의학박사 학위논문

Efficacy of A Chimeric Anti-human ICAM-1 Monoclonal Antibody, MD-3, for Kidney Transplantation in Rhesus Monkeys

2016년 2월

서울대학교 대학원

의학과 외과학 전공

민상일

A thesis of the Degree of Doctor of Philosophy

Rhesus 원숭이 동종 신장이식 모델에서 **MD-3** 항체의 효과에 대한 연구

2016년 2월

서울대학교 대학원

의학과 외과학 전공

민상일

Efficacy of A Mouse Anti-human ICAM-1 Monoclonal Antibody, MD-3, for Kidney Transplantation in Rhesus Monkeys

지도 교수 하 종 원

이 논문을 의학박사 학위논문으로 제출함
2015년 10월

서울대학교 대학원
의학과 외과학전공
민상일

민상일의 의학박사 학위논문을 인준함
2015년 12월

위 원 장 _____ (인)
부위원장 _____ (인)
위 원 _____ (인)
위 원 _____ (인)
위 원 _____ (인)

Efficacy of A Mouse Anti-human ICAM-1 Monoclonal Antibody, MD-3, for Kidney Transplantation in Rhesus Monkeys

by
Sang-il Min

A thesis submitted to the Department of Surgery in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Medical Science (Surgery) at Seoul National University College of Medicine

December 2015

Approved by Thesis Committee:

Professor _____Chairman
Professor _____Vice chairman
Professor _____
Professor _____
Professor _____

Abstract

Introduction: The MD-3 is a newly developed chimeric anti-human intracellular adhesion molecule 1 (ICAM-1) monoclonal antibody (mAb). The effects of this monoclonal antibody in solid organ transplantation have not been elucidated.

Methods: Five rhesus monkey renal allograft recipients treated with MD-3 induction and one recipient without MD-3 treatment were studied for the incidence of acute rejection and donor-specific antibody development. Cellular immune response was tested with ELISPOT assay. Development of anti-drug antibody was also tested. Maintenance immunosuppression included anti-CD154 mAb (hu5C8), mycophenolate mofetil and corticosteroid.

Results: No recipient monkey developed acute rejection as long as 8 months after transplantation. Donor-specific antibody was not developed in monkeys treated with MD-3. A monkey without MD-3 induction started to produce donor-specific antibody 3 weeks after cessation of anti-CD154mAb and persisted. No anti-drug antibody was detected during the study period.

Conclusions: MD-3 can be a highly promising agent for clinical use in human allotransplantation and MD-3 treatment can allow optimization of kidney transplant outcome. Further studies for refining dosages and dosing

schedule of MD-3 in kidney transplantation are required.

Keywords: Primate models, MD-3, Renal transplantation, Acute rejection, Donor-specific antibody

Student Number: 2009-31080

Contents

List of Figures

List of abbreviations

Ada: anti-drug antibody

DC: dendritic cell

DSA: donor-specific antibody

ICAM-1: intercellular adhesion molecule 1

mAb: monoclonal antibody

PBMC: peripheral blood mononuclear cell

POD: postoperative day

Introduction

Enormous progress has been made in the field of transplantation during the past four decades and kidney transplantation is the accepted standard treatment for the patients with end stage renal disease. This is due large in part to availability of effective immunosuppressive drugs such as cyclosporine, tacrolimus, mycophenolate mofetil, antithymocyte globulin and basiliximab. All of these agents suppress the immune response nonspecifically and has been widely adopted to prevent graft rejection (1). While immunosuppression has progressively reduced the incidence of acute rejection and markedly reduced the mortality of organ failure (2), the use of more potent immunosuppressive agents has incurred significant costs. In addition, current nonspecific immunosuppressants are associated with myriad toxicities such as diabetes, hypertension, dyslipidemia, osteoporosis, infection, nephrotoxicity and cancer, and these side effects tempered the substantial benefits of organ transplantation (3-8). Current therapies have also failed to prevent the chronic rejection in most organs. Therefore, more specific and less toxic therapies are needed to optimize transplantation.

More than ten methods have been demonstrated the potential of producing tolerance in rodent allograft models, and several strategies have showed success when applied to higher mammals (9). Two most promising of these protocols include mixed chimerism through donor bone marrow

transplantation, and costimulation blockade (10, 11). In the past decade, these strategies have matured to the point of clinical application. However, apparent inability to eliminate toxic effects of myeloconditioning regimen or side effects of blocking antibodies was clinically unacceptable in most transplant patients.

The MD-3 is a newly developed chimeric anti-human intercellular adhesion molecule 1 (ICAM-1) monoclonal antibody. In the previous study, we have demonstrated that treatment with the MD-3 directed against domain 2 of human and non-human primates ICAM-1 molecules prevents the maturation of human myeloid dendritic cells in response to lipopolysaccharide stimulation, without affecting critical functions of ICAM-1, such as adhesion of leukocytes to endothelial cells (12). Humanized mice rendered diabetic by streptozotocin and injected intravenously with three doses of MD-3 antibody, nine days prior to porcine islet transplantation (3 days intervals and the dose of 300μg per mouse), showed no evidence of xenograft rejection up to 42 days. Ex-vivo analysis indicated that the lack of graft rejection was because MD-3 treatment led to arrest of human dendritic cells (DCs) in a semimature stage and these DCs develop antigen-specific T cell tolerance. In rhesus macaques rendered diabetic by streptozotocin injection, MD-3 antibody monotherapy before porcine islet transplantation promoted the induction of T cell tolerance to porcine islet, although the islet grafts were rejected within 47 days. However, long-term graft survival to

>140 days was achieved when MD-3 antibody therapy was combined with low-dose rapamycin and chimeric anti-CD154 blocking antibody (5C8; National Institutes of Health, Bethesda).

In the scope of clinical application, we conducted this study to elucidate the immunosuppressive effects of MD-3 in the renal transplantation model in nonhuman primates. In this study, we show that MD-3 therapy combined with mycophenolate mofetil and chimeric anti-CD154 blocking antibody can prevent allograft rejection and inhibit donor-specific antibody development.

Materials and Methods

Animals and donor-recipient pair selection

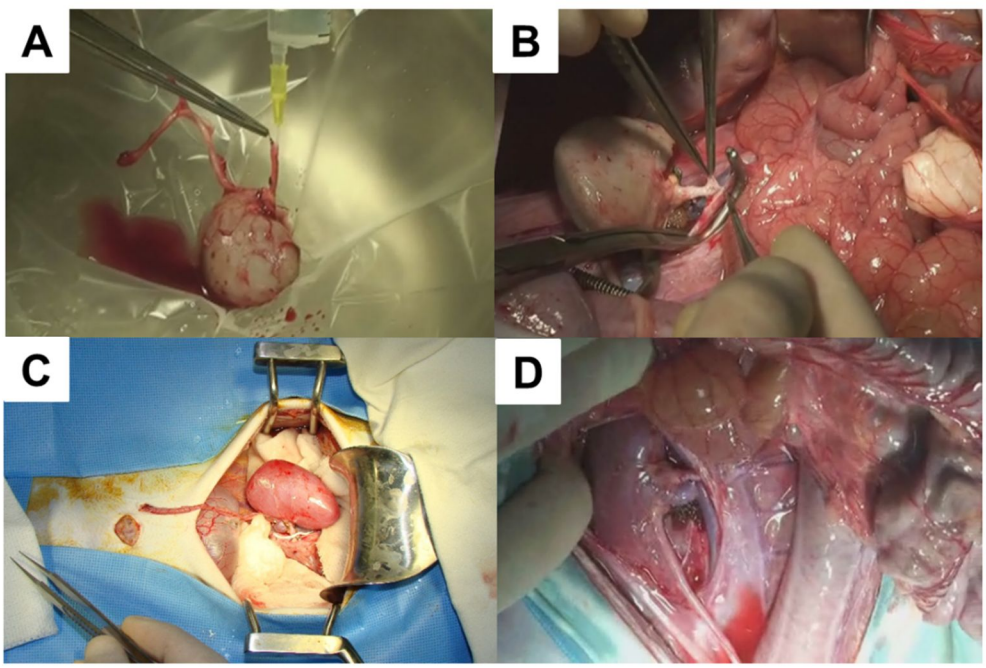
Rhesus macaques (Macaca mulatta; 4 – 5 years old and 4.5 - 6.0 kg) were obtained and maintained at the SNU Hospital Non-Human Primate Center. All animals were seronegative for Hepatitis B virus, but seropositive for cytomegalovirus. The experimental protocol and all procedures were approved by the Institutional Animal Care and Use Committee, and conducted in accordance with the National Institutes of Health guidelines. The donor-recipient combinations were chosen according to blood group compatibility, absence of preformed donor-specific alloantibodies (DSA) and MHC mismatching by DRB MHC class II locus typing and verification of MHC incompatibility by mixed lymphocyte reaction (stimulatory index >3).

