Tacrolimus loading-dose	L-291	7.4
	L-292	3.3
	L-293	7.7
	L-294	5.0

SI was calculated by dividing the proliferative response (3H-thymidine incorporation) in stimulated recipient cells by the background proliferative response in the one-way mixed lymphocyte reaction.

2.3. Immunosuppressive regimen

The eight monkeys were divided into two treatment groups of four animals each, a tacrolimus maintenance-dose group and a tacrolimus loading-dose group based on the initial dose after transplantation. In both groups, immunosuppressive treatment was initiated on the day of the kidney transplantation. All recipients received tacrolimus (solid dispersion formulation containing 20% active ingredient, prepared at Astellas Pharma Inc. Tokyo, Japan) orally in combination with 15 mg/kg mycophenolate mofetil (MMF; Cellcept® Intravenous, Roche Laboratories Inc., Nutley, NJ, USA) subcutaneously, once a day. The tacrolimus suspension was prepared in distilled water before use. The maintenance-dose group received 1 mg/kg tacrolimus from the first dose. The loading-dose group received 2 mg/kg tacrolimus until day 21, followed by daily doses of 1 mg/kg. The tacrolimus dose was increased if recipients required anti-acute rejection therapy as described below.

2.4. Tacrolimus trough levels in whole blood

Pre-dose blood samples were collected on day 22 or 23 after kidney transplantation in the tacrolimus maintenance-dose group and on day 16 or 17 in the tacrolimus loading-dose group. Whole blood trough levels of tacrolimus were measured using liquid chromatography-tandem mass spectrometry (AB SCIEX, Tokyo, Japan).

2.5. Anti-acute rejection therapy

When pCr levels increased to > 2 mg/dL, recipients had their daily tacrolimus dose increased to 2 mg/kg and received a 3-day steroid pulse therapy (10 mg/kg, qd, methylprednisolone; Pridol®, Toho Holdings Co., Ltd., Tokyo, Japan.) until pCr levels returned to below 2 mg/dL. Once pCr levels dropped to 2 mg/dL, the steroid was decreased to 1 mg/kg.

2.6. Measurement of donor-specific antibodies

Donor-specific antibody (DSA) levels were determined using flow cytometry, as described previously with minor modifications [28,29]. Briefly, plasma samples from recipients were incubated with peripheral blood mononuclear cells from the donor, which were prepared beforehand and kept frozen at −80 °C. The mixture was subsequently incubated with FITC-anti-human IgG (DAKO, Glostrup, Denmark). Cells were washed, fixed, and analyzed using flow cytometry (BD Biosciences, CA, USA). Mean fluorescence intensity (MFI) was measured and used to calculate DSA levels, and expressed as ΔMFI of IgG at each measurement point. ΔMFI was calculated using the following equation: ΔMFI = MFIR - MFID, where MFIR is the MFI of the sample containing recipient plasma and MFID is the MFI of the sample containing donor plasma. ΔMFI values that were lower than the mean ΔMFI of pre-transplant monkeys plus two standard deviations were considered DSA-negative.

2.7. Histopathology

Specimens of the kidney allograft were obtained at autopsy for histopathological examination. Allografts were isolated, cut into small pieces, fixed with 10% neutral-buffered formalin, dehydrated through a graded ethanol series, and embedded in paraffin. A proportion of sections of 3-μm thickness were stained with hematoxylin and eosin (HE) and Periodic acid-Schiff (PAS). The remaining sections were stained with C4d antibody. Sections were deparaffinized and treated with ImmunoSaver® (catalog number 333, Nisshin EM, Tokyo, Japan) for antigen retrieval before incubation with rabbit anti-human C4d antibody (catalog number 0300–0230, Serotec, Kidlington, UK) for 16 h at 4 °C. After washing, sections were incubated with Envision+ System-HRP Labelled Polymer Anti-Rabbit (catalog number K4002, Dako, Glostrup, Denmark) according to the manufacturer's instructions and reacted with diaminobenzidine tetrahydrochloride (DAB). The sections were counterstained with hematoxylin. Pathological diagnosis and grading were performed according to the Banff classification (Banff 2013) [30].

