SUPPLEMENTAL DIGITAL CONTENT

Materials and Methods

Patients

All patients with end stage kidney disease and infected with HIV were evaluated for kidney transplant if they had CD4+ T-cell counts of ≥200 cells per cubic millimeter and undetectable plasma HIV type-1 (HIV-1) RNA levels (<400 copies per milliliter on the standard polymerase-chain-reaction assay version 1.5 or <48 copies per milliliter on the ultrasensitive Taqman polymerase chain reaction assay, Amplicor-1 HIV Monitor Test, Roche) while adherent on a stable HAART regimen. All 11 patients reported in this study had a sustained CD4+ T-cell count ≥200 cells per cubic millimeter for a median of 20 months prior to transplant (range 2-83 months) and a sustained undetectable plasma HIV type 1 RNA level for a median of 20 months prior to transplant (range 5-73 months). All were maintained on HAART medications for a median of 69 months prior to transplant (range 17-147 months). Those with (i) history of progressive multifocal leukoencephalopathy, chronic intestinal cryptosporidiosis, primary central nervous system lymphoma, or visceral Kaposi's sarcoma, (ii) severe and significant coronary artery disease, (iii) history of medication non-adherence, (iv) current recreational drug use, (v) metastatic cancer or on active chemotherapy, (vi) significant neurocognitive impairment, (vii) active systemic infection and (viii) body mass index of ≥40 were not listed for a transplant. Patients with co-infection with HBV or HCV were not excluded provided they had no detectable HBV surface antigen or cirrhosis. Patients were eligible to receive living or deceased donor kidney graft.

Medications

Immunosuppression consisted of induction with either 5 doses (1.5 mg/Kg/day) of rabbit anti-

thymocyte globulin (Thymoglobulin®, Genzyme Corporation, USA) or 2 doses (20 mg/day on days 0 and 4) of basiliximab (Simulect®, Novartis Pharmaceuticals, USA). Intravenous methylprednisolone was given in tapering doses (500 mg IV day 0, 250 mg day 1, 125 mg day 2 and 60 mg days 3-4) along with the induction agents and were discontinued by day-4 of transplant. Maintenance immunosuppression included tacrolimus (drug level 6-11 ng/ml) and mycophenolate (500 mg bid [1000 mg bid in African-Americans]). The infection prophylaxis regimen included trimethoprim/sulfamethoxazole, valganciclovir and clotrimazole. There was no restriction on the use of HAART medications.

Data Collection

Electronic medical records were reviewed for each patient and all relevant demographic, clinical, medication, biochemical, microbiological, radiological and pathology data were obtained. One year kidney graft and patient survival were the primary outcome measures. All patients were followed up at regular intervals in the transplant clinic as per our center's protocol. Delayed graft function was defined as the need for dialysis treatment within the first week of transplant. Allograft biopsies were performed under songraphic guidance for clinical indications. Graft failure was defined as return to maintenance dialysis. Patients who did not reach their primary outcome were censored at their last follow up.

Tacrolimus Pharmacokinetic Studies

Blood Tacrolimus levels were measured by the microparticle enzyme immunoassay II using Abbott IMx immunoassay analyzer (Abbott Diagnostics, Abbott Park, IL, USA) and the minimal detection level is 2 ng/mL. We performed pharmacokinetic profiling of tacrolimus on two kidney

transplant recipients who were on PI based HAART regimen. Patient-1 was on atazanavir and has been reported previously *(1)*. After several attempts to determine the correct dose and dosing interval of tacrolimus to achieve therapeutic drug level, pharmacokinetic profiling was done on the third post-transplant week. Patient-2 was on tipranavir and ritonavir and the profiling study was done on the first post-transplant week.

Urinary Cell mRNA Expression Studies

We obtained 50 ml urine from all the 9 HIV-infected kidney recipients with functioning and stable allograft function at the time of their routine clinic visit (median: 293 days after transplant, range: 190-980). We isolated total RNA from urine-cell pellets and reverse transcribed to complementary DNA. Using gene-specific oligonucleotide primers and fluorogenic probes designed and validated in our laboratory, we measured absolute levels of mRNAs of Toll-like receptor-4 (TLR-4) (CD284), complement component 3 (C3), C5, complement factor B, properdin, complement regulatory proteins membrane cofactor protein CD46 and decay accelerating factor CD55. Anti-thymocyte globulin induction is associated with a greater depletion of peripheral blood immune cells compared to biologics such anti-CD25 mAbs. We investigated urinary cell levels of mRNA encoding cell lineage specific proteins T cell CD3, CD4, CD8, mRNA for T cell co-inhibitory receptor CTLA4, mRNA for CD25, mRNA for regulatory T cell specification factor Foxp3 and mRNA for B cell CD20.