Non-life supporting renal transplantation

Left kidney was exchanged in one pair. Intraabdominal kidney transplantation was performed as described previously (13). All animals were anesthetized initially with intravenous injection of midazolam (0.01 mg/kg) and maintained by isoflurane. Animals were heparinized (100U/kg) during organ procurement and implantation. Donor kidneys were perfused ex vivo with HTK solution. Renal allograft was implanted by end-to-side anastomoses with 8.0 prolene® polypropylene suture (Johnson & Johnson

Medical Korea, Seoul, Korea) between the donor renal artery and the recipient distal aorta, and between the donor renal vein and the recipient vena cava under magnification view (3.5x). For the ureteroneocystostomy, Single-stitch technique (13) or Lich-Gregoir technique were used and 4.7Fr Double-J ureteral stent (Cook Medical Korea, Seoul, Korea) was placed (Figure 1). 7.0Fr Flexible ureteroscopy (Karl Storz Endoscopy Korea Co., Seoul, Korea) was used in cases with stent removal. Right nephrectomy was not performed and right kidney remained left in the recipient. The animals were given cefazolin (25mg/kg) for five days. Ranitidine (2mg/kg bid) was used and intravenous fluid was given for 3 days during fasting period. Clopidogrel (7.5mg qd) was used for thrombo-prophylaxis for 28 days after transplantation. For pain control, ketorolac (15mg bid) were used for 3 days. All transplanted animals were monitored with daily clinical assessment, serial laboratory tests, and ultrasonography with color Doppler at 1, 3 day and every week thereafter.

Figure 1. Kidney transplantation in non-human-primate model. Donor kidney is perfused with HTK solution via 24G angiocath (A). Renal artery and vein are anastomosed with recipient aorta and inferior vena cava in end-to-side fashion (B). Donor kidney is reperfused after vessel anastomosis (C). Retroperitoneal positioning of kidney allograft (D).



NHP experimental groups and immunomodulation

A total of six rhesus monkeys in two experimental groups were transplanted in this study. Experimental group (n = 5) used MD-3 mAb, anti-CD154 mAb (5C8, National Institutes of Health, Besthesda), mycophenolate mofetil (CellCept® suspension, Roche, Canada) and methylprednisolone (Solumedrol®, Pfizer Pharmaceuticals Korea Ltd., Seoul, Korea) as immunosuppressants. Control group (n =1) animal received anti-CD154 mAb, mycophenolate mofetil and methylprednisolone. MD-3 mAb (8mg/kg) was given on days -9, -6 and -3 prior to kidney transplantation. Anti-CD154 blocking antibody (20mg/kg, iv) was given on days -10, -7, -4, 0, 3, 7, 12, 17, 22, 28, weekly for 1 month, biweekly for 1 month, and monthly thereafter. Mycophenolate mofetil (20mg/kg, po, bid) was used from -1 day. Methylprednisolone was rapidly tapered and discontinued at 6 month after transplantation. (1 mg/kg for 7 days, 0.75mg/kg for 7 days, 0.5mg/kg for 14 days, 0.3mg/kg for 4 weeks, 0.15mg/kg for 4 weeks, and 0.08mg/kg for 12 weeks)

To determine the effect of MD-3 mAb in inhibiting DSA development, anti-CD154 blocking antibody was discontinued at 133 days after transplantation in one animal of experimental group and one animal of control group.

Histopathology

Routine renal biopsies were performed around postoperative days (PODs) 90 and 180, and whenever graft rejection was suspected by using the 16-gauge core biopsy needle. Autopsy was performed immediately after euthanasia to evaluate abnormality of the graft and organs. Standard hematoxylin and eosin staining was performed on all allograft biopsy and nephrectomy specimens. Graft histopathology was evaluated based on the Banff classification (14, 15) by a single pathologist without knowledge of the treatments or clinical findings.

Antibodies and flow cytometric analysis

Peripheral blood T cell phenotypes were characterized serially from pretransplantation to assess changes in CD3, CD4 and CD8 T cell subsets with particular attention to $CD28^{+}95^{-}$ naïve T (T_N) cells, $CD28^{+}CD95^{+}$ central memory (T_{CM}), $CD28^{-}CD95^{+}$ effector memory (T_{EM}), and $CD4^{+}CD25^{+}FoxP3^{+}$ regulatory T (T_{REG}) cells (16).T cells were quantified by flow cytometric analysis. Fresh peripheral blood mononuclear cells (PBMCs) were isolated by ficoll density gradient centrifugation (BD, Franklin Lakes, NJ) and stained with the following mononuclear antibodies. Immunophenotyping was performed on fresh peripheral whole blood anticoagulated with EDTA. Monoclonal antibodies against CD3 (PerCP-Cy5.5, BD), CD4 (PE-Cy7, BD), CD8 (APC-Cy7, BD), CD11c(PerCp-Cy5.5, BioLegend, San Diego, CA),

CD28(PE, BD), CD14(APC, BD), CD16(APC, BD), CD20(FITC, BD), CD56(PE, BD), CD95(APC, BD), CD123(PE, BD), HLA-DR(PE-Cy7, BD) were added directly to 100 μl aliquots of the whole blood and incubated at room temperature for 10 minutes. Red blood cells were lysed using Cal-lyse lysing solution (Molecular Probes) for non-T cells analysis, and intracellular Foxp3 (PE, eBioscience, San Diego, CA) was stained for regulatory T cells analysis. Stained cells were analyzed on a FACSCalibur (Becton Dickinson, San Joes, CA) and a LSRFortessa (Becton Dickinson). Data were analyzed using FlowJo software (Ver. 10.0.6). Events were gated on lymphocytes based on forward scatter vs. side scatter and on B lymphocytes based on the expression of CD20.

Donor–specific IgG was assessed on whole blood samples preoperatively, every week for a month, and every month thereafter postoperatively. Donor whole blood was incubated with undiluted recipient sera for 30 min at 4℃, washed in PBS and stained with mouse anti-monkey IgG-FITC (Santa Cruz Biotechnology, Inc., Dallas, Texas) for 30 min. Third-party and autologous sera and cells were used as negative controls. Cells were then counterstained with antibody against CD3-PE to segregate antibody responsiveness to resting T cells from that of generalized donor-specific antibody. Red cells were lysed with ACK lysis buffer before analysis on a BD FACScan.

ELISPOT

The frequencies of IFN-γ-secreting antigen-specific T cells in peripheral blood of nonhuman primates were measured using an ELISPOT kit (Mabtech, Sweden) as described previously (12). Anti-IFN-γ capture antibody-coated plates were washed four times with sterile PBS (200 μl/well) and blocked for 30 min with 10% human serum-supplemented RPMI 1640 media at room temperature. After removing media, responding cells (5×10^5) were added in RPMI 1640 media supplemented with 10% human serum for 40h at 37℃ in a 5% CO_2 incubator. The responding PBMCs of recipients were cocultured with irradiated stimulating donor PBMCs (3×10^5), or unstimulated in medium alone (3×10^5), or with anti-CD3 monoclonal Ab. The irradiated donor PBMCs (5×10^5) was also cultured in medium alone in order to investigate stability of stimulating cells. After 40h culture, cells were removed and the plates were washed five times with PBS (200μl/well). Alkaline phosphatase-conjugated detecting antibody diluted at 1:1,000 in 100 μl PBS containing 0.5% fetal bovine serum was then added and incubated for 2 h at room temperature. The plates were washed five times with PBS, and 100 μl BCIP/NBP substrate was added. Color development was stopped by washing with tap water. The resulting spots were counted on a computer-assisted ELISPOT Reader System (AID, Germany).