3. Results

3.1. Graft survival

Graft survival in the tacrolimus maintenance-dose group and loading-dose group are shown in Table 2. Graft survival was > 100 days in all recipients. In the maintenance-dose group, three of four recipients exhibited graft rejection on day 100 or 118, while the graft in one recipient (M-329) survived for over 177 days. In the tacrolimus loading-dose group, grafts in three of four recipients survived for over 178 days, while one (L-292) was rejected on day 135.

3.2. Body weight

The percentage change in body weight for each recipient is shown in Fig. 1. Body weights of three recipients (M-328, M-329 and M-330) in the maintenance-dose group and two recipients (L-291and L-292) in the loading-dose group decreased with time.

3.3. Tacrolimus trough levels in whole blood

Table 3 shows the blood tacrolimus trough levels of each recipient. The trough level ranged from 1.6 to 9.1 ng/mL and from 3.0 to 6.0 ng/mL in the maintenance-dose group and the loading-dose group, respectively.

The maintenance-dose group received 1 mg/kg tacrolimus from the day of the kidney transplantation, while the loading-dose group received 2 mg/kg tacrolimus until day 21, followed by daily doses of 1 mg/kg.

Pre-dose blood samples were collected on day 22 or 23 after kidney transplantation in the tacrolimus maintenance-dose group and on day 16 or 17 in the tacrolimus loading-dose group.

3.4. Plasma creatinine levels

Changes in pCr levels and the details of anti-rejection therapy are shown in Fig. 2. In the maintenance-dose group, three recipients (M-328, M-329 and M-330) exhibited increased pCr levels within 20 days after transplantation and required anti-acute rejection therapy. The other recipient (M-327) had an intermittent increase in pCr levels to over 3 mg/dL. All recipients in the maintenance-dose group were ultimately treated with 2 mg/kg tacrolimus.

In the loading-dose group, two recipients (L-291 and L-292) required anti-rejection therapy. One recipient (L-291) exhibited a sudden increase in pCr levels at around 60 days after transplantation, but such acute rejection episodes were successfully managed with anti-rejection therapy. The other recipient (L-292) also required acute rejection therapy; however, the pCr levels continued to gradually increase and the animal did not survive until the end of the observation period. Creatinine levels in the other two recipients (L-293 and L-294) remained below 3 mg/dL throughout the observation period except at around day 156 for L-294, when pCr levels increased sharply due to a

Table 2
Graft survival.

Group	Survival, days (animal ID)			
Tacrolimus maintenance-dose	100 (M-330)	118 (M-327)	118 (M-328)	> 177 (M-329)
Tacrolimus loading-dose	135 (L-292)	> 178 (L-291)	> 178 (L-293)	> 178 (L-294)

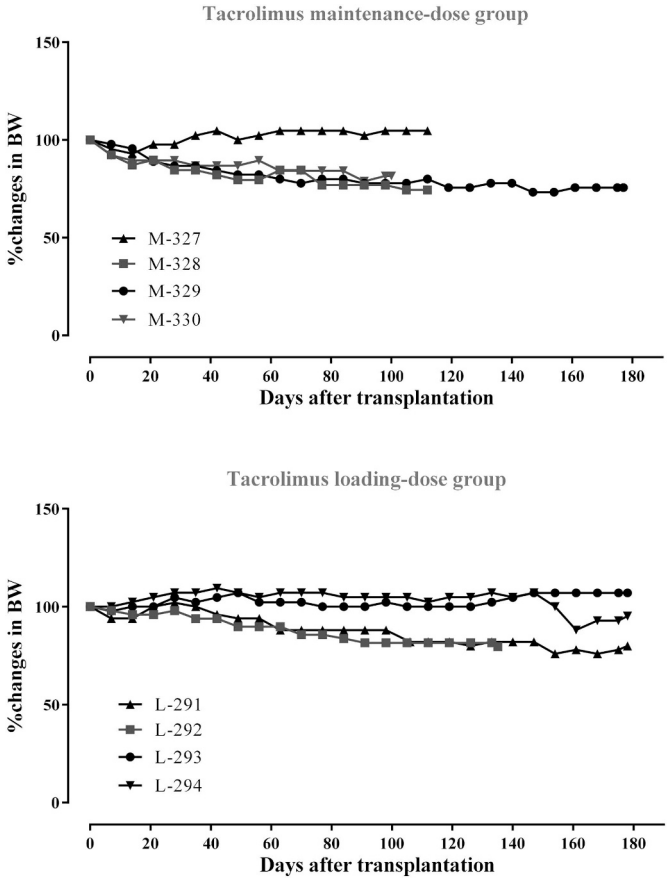

Fig. 1. Percentage change in body weight.
Changes in body weight of each recipient are expressed as a percentage of body weight measured on the day of kidney transplantation.
BW, body weight.