An intriguing and unexpected finding is that the incidence of acute rejection is more frequent in HIV-infected recipients than in HIV-negative recipients of renal allografts *(2-5).* We have reported that urinary cell levels of mRNA for the chemokine IP-10 and mRNA for the cytotoxic attack molecules granzyme B and perforin are diagnostic of acute rejection of renal allografts and predictive of the future development of acute rejection *(6-8).* In this study, we examined

whether the levels of mRNAs for IP-10, granzyme B and perforin are higher in the HIV-infected recipients compared to the HIV-negative Stable group. A major concern in HIV-infected recipients of allografts is over immunosuppression and resultant reactivation of latent virus. We examined whether BKV replication, potential index of over immunosuppression, is more frequent (as assessed by measuring urinary cell BKV VPI-mRNA levels *[9]*) in HIV-infected recipients and the HIV-negative Stable group.

PCR analysis was performed as previously described *(10).* Transcript levels were calculated by standard curve method *(11)* and expressed as a ratio target gene copy number per microgram of RNA to the reference gene 18S rRNA copy number per femtogram of RNA. We compared the urinary cell gene expression profile of HIV-infected kidney graft recipients with a cohort of 22 HIV-negative kidney graft recipients with stable graft function and normal biopsy results and treated with the early corticosteroid withdrawal immunosuppression regimen. Collection of urine specimen, RNA isolation and reverse transcription were performed using an identical protocol. This cohort was selected from our Institutional Review Board approved study of urinary cell mRNA profiling of kidney transplant recipients. Urine specimens were obtained at the time of surveillance biopsies (median: 44 days after transplant, range: 30-451; N=22, 13 men and 9 women; 8 white, 4 black and 10 with other racial or ethnic backgrounds; with 18 living and 4 deceased donors; 18 with antithymocyte globulin and 4 with basiliximab induction; mean [±SD] creatinine level, 1.42±0.32 mg per deciliter).

Supplementary References

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SUPPLEMENTAL DIGITAL CONTENT

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SUPPLEMENTAL DIGITAL CONTENT

Table S1. Cardiovascular risk profile of kidney transplant recipients infected with HIV

Variable, *median (inter quartile range)*	**Pre-Transplant**	**Post-Transplant**			**P value**
		6 months	**12 months**	**24 months**	Repeated Measures ANOVA
Weight, *Kg*	82 (76-88)	81 (72-90)	81 (71-86)	82 (71-91)	0.97
Systolic blood pressure, *mmHg*	139 (126-151)	130 (124-140)	130 (115-138)	131 (120-138)	0.24
Diastolic blood pressure, *mmHg*	79 (72-88)	79 (72-80)	78 (71-80)	79 (72-90)	0.82
Glucose, *mg/dL*	113 (104-135)	102 (89-117)	98 (92-109)	97 (88-108)	0.09
Total cholesterol, *mg/dL*	181 (167-219)	194 (178-223)	192 (173-228)	217 (172-223)	0.96
LDL cholesterol, *mg/dL*	95 (81-125)	116 (92-137)	105 (93-120)	122 (108-128)	0.86
HDL cholesterol, *mg/dL*	34 (27-51)	44 (35-50)	41 (39-46)	38 (32-50)	0.27
Triglycerides, *mg/dL*	239 (178-287)	187 (93-209)	182 (115-199)	132 (105-243)	0.17
# of blood pressure medicines	1.5 (1-2)	1.5 (1-2)	2.0 (1-2)	1.5 (1-2)	0.85
Lipid lowering medicines[a]**,** *(%)*	55	40	50	55	0.60[b]

a: Percentage of patients who are on lipid lowering medicines

b: Chi-square test

SUPPLEMENTAL