Anti-MD-3 antibody

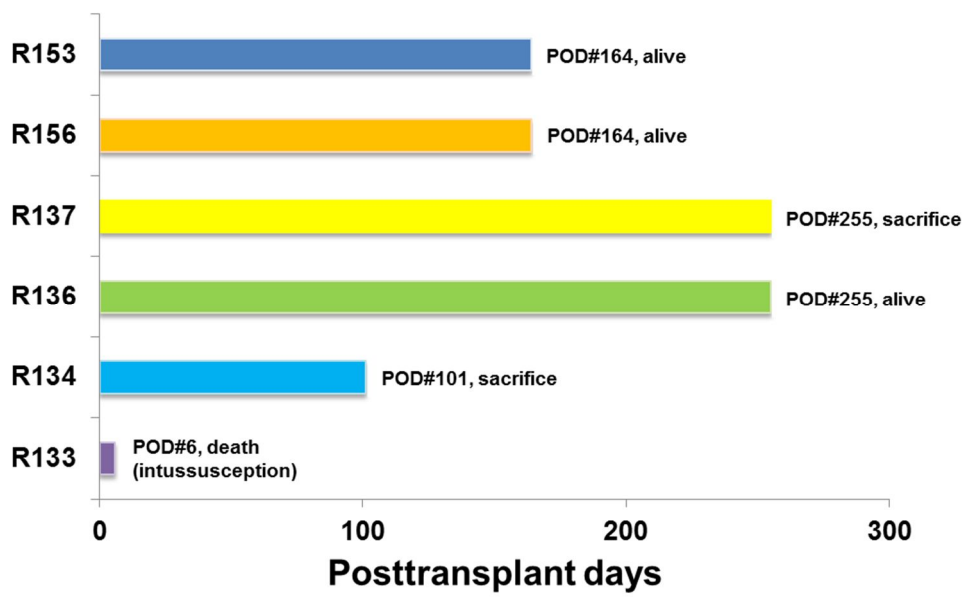
The anti-MD-3 Ab was examined by antigen bridge type sandwich ELISA using the blood samples of transplant recipients collected monthly and at the time of autopsy. DNP003 was used as a capture antibody and detector was a biotin conjugated DNP003. Anti-drug antibodies (ADA) assay was conducted in DINONA Inc (seoul, Korea).

Results

Animal survival and surgical complication

One (R133, experimental group) of six animals died from small bowel intussusception and two animals (R134 and R137, experimental group) were sacrificed on POD 101 day and 255 day due to general weakness. Other two experimental animals (R136 and R156) and one control animal (R153) are alive at last follow-up (255, 164, and 164 days). (Figure 2)

Figure 2. Monkey and allokidney survival.



Kidney ultrasonography and color Doppler analysis were done at POD 1, 3 and every week thereafter (Figure 3). Resistance index throughout the follow-up is illustrated in (Figure 4).

Figure 3. Posttransplant doppler exam. Color doppler signal of kidney allograft at posttransplant day 3 in R136 (A). Hydropelvis detected in follow-up ultrasonography at posttransplant day 136 in R136 (B).

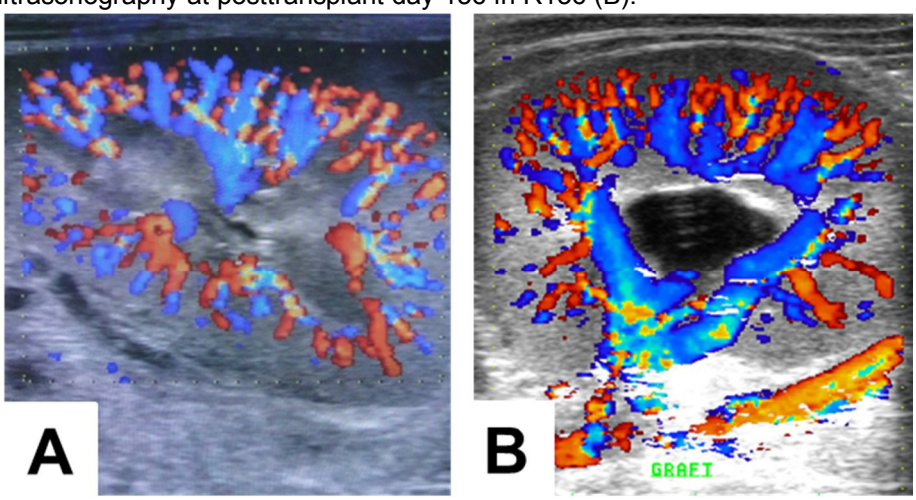
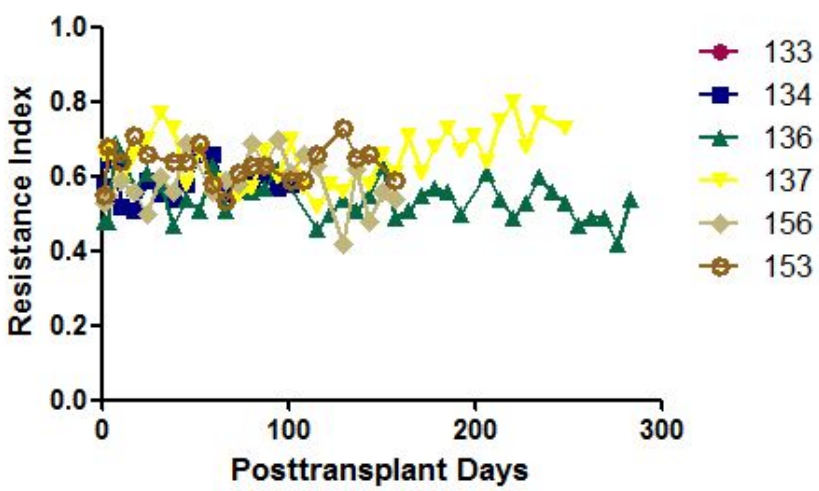


Figure 4. Resistance index of kidney allograft



Neither arterial bleeding nor stenosis occurred. No urine leakage occurred. Ureteral stenosis was the only surgical complication developed in animals. Although 4.7Fr Double-J ureteral stent was placed after ureteral implantation, all animals showed hydropelvis and hydroureter in graft kidney after 1 week. In one animal (R137), Double-J stent was removed at POD 21. However, it needed ureter reanastomosis with stent at POD 105. At POD 200, DJ stent was removed with cystoscope. In another animal (R136), Double-J stent was spontaneously removed at POD 3. Ureteral stent was re-inserted at POD 105. In animals R156 and R153, ureteral stent was removed at POD 68 with a flexible ureteroscopy.

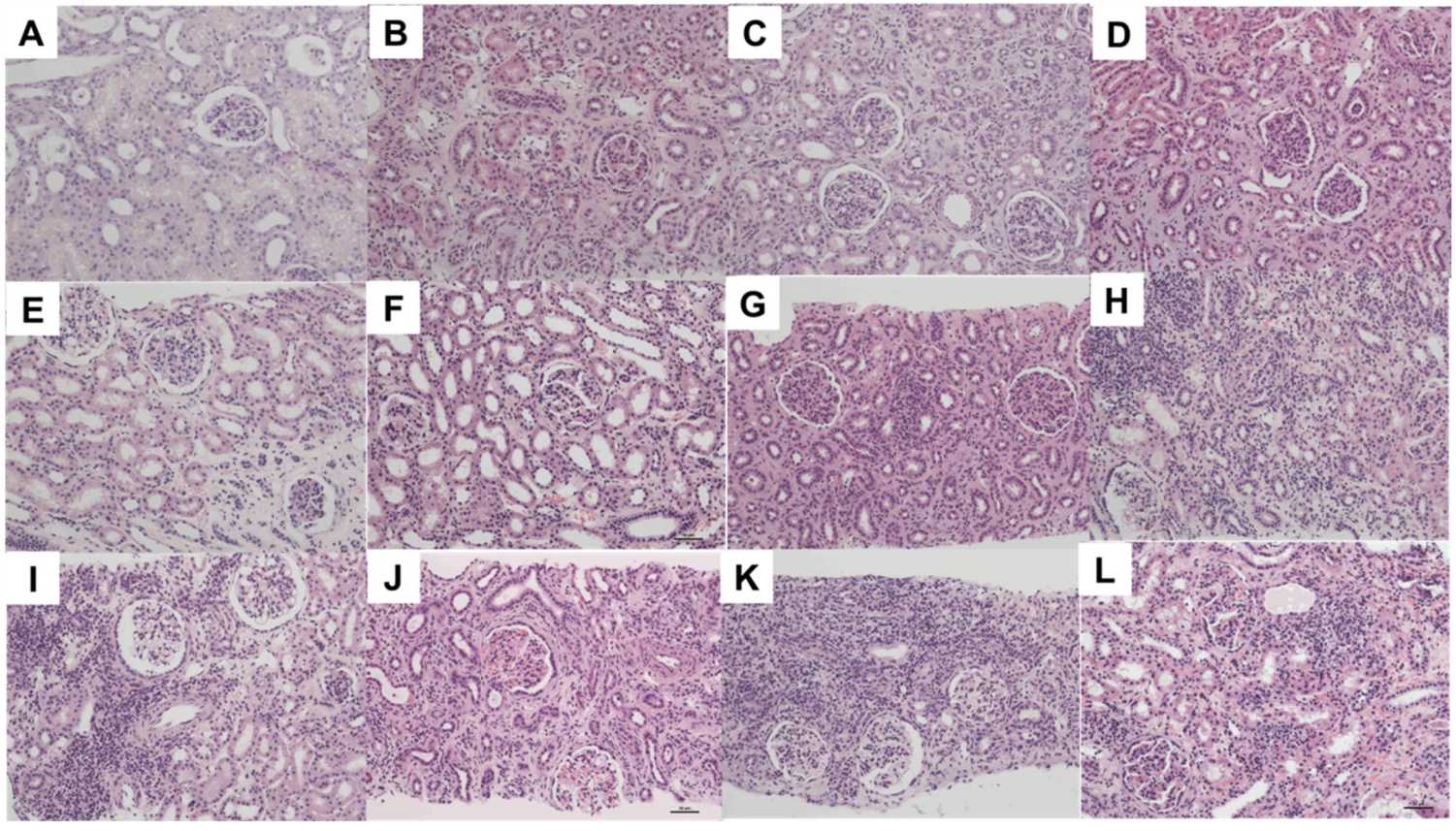
Histopathological study (Figure 5)