Table 3
Tacrolimus blood trough level.

Group	Animal ID	Trough level of tacrolimus (ng/mL)
Tacrolimus maintenance-dose	M-327	3.5
	M-328	1.6
	M-329	2.2
	M-330	9.1
Tacrolimus loading-dose	L-291	5.0
	L-292	5.7
	L-293	6.0
	L-294	3.0

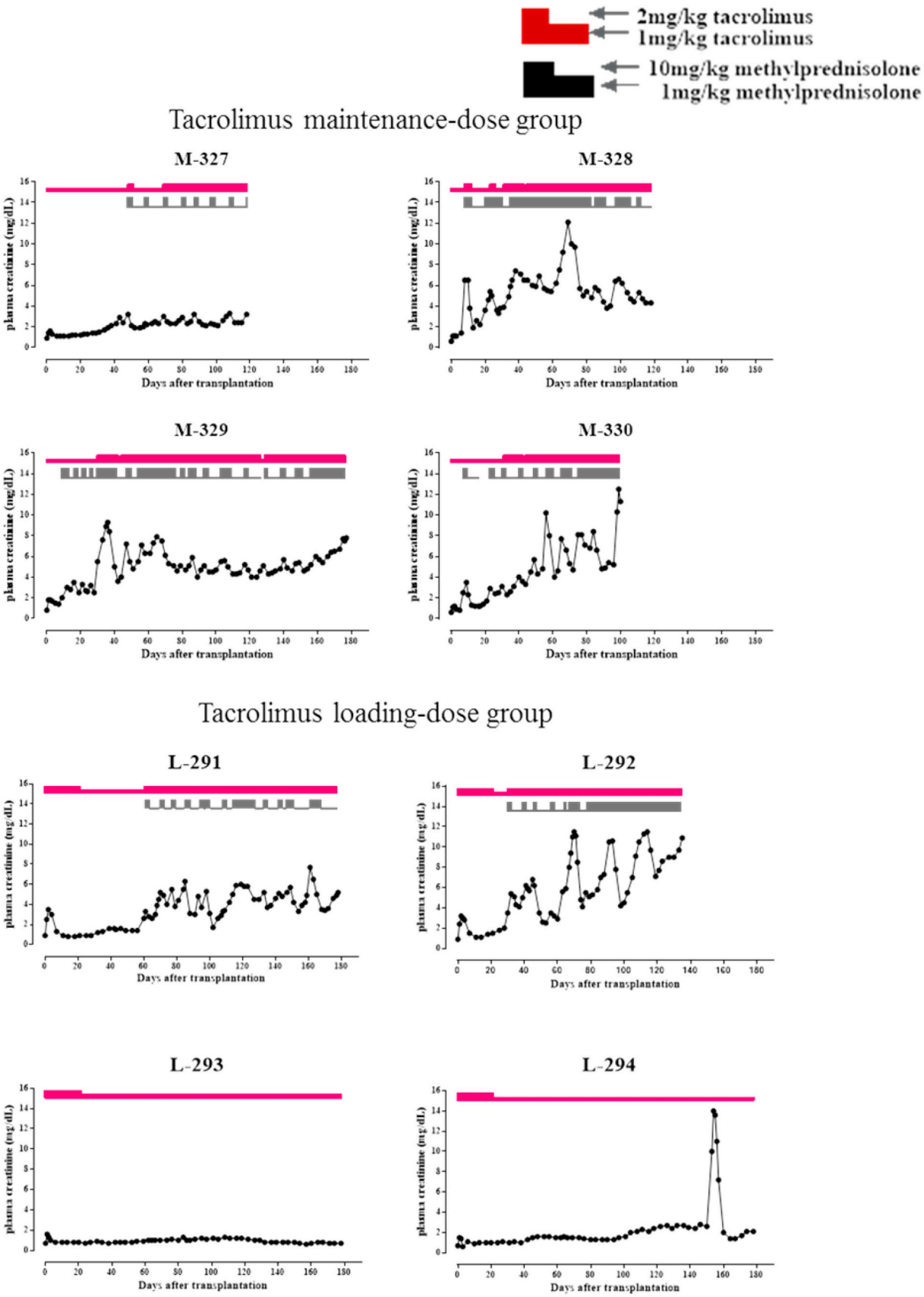

Fig. 2. Treatment details and plasma creatinine levels in individual monkeys.
In the maintenance-dose group, M-328, M-329 and M-330, but not M-327, required anti-acute rejection therapy within 20 days after transplantation. All monkeys in the maintenance-dose group were ultimately treated with 2 mg/kg tacrolimus. In the tacrolimus loading-dose group, no monkeys required anti-acute rejection therapy by day 29.

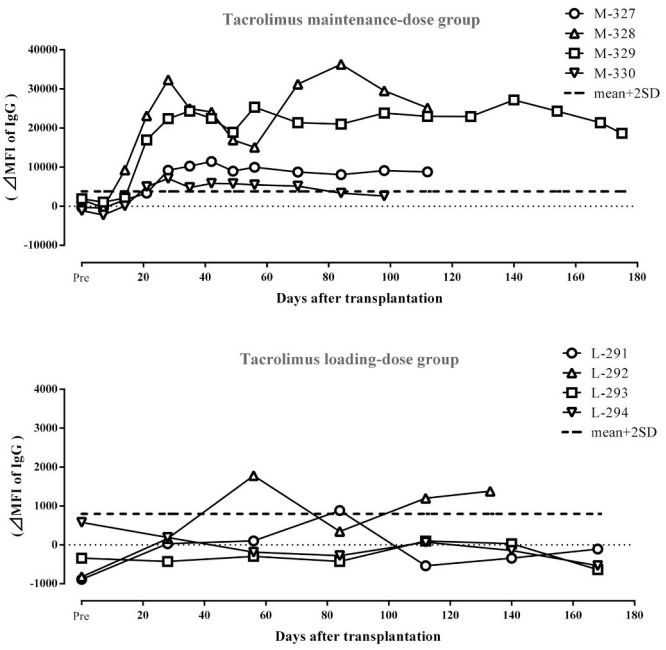

Fig. 3. Changes in plasma DSA levels.

ureter obstruction. The pCr levels promptly decreased to < 3 mg/dL after stent placement.

3.5. Plasma donor-specific antibody levels

The changes in DSA levels are shown at Fig. 3. In the maintenance-dose group, all recipients showed DSA production approximately 20 days after kidney transplantation, and remained positive for DSA throughout the survival period, except at the final measurement point in one recipient (M-330). In the loading-dose group, two recipients (L-291 and L-292) showed an inconsistent rise in DSA levels. DSA production in the recipients of the maintenance-dose group was more pronounced than that in the loading-dose group.

3.6. Histopathological changes in kidney allografts

Details of the histopathological features of kidney allografts are summarized in Table 4. In the maintenance-dose group, all recipients showed mild to moderate arteritis (v1 to v2), moderate mononuclear cell infiltration (i1 to i2), moderate to severe interstitial fibrosis (ci2 to ci3), moderate to severe tubular atrophy (ct2 to ct3) and mild to moderate duplication of the glomerular basement membrane (cg1 to cg2), which is a characteristic feature of transplant glomerulopathy. C4d deposition was observed in all recipients, at mild to moderate (+ to + +) levels in the glomeruli and mild to severe (+ to + + +) levels in the peritubular capillaries (PTC). All recipients were diagnosed with chronic active ABMR, chronic active T cell-mediated rejection (TCMR), and moderate to severe interstitial fibrosis/tubular atrophy (IF/TA; grade II to III).