At 3-month protocol biopsies, three (R134, R136, and R137) of experimental group showed only minimal infiltration of lymphocyte, a few eosinophils without vasculitis and equivocal tubulitis. One animal in both experimental and control group (R153 and R156) did not show feature of immunologic rejection but pyelonephritis such as numerous lymphocyte filling renal tubules at 3-month protocol biopsies. At 6-month protocol biopsies, R136 showed normal glomeruli and minimal infiltration of lymphocyte. R137 showed pyelonephritis sign with normal glomeruli. R136 showed normal histology at 8-month biopsies.

One animal in both experimental and control group (R153 and R156) had ‘for cause‘ biopsies at POD 46. Both animals showed dense accumulation of PMNs that destroy tubules and expand the interstitium forming a microabscess and diagnosed as acute pyelonephritis.

Autopsy was performed in two animals in experimental group (R133 and R134). R133 showed normal renal histology at POD6. R134 showed minimal infiltration of lymphocytes in interstitium without tubulitis.

Figure 5. Microscopic appearance of renal allograft. Photographs show histology of graft biopsy specimen (H&E staining, magnification x200). Graft biopsies at autopsy in R133 (posttransplant day 6, A) and in R134 (posttransplant day 102, C) show minimal interstitial inflammation without tubulitis. Graft biopsies in R134 (B), R136 (D), and R137 (G) show minimal iinfiltration of lymphocytes with equivocal tubulitis at 3-month protocol biopsies. At 6-month protocol biopsies, graft biopsy in R136 (E) shows normal histology and graft biopsy of R137 (H) show pyelonephritis. R153 (I) and R156(K) show severe pyelonephritis in indication biopsy at posttransplant day 46. R153 (J) and R156 (L) also show pyelonephritis feature without rejection at 3-month protocol biopsies.


A
B
C
D
E
F
G
H
I
J
K
L

Donor-specific antibody production

All animals were seronegative for DSA before transplantation in both experimental and control groups (Figure 6). DSA was not detected during therapy with maintenance immunosuppression including anti-CD154 blocking antibody (Figure 7). However, after cessation of anti-CD154 blocking antibody, transplant recipient (R153, control group) started to develop DSA after 3 weeks, and anti-donor IgG Abs remained present thereafter while R156 (experimental group) remained seronegative for DSA after cessations of anti-CD154 blocking antibody (Figure 8).

Figure 6. Donor-specific antibodies prior to transplantation. Donor-specific antibodies were not detected prior to transplantation in all animals.

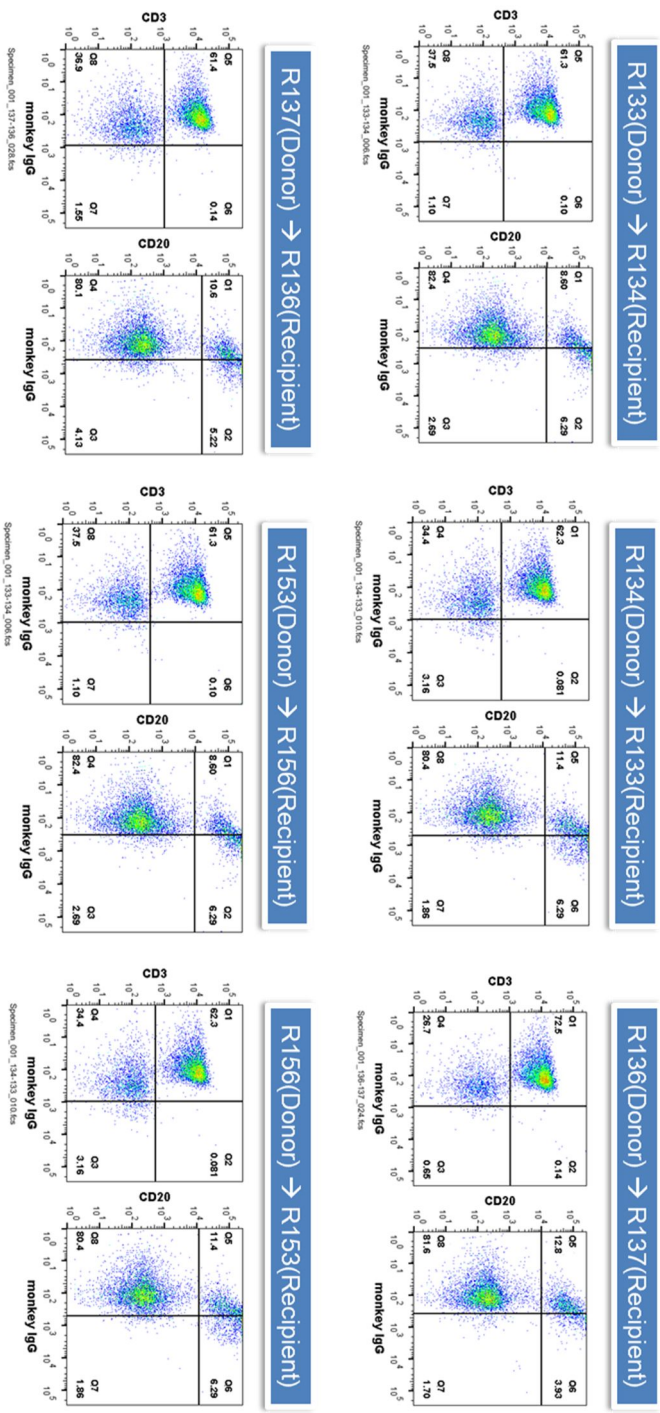


Figure 7. Donor-specific antibodies production after transplantation. Donor-specific antibodies were also not detected during therapy in all animals. R133 and R134 were not checked because R133 was early sacrificed.