In the loading-dose group, all recipients showed mild to moderate mononuclear cell infiltration (i1 to i2), mild and severe interstitial fibrosis (ci1 and ci3), and mild to severe tubular atrophy (ct1 to ct3), while one recipient (L-292) also showed moderate arteritis (v2) and mild duplication of the glomerular basement membrane (cg1). Although mild to moderate C4d deposition was observed in the glomeruli (+ to + +) of all recipients, only two recipients showed mild C4d deposition in the PTC. From these findings, one recipient (L-292) was diagnosed with acute TCMR type IIB, chronic active ABMR and severe IF/TA (grade III) and another (L-291) was diagnosed with

Table 4
Histopathological scores and diagnosis of the recipients.

Group	Animal ID	Arteritis	Mononuclear Cell Infiltration	Interstitial Fibrosis	Tubular Atrophy	Duplication of GBM	C4d Deposition in Glomerular	C4d Deposition in PTC	Diagnosis
Tacrolimus maintenance-dose	M-327	v2	i2	ci2	ct2	cg1	+	+	Chronic active ABMR, Chronic active TCMR, IF/TA II
	M-328	v1	i1	ci3	ct3	cg2	+	+ + +	Chronic active ABMR, Chronic active TCMR, IF/TA III
	M-329	v2	i2	ci3	ct3	cg2	+ +	+ + +	Chronic active ABMR, Chronic active TCMR, IF/TA III
	M-330	v1	i2	ci3	ct3	cg2	+	+ +	Chronic active ABMR, Chronic active TCMR, IF/TA III
Tacrolimus loading-dose	L-291	0	i1	ci3	ct2	0	+	-	Chronic ABMR, Borderline changes, IF/TA III
	L-292	v2	i2	ci3	ct3	cg1	+ +	+	Chronic active ABMR, Acute TCMR type IIB, IF/TA III
	L-293	0	i1	ci1	ct1	0	+	-	Borderline changes, IF/TA I
	L-294	0	i2	ci1	ct1	0	+	+	Borderline changes, IF/TA I

Pathological diagnosis (v, i, ci, ct, and cg) and grading (0, 1, 2 and 3) were performed according to the Banff classification (Banff [30]).
v, arteritis score; i, interstitial inflammation score; ci, interstitial fibrosis score; ct, tubular atrophy score; cg, glomerular double contours score.
C4d deposition scores were graded on a four-level scale: -: none, +: mild, + +: moderate, + + +: severe.
GBM, glomerular basement membrane; PTC, peritubular capillaries; ABMR, antibody-mediated rejection; TCMR, T cell-mediated rejection; IF/TA, interstitial fibrosis/tubular atrophy.