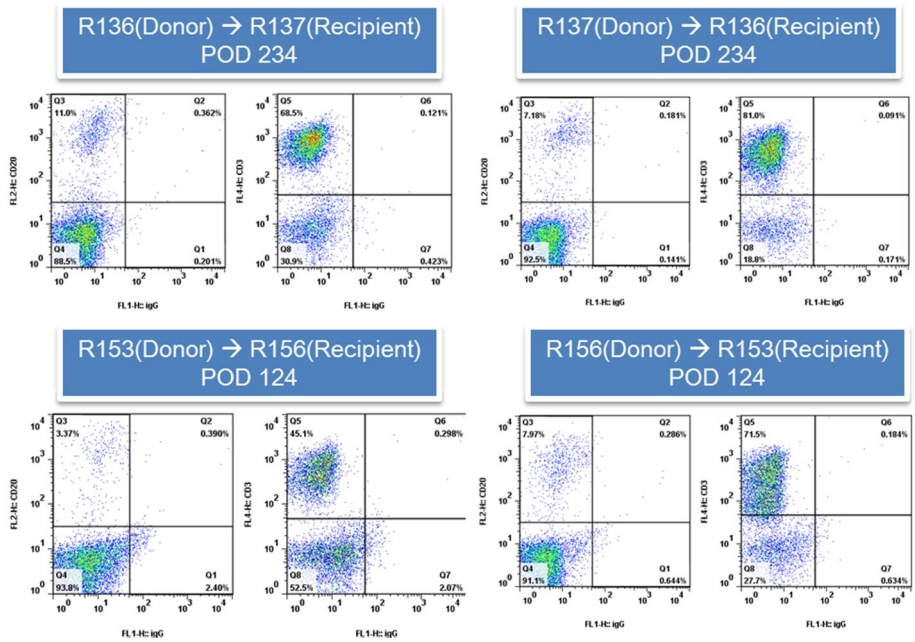
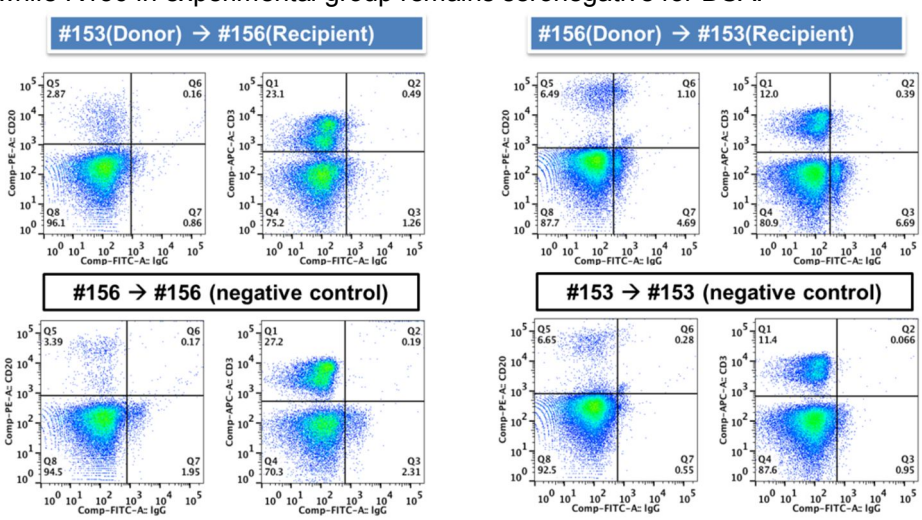


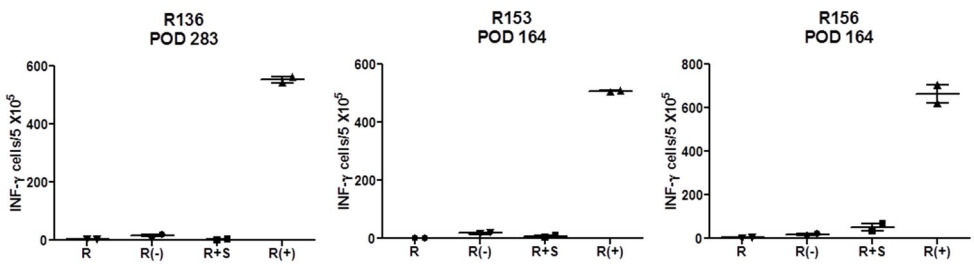
Figure 8. Donor-specific antibody (DSA) development 4 weeks after cessation of anti-CD154 blocking antibody. R153 in control group shows production of DSA while R156 in experimental group remains seronegative for DSA.



Cellular immune response

Anti-donor cellular responses were examined by assessing the frequency of IFN-γ-secreting alloreactive T cells in the periphery. ELISPOT analysis of PBMCs isolated at 3-month from recipient animals revealed near-complete suppression of IFN-γ responses to donor PBMCs, whereas an intact immune response to a third party of alloantigen was maintained (Figure 9).

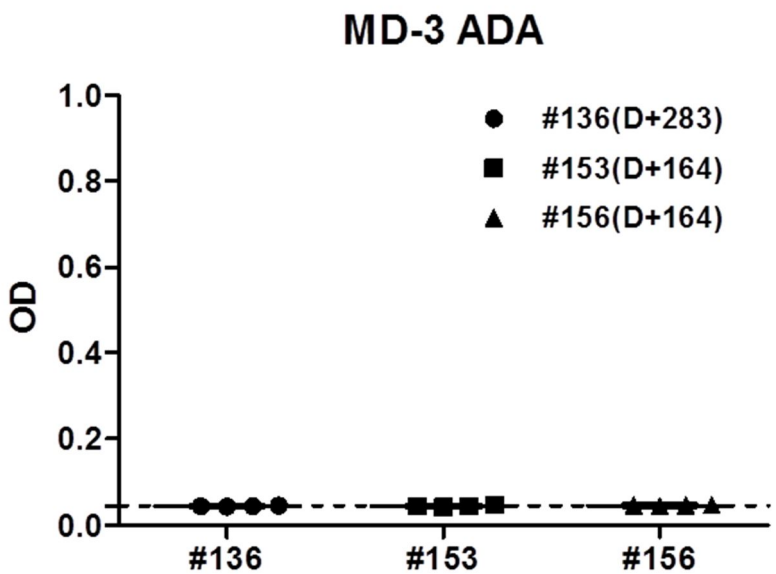
Figure 9. ELISPOT analysis of IFN-γ-secreting alloreactive T cells at 3 month.



Anti-MD-3 antibody production

Antibodies against MD-3 were not detected in any animal during the study (Figure 10). In addition, anti-MD-3 antibody did not develop in animal with cessation of anti-CD154 mAb (R156).

Figure 10. Anti-MD-3 antibody production. Antibodies against MD-3 were not detected in long-term survived animals throughout the study.



Hematology

No significant changes in red blood cells (RBC), hematocrit, and platelet counts were noted in all animals (data now shown). Recipient peripheral blood leukocytes were serially 'phenotyped' at different posttransplant time points. After initiation of MD-3 therapy in experimental group, the absolute number of $CD3^+$, $CD4^+$ and $CD8^+$ T cells immediately increased above baseline and decreased shortly to the baseline (Figure 11). This phenomenon involved in all of T cell phenotypes (naïve, effector memory, and central memory T cells, Figure 12 and 13). In control animal (R153), this increase of the number of T cells was absent. In both groups, the absolute number of $CD3^+$, $CD4^+$ and $CD8^+$ T cells remained stable throughout therapy, with no evidence of T cell depletion.

Regarding the regulatory T cells (Tregs), there was no definite effect of MD-3 on the Tregs, with the percent remaining stable throughout therapy (Figure 14).

Figure 11. Changes in absolute number of $CD3^+$, $CD4^+$ and $CD8^+$ T cells. All animals in experimental group show immediate increase in number of peripheral T cells after injection of MD-3 and return to baseline in short-term (arrow).