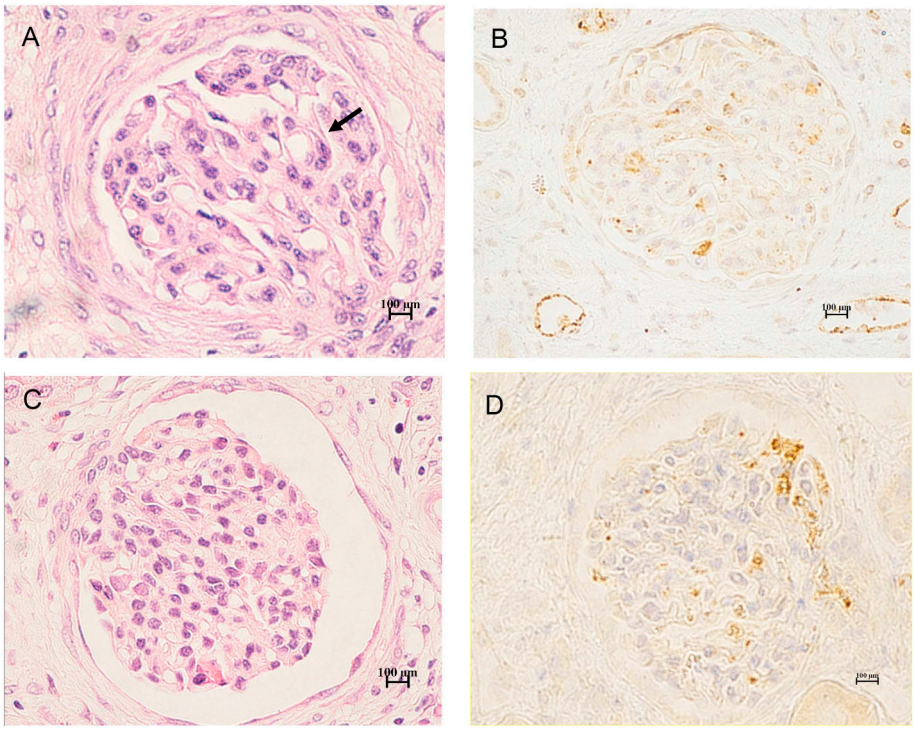

Fig. 4. Histopathological changes in kidney allografts.
Sections through glomeruli were stained with hematoxylin and eosin (A, C) or C4d antibody (B, D). Glomerular sclerosis was observed in a recipient in the maintenance-dose group (M-329) (A, B) and a recipient in the loading-dose group (L-293) (C, D). The arrow in A indicates duplication of the glomerular basement membrane, which is a typical feature of transplant glomerulopathy.

chronic ABMR, borderline changes, and severe IF/TA (grade III). The remaining two recipients (L-293 and L-294) were diagnosed with borderline changes and mild IF/TA (grade I).

Fig. 4 shows representative micrographs of transplant glomerulopathy and C4d deposition in the glomeruli. A glomerulus from a recipient in the maintenance-dose group (M-329) exhibited glomerular basement membrane double contours, a typical feature of transplant glomerulopathy (Fig. 4A), as well as linear C4d staining in a PTC and focal C4d staining (Fig. 4B). In contrast, a glomerulus from a recipient in the loading-dose group (L-293) showed no duplication of the glomerular basement membrane (Fig. 4C), but showed some C4d staining (Fig. 4D).

4. Discussion

ABMR due to DSA is responsible for many cases of late graft loss in kidney transplantation and remains a largely unsolved medical problem. Here, we studied the effect of tacrolimus using two dosage regimens, a maintenance-dose regimen and a loading-dose regimen, on DSA production and consequent ABMR in a monkey kidney transplant model.

Recipients in the maintenance-dose group who received 1 mg/kg tacrolimus in combination with MMF survived > 90 days, which is markedly longer than that observed in a previous study in which animals treated with 1 mg/kg tacrolimus alone showed a median survival time of 21 days [31]. Combination treatment with tacrolimus and MMF prolonged graft survival; however, AR episodes, as indicated by an acute increase in pCr to > 2 mg/dL, occurred frequently in three of the four recipients. In particular, recipients (M-328 and M-329) whose tacrolimus trough levels were < 3 ng/mL, which is below the recommended target blood level for renal transplantation patients [32], on day 22 or 23 experienced AR episodes in the early phase after kidney transplantation. Three of the four recipients in the maintenance-dose group did not survive until the end of the designated observation period of 178 days despite repeated anti-acute rejection therapy for AR episodes. In contrast, in the loading-dose group, which was treated with 2 mg/kg tacrolimus for 21 days in combination with MMF, three of the four recipients survived until the end of the observation period. One recipient, L-292, showed severe and frequent AR episodes after day 22 when the dose of tacrolimus was reduced from 2 mg/kg to 1 mg/kg and did not survive until the end of the designated observation period despite repeated anti-acute rejection therapy (survival: 135 days). Two recipients (L-293 and L-294) showed no AR episodes throughout the observation period. Therefore, the loading-dose regimen had a marked effect on graft survival as well as the occurrence of AR episodes compared to the maintenance-dose regimen. These findings suggest that a high frequency of AR episodes may be a risk factor for long-term graft failure, even following treatment with anti-AR therapy.

Recipients who did not experience AR episodes had consistent body weight throughout the observation period. In contrast, recipients who experienced frequent AR episodes lost weight with time. The frequent AR episodes that were associated with the decline in renal function may be a cause of the body weight loss; however, increasing the dose of tacrolimus in frequent anti-AR therapies may also cause weight loss. Adverse gastrointestinal effects such as persistent diarrhea are a major concern of MMF administration [33]. In the present study, while unformed stools were observed in some recipients, the incidence was infrequent and did not continue for > 11 days (data not shown). The occurrence of unformed stools was therefore thought to be unlikely related to MMF.