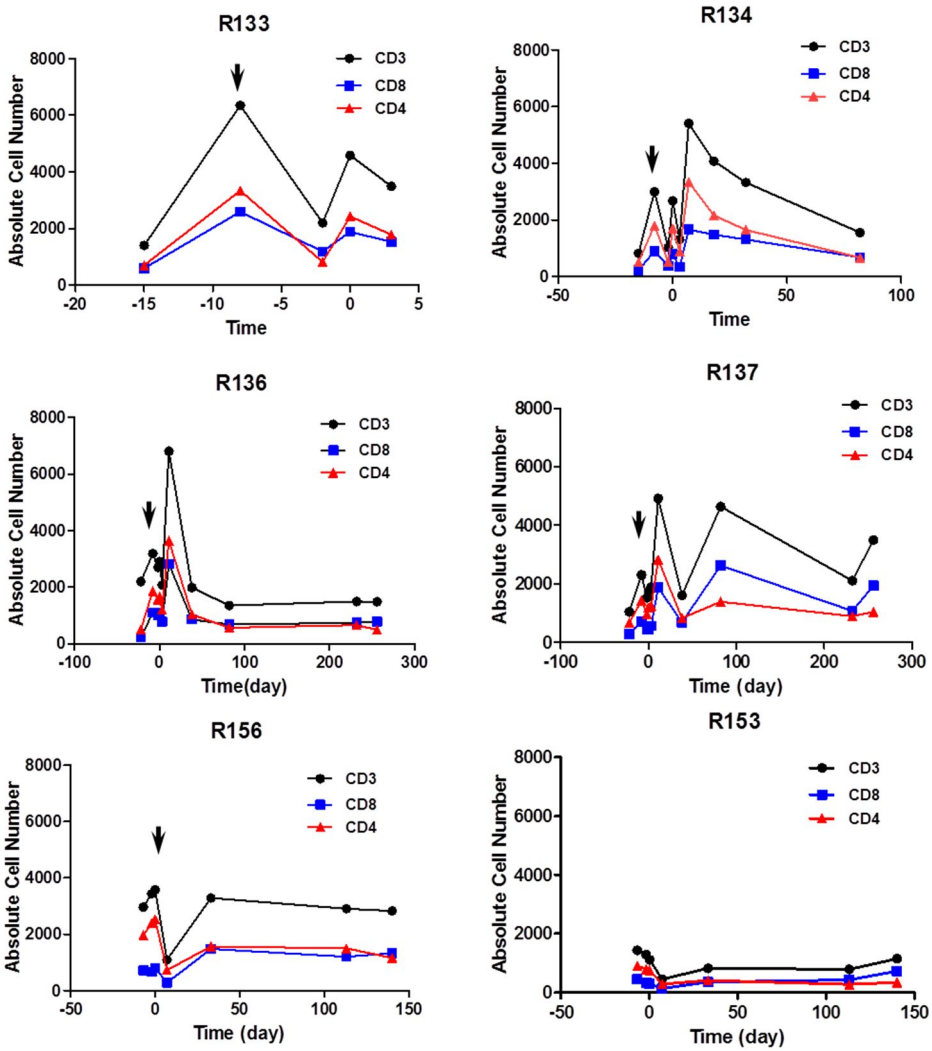


Figure 12. Changes in absolute number of $CD4^+$ T cells phenotype. All animals in experimental group show immediate increase in number of peripheral $CD4^+$ naïve (TN), central memory (TCM), and effector memory (TEM) T cells after injection of MD-3 and return to baseline in short-term (arrow).

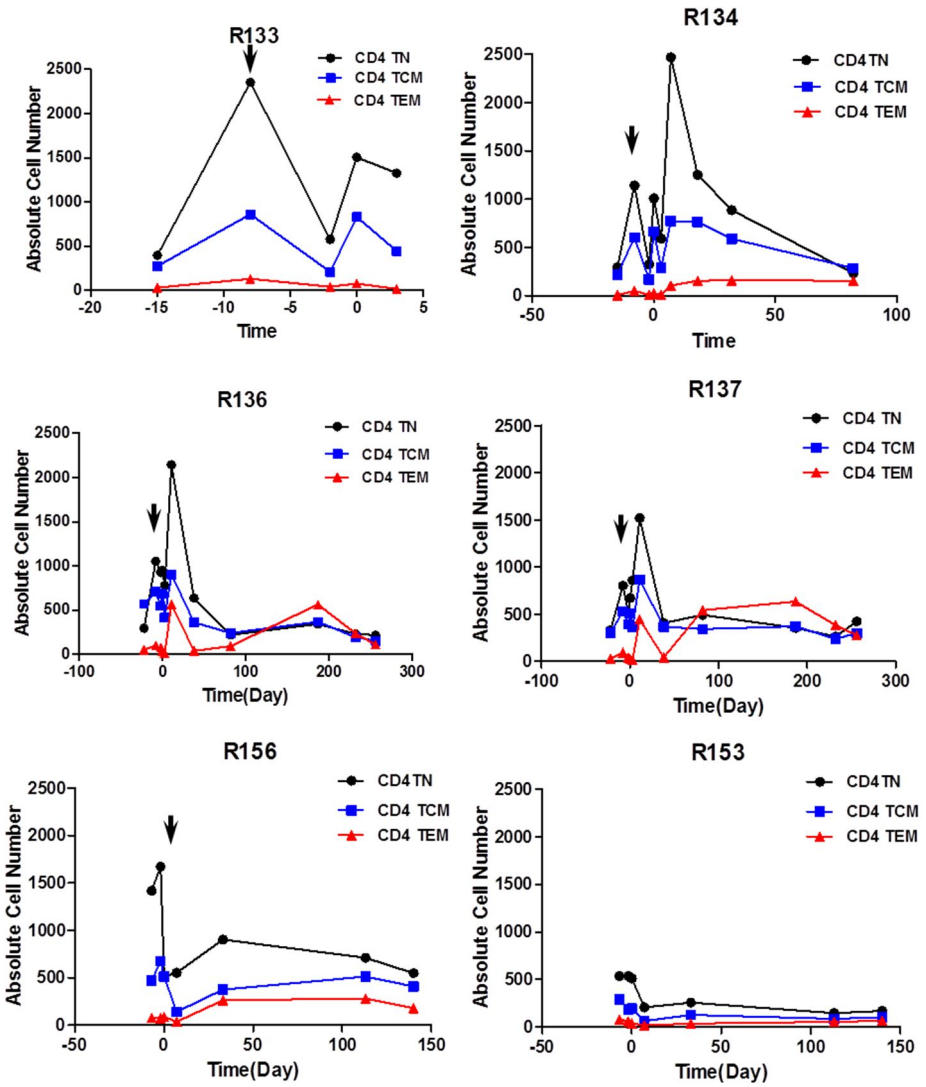


Figure 13. Changes in absolute number of $CD8^+$ T cells phenotype.

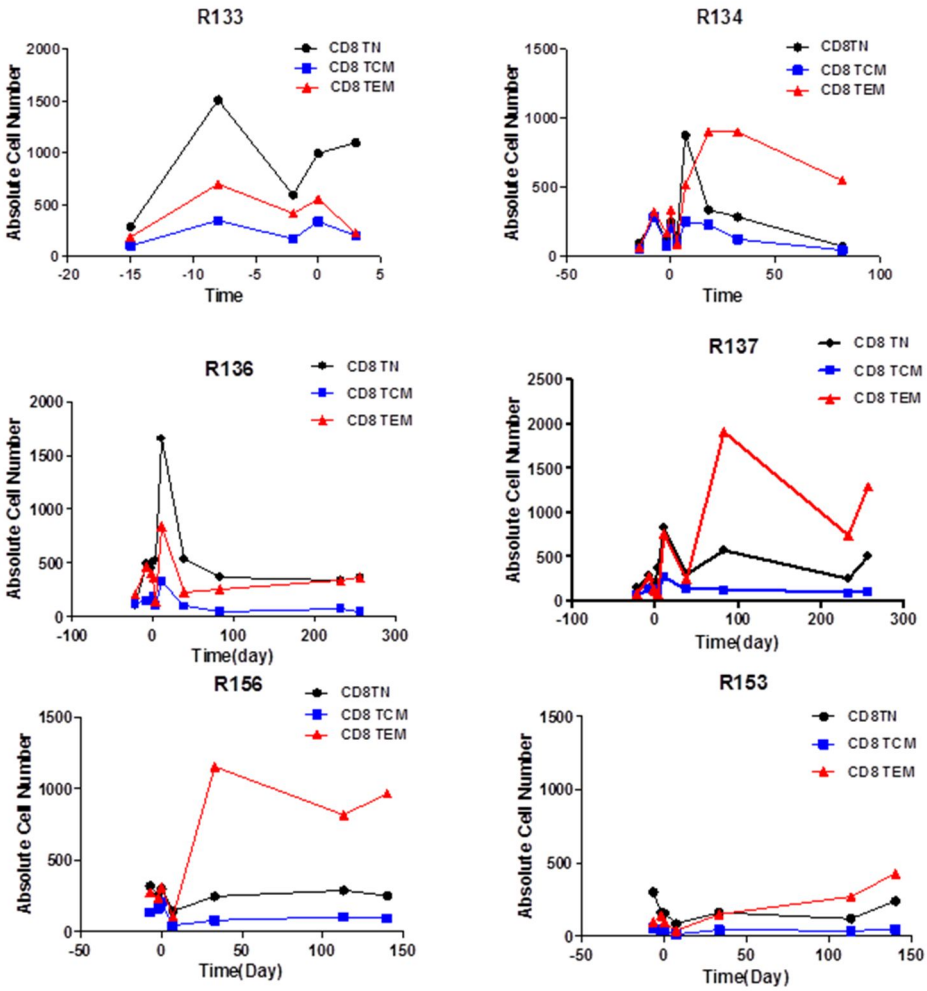
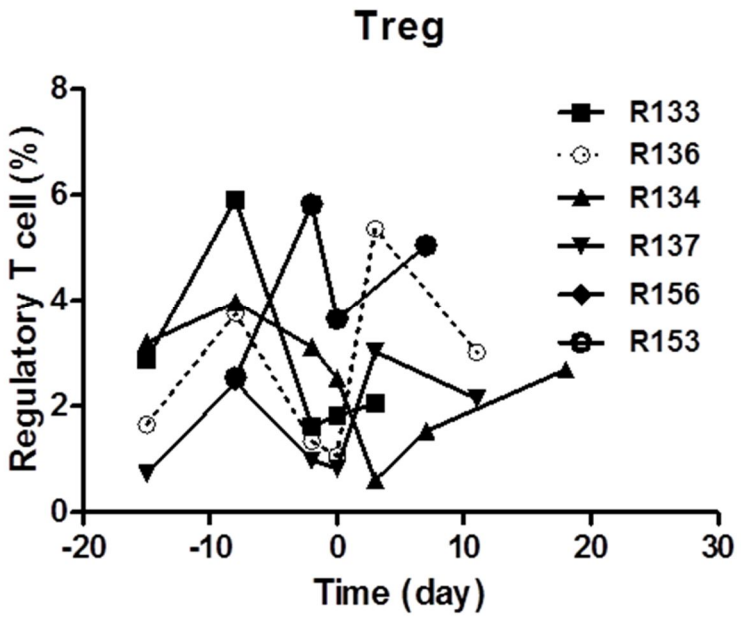


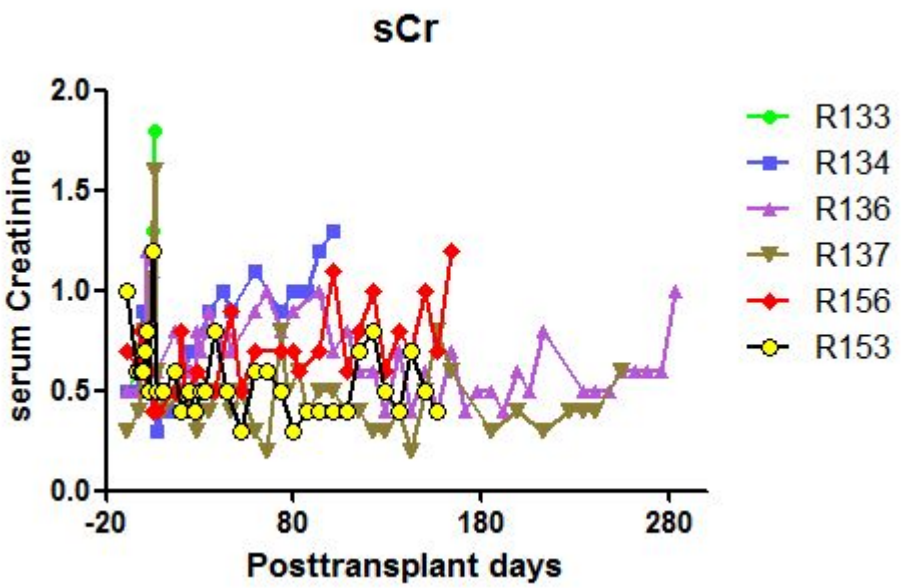
Figure 14. Changes in percentage of regulatory T cells in early period after MD-3 mAb injection



Biochemistry

Serum liver enzyme, pancreatic enzyme, electrolyte, glucose and all other biochemical parameters determined were unaffected in all of MD-3 mAb-treated animals (data now shown). Serum Creatinine increased in some animals in various time points, which reflected urinary tract infection and resultant general weakness (Figure 15).

Figure 15. Changes in serum creatinine (sCr) in animals. Increase in sCr reflects urinary tract infection or general weakness.



Discussion

Nonhuman primates provide an optimal model for the evaluation of new immunosuppressive drugs and tolerance in the preclinical setting. The surgery is similar to renal transplants in small infants. Same as in clinical practice, surgical complication including renal vessel thrombosis, urine leakage, and ureteral stenosis is common in nonhuman primate kidney transplantation model (17). A special expertise in both surgical technique and postoperative management is critical to a successful experiment (18-21). The vascular anastomosis technique is similar to what is used in infants. However, the routine use of techniques developed in microvascular surgery including magnification (3.5x) and 8.0 Prolene® polypropylene suture should be adopted. It has also been believed critical that the nonhuman primates are anticoagulated with heparin prior to performing vascular anastomoses and receive antiplatelet agent postoperatively. Following this guideline, no renal vessel thrombosis has occurred in our experimental animals.

The Achilles heel in renal transplantation in rhesus monkeys is the ureteroneocystostomy. A significant incidence of ureteral stenosis (approximately 50% of long-term survivors) was reported when a Leadbetter-Politano technique was used and this complication can be reduced with routine use of ureteral stent (13, 22). To reduce the incidence of ureteral stenosis or stricture, we used single stitch technique in four

animals and a Lich-Gregoir technique in two, and ureteral stent was placed in all animals. However, all animals developed ureteral stenosis detected by B-mode ultrasonography which is known to be very helpful to reveal hydronephrosis and establish the diagnosis of ureteral stenosis/stricture (23). Because 3.0 Fr Ureteral stent is small enough to fit in the rhesus monkey ureter but is not readily available, we had to use 4.7 Fr stent which is relatively large to the monkey ureter. This may lead to 'functional obstruction' of ureter, and resultant hydroureter and hydronephrosis. The recurrent episode of urinary tract infection caused by hydronephrosis precluded long-term survival of kidney allograft and evaluation of novel immunosuppressive drug in some monkeys, therefore improvement in technique should be critical.

Modern allotransplantation requires the daily administration of nonspecific immunosuppressive agents to prevent T cell-mediated acute rejection (24). The agents commonly used are calcineurin inhibitors such as tacrolimus and cyclosporine, antiproliferative agents such as mycophenolate mofetil, and corticosteroid (1). Although these agents have considerably improved organ transplant outcome, their therapeutic effects are transient and all have substantial toxicity. Therefore, more specific and less toxic immunosuppression is required to optimize transplant outcome. An induction and a prolonged maintenance therapy with a humanized monoclonal antibody specific for CD154 (hu5C8) could safely prevent the

development of acute allograft rejection and achieve extended rejection-free survival in rhesus monkeys (25-29). The previous studies also described the use of anti-CD154 antibody as a rescue agent for acute rejection (30). However, allospecific antibody production was not eliminated by anti-CD154 antibody therapy. Donor-specific IgG antibodies started to develop between 30 and 60 days after transplantation and persisted in all monkeys treated with hu5C8 (25). Considering that the primary responsible cause of kidney allograft dysfunction and loss is donor-specific antibody produced by recipient's immune system and resultant acute and/or chronic antibody-mediated rejection (31-33), the inability of to suppress DSA development along with thrombophilia is inherent critical weakness of anti-CD154 blocking antibody. In this study, we have clearly shown that donor-specific antibody does not develop in animals with MD-3 treatment in combination with anti-CD154 blocking antibody (Figure 7). In addition, MD-3-treated animals remained seronegative for donor-specific antibody even after cessation of anti-CD154 blocking antibody while rhesus monkey without MD-3 therapy developed DSA. Along with the MD-3-induced antigen-specific T cell hyporesponsiveness (Figure 9), effect of MD-3 to suppress DSA development is critically important in clinical field to achieve optimal kidney transplant outcome in long-term. By using MD-3 with a combination of anti-CD154 antibody, we can inhibit donor-specific antibody injury as well as acute T-cell mediated rejection.

The interesting finding is that all recipients were seronegative for anti-drug antibody throughout the study period. Considering that incidence of retransplantation has grown significantly during last decades, MD-3 treatment can be successfully used in highly sensitized retransplant patients.

In this study, we demonstrated that MD-3 treatment in combination with anti-CD154 antibody induced antigen-specific immunosuppression rather than nonspecific generalized immunosuppression in solid organ transplantation in non-human primates. The inhibition of DSA development in MD-3 therapy can also reduce antibody-mediated rejection in clinical solid organ transplantation and be a highly promising strategy to prolong the graft survival rate. Further refinement study for dosages of MD-3 and its dosing schedule, for example prolonged induction or maintenance regimen, should be warranted.

References

1. Korean Network for Organ Sharing (KONOS). 2013 Annual Data Report [Internet].

Seoul: KONOS; 2014 [cited 2015 Feb 24]. Available from: http://konos.go.kr.

2. Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 1996. N Engl J Med. 2000 Mar 2;342(9):605-12.

3. Penn I. Cancers in renal transplant recipients. Adv Ren Replace Ther. 2000 Apr;7(2):147-56.

4. Tanphaichitr NT, Brennan DC. Infectious complications in renal transplant recipients. Adv Ren Replace Ther. 2000 Apr;7(2):131-46.