A substantial difference in DSA production was observed between the maintenance-dose and loading-dose groups. Although the same dose of MMF was given to all recipients in both groups, marked DSA production was observed in recipients in the maintenance-dose group but not in the loading-dose group. In the maintenance-dose group, DSA production was observed in all recipients. Two recipients (M-328 and M-329) showed high DSA production until day 20 after kidney transplantation, and DSA levels remained positive throughout the observation period. In contrast, while two recipients (L-291 and L-292) in the loading-dose group showed DSA production, the increase in DSA levels was marginal. Considering the graft survival in the two groups, these findings suggest that DSA level might be inversely correlated with the

long-term outcome. Similar findings were reported in clinical studies [34]. Terasaki et al. suggested that human leukocyte antigen antibodies are a major cause of graft failure such as graft loss [35]. Halloran et al. reported that long-term graft failure was associated with DSA production in patients with non-adherence to medication [16,36]. Gaston et al. reported that graft loss within three years after transplantation is rarely observed in patients without DSA production [37]. Further, Djamali et al. demonstrated that the survival rate of patients with ABMR featuring DSA production within 1 year is 3-fold lower than that for patients with ABMR after 3 years [23]. Therefore, it is highly probable that DSA production is strongly correlated with long-term graft failure.

We observed that DSA production was more effectively prevented in the loading-dose group than in the maintenance-dose group even after the tacrolimus dose reduction on day 22. These findings suggest that a loading dose of tacrolimus may inhibit DSA production via strong inhibition of T cell activation. A previous report demonstrated that initial high frequency priming through the direct pathway, initiated by donor dendritic cells presenting donor major histocompatibility complex molecules, may result in a residual population of donor-reactive memory T cells [38]. Several studies have demonstrated that such memory T cells promote early AR episodes, which often lead to increased risk of DSA production [39] and chronic rejection [40]. Given that T cell activation during early rejection episodes may lead to the formation of memory T cells that contribute to late graft loss [40], we speculate that a loading-dose of tacrolimus might prevent the development of memory T cells and consequently reduce both DSA production and the frequency of AR episodes, even after a tacrolimus dose reduction. Further studies are required to investigate the role of memory T cells in DSA production and the occurrence of AR episodes.

C4d deposition, which occurs via C1q activation and tight anchoring of C4d to the tissue by covalent bonds, is a distinct pathological marker of ABMR [41,42]. In the maintenance-dose group, all recipients were diagnosed with chronic active ABMR, with three of the four recipients (M-328. M-329 and M-330) showing moderate to severe C4d deposition in the PTC and mild duplication of the glomerular basement membrane. A similar relationship between C4d deposition in PTC and duplication of the glomerular basement membrane was observed in a kidney transplantation study in monkeys examining the effects of cyclosporine A [43]. Therefore, C4d deposition in PTC may be closely associated with duplication of the glomerular basement membrane.

Of those that showed moderate and severe interstitial fibrosis, all of the recipients in the maintenance-dose group and two (L-291 and L-292) in the loading-dose group also exhibited frequent AR episodes. It is highly probable that the interstitial fibrosis was caused by the frequent AR episodes, since interstitial fibrosis in a graft after transplantation has been reported to represent a repair process following AR episodes [39]. However, Kinugasa et al. demonstrated that oral treatment with 2 mg/kg tacrolimus prolonged survival, with median survival days of > 90 days following kidney transplantation in cynomolgus monkeys, although some interstitial fibrosis was detected in the grafts [31]. Therefore, it is likely that repeated anti-acute rejection therapy with 2 mg/kg tacrolimus might in part cause severe interstitial fibrosis.

While the present study demonstrated that a loading-dose of tacrolimus improved long-term graft survival by preventing frequent AR events, DSA production and ABMR, our observation period of 178 days is shorter than that in clinical settings. Further studies are needed to elucidate the therapeutic benefits of a loading dose of tacrolimus in the long term.

In summary, the present study demonstrated that a loading dose of tacrolimus prevented DSA production, occurrence of AR episodes and ABMR, and prolonged graft survival in a cynomolgus monkey kidney transplantation model. As non-human primate models have been shown to mimic transplantation in clinical settings [44], our results may be translatable to clinical practice. Therefore, our findings may provide further insight into the therapeutic potential of tacrolimus for long-term graft survival after kidney transplantation.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation. All authors were employees of Astellas Pharma Inc. when this study was conducted and have no further conflicts of interest to declare. Part of the study was presented at the American Transplant Congress 2013.

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