5. Schwenger V, Zeier M, Ritz E. Hypertension after renal transplantation. Ann Transplant. 2001;6(4):25-30.

6. Pannu HS, Singh D, Sandhu JS. Lipid profile before and after renal transplantation--a longitudinal study. Ren Fail. 2003 May;25(3):411-7.

7. Sprague SM, Josephson MA. Bone disease after kidney transplantation. Semin Nephrol. 2004 Jan;24(1):82-90.

8. Pilmore HL, Dittmer ID. Calcineurin inhibitor nephrotoxicity: reduction in dose results in marked improvement in renal function in patients with coexisting chronic allograft nephropathy. Clin Transplant. 2002 Jun;16(3):191-5.

9. Kirk AD. Transplantation tolerance: a look at the nonhuman primate

literature in the light of modern tolerance theories. Crit Rev Immunol. 1999;19(5-6):349-88.

10. Verbinnen B, Van Gool SW, Ceuppens JL. Blocking costimulatory pathways: prospects for inducing transplantation tolerance. Immunotherapy. 2010 Jul;2(4):497-509.

11. Kawai T, Cosimi AB, Sachs DH. Preclinical and clinical studies on the induction of renal allograft tolerance through transient mixed chimerism. Curr Opin Organ Transplant. 2011 Aug;16(4):366-71.

12. Jung KC, Park CG, Jeon YK, Park HJ, Ban YL, Min HS, et al. In situ induction of dendritic cell-based T cell tolerance in humanized mice and nonhuman primates. J Exp Med. 2011 Nov 21;208(12):2477-88.

13. Eckhoff DE, Haisch CE, Contreras JL, Thomas FT, Robbins M, Frenette L, et al. Experience with renal transplantation in the nonhuman primate: a modified ureteroneocystostomy to prevent urologic complications. J Invest Surg. 2000 Sep-Oct;13(5):247-51.

14. Racusen LC, Solez K, Colvin RB, Bonsib SM, Castro MC, Cavallo T, et al. The Banff 97 working classification of renal allograft pathology. Kidney Int. 1999 Feb;55(2):713-23.

15. Solez K, Colvin RB, Racusen LC, Sis B, Halloran PF, Birk PE, et al. Banff '05 Meeting Report: differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). Am J Transplant. 2007 Mar;7(3):518-26.

16. Lo DJ, Anderson DJ, Weaver TA, Leopardi F, Song M, Farris AB, et al. Belatacept and sirolimus prolong nonhuman primate renal allograft survival without a requirement for memory T cell depletion. Am J Transplant. 2013 Feb;13(2):320-8.

17. Borie D, Hausen B, Larson M, Klupp J, Stalder M, Birsan T, et al. A life-supporting technique of renal allotransplantation in Macaca fascicularis to evaluate novel immunosuppressive drugs in nonhuman primates. J Surg Res. 2002 Sep;107(1):64-74.

18. Neuhaus P, Neuhaus R, Wiersema HD, Borleffs JC, Balner H. The technique of kidney transplantation in rhesus monkeys. J Med Primatol. 1982;11(3):155-62.

19. Shapiro ME, Reed MH, Strom TB, Carpenter CB, Milford EL, Kirkman RL. The role of a primate model of renal transplantation in the development of new monoclonal antibodies. Am J Kidney Dis. 1989 Nov;14(5 Suppl 2):58-60.

20. Cecka JM, Gjertson DW, Terasaki PI. Pediatric renal transplantation: a review of the UNOS data. United Network for Organ Sharing. Pediatr Transplant. 1997 Aug;1(1):55-64.

21. Salvatierra O, Alfrey E, Tanney DC, Mak R, Hammer GB, Krane EJ, et al. Superior outcomes in pediatric renal transplantation. Arch Surg. 1997 Aug;132(8):842-7; discussion 7-9.

22. Wilson CH, Bhatti AA, Rix DA, Manas DM. Routine intraoperative

ureteric stenting for kidney transplant recipients. Cochrane Database Syst Rev. 2005(4):CD004925.

23. Cosgrove DO, Chan KE. Renal transplants: what ultrasound can and cannot do. Ultrasound Q. 2008 Jun;24(2):77-87; quiz 141-2.

24. Knoll G. Trends in kidney transplantation over the past decade. Drugs. 2008;68 Suppl 1:3-10.

25. Kirk AD, Burkly LC, Batty DS, Baumgartner RE, Berning JD, Buchanan K, et al. Treatment with humanized monoclonal antibody against CD154 prevents acute renal allograft rejection in nonhuman primates. Nat Med. 1999 Jun;5(6):686-93.

26. Cho CS, Burkly LC, Fechner JH, Jr., Kirk AD, Oberley TD, Dong Y, et al. Successful conversion from conventional immunosuppression to anti-CD154 monoclonal antibody costimulatory molecule blockade in rhesus renal allograft recipients. Transplantation. 2001 Aug 27;72(4):587-97.

27. Pearl JP, Xu H, Leopardi F, Preston E, Kirk AD. CD154 blockade, sirolimus, and donor-specific transfusion prevents renal allograft rejection in cynomolgus monkeys despite homeostatic T-cell activation. Transplantation. 2007 May 15;83(9):1219-25.

28. Kirk AD, Harlan DM, Armstrong NN, Davis TA, Dong Y, Gray GS, et al. CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. Proc Natl Acad Sci U S A. 1997 Aug 5;94(16):8789-94.

29. Kawai T, Sogawa H, Boskovic S, Abrahamian G, Smith RN, Wee

SL, et al. CD154 blockade for induction of mixed chimerism and prolonged renal allograft survival in nonhuman primates. Am J Transplant. 2004 Sep;4(9):1391-8.

30. Xu H, Tadaki DK, Elster EA, Burkly LC, Berning JD, Cruzata F, et al. Humanized anti-CD154 antibody therapy for the treatment of allograft rejection in nonhuman primates. Transplantation. 2002 Oct 15;74(7):940-3.

31. Jordan SC, Vo AA. Donor-specific antibodies in allograft recipients: etiology, impact and therapeutic approaches. Curr Opin Organ Transplant. 2014 Dec;19(6):591-7.

32. Gaston RS, Cecka JM, Kasiske BL, Fieberg AM, Leduc R, Cosio FC, et al. Evidence for antibody-mediated injury as a major determinant of late kidney allograft failure. Transplantation. 2010 Jul 15;90(1):68-74.

33. Sellares J, de Freitas DG, Mengel M, Reeve J, Einecke G, Sis B, et al. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. Am J Transplant. 2012 Feb;12(2):388-99.

초 록

서론: MD-3 항체는 최근에 개발된 사람의 세포내 접착분자 1 (intracellular adhesion molecule 1, ICAM-1)에 특이적인 키메라항체이다. 본 연구에서는 이 MD-3 항체의 고형장기이식에서의 효과를 확인해보고자 하였다.

방법: 리저스 원숭이 간의 신장이식 모델을 확립한 후, 실험군 다섯 마리에서는 MD-3를 이용한 유도요법을 시행하였고 대조군 한 마리는 MD-3 항체를 주입하지 않았다. 유지면역억제 요법으로는 anti-CD154 mAb (hu5C8), mycophenolate mofetil 및 스테로이드를 사용하였다. 각 원숭이에서 이식한 신장의 급성 거부반응 및 기증자 특이 항체의 발생 여부를 검사하였다. ELISPOT 검사로 세포성 면역 반응을 확인하였고 MD-3에 대한 항체의 발생 여부도 검사하였다.

결과: 최장 이식 후 8개월까지 관찰한 원숭이 수혜자에서 급성 거부반응은 확인되지 않았다. 기증자 특이 항체 역시 MD-3를 투여받은 수혜 원숭이에서 발생하지 않았다. MD-3를 투여받지 않은 대조군 원숭이에서는 anti-CD154 mAb를 중단한 후 3주경부터 기증자 특이 항체가 발생하기

시작하였으며 이후 지속되었다. MD-3에 대한 항체는 연구 기간 동안 모든 원숭이에서 확인되지 않았다.

결론: MD-3는 사람의 동종 장기이식에서 급성 거부반응 및 기증자 특이 항체 발생을 예방하여 장기 이식 성과를 향상시킴으로써 임상적으로 매우 유용한 면역억제제가 될 것으로 예측된다. 향후 적절한 MD-3의 용량 및 투여 스케쥴 등을 결정하기 위한 추가적인 연구가 필요하다.

주요어: 영장류 모델, MD-3, 신장이식, 급성 거부반응, 기증자 특이 항체

학 번: 2009-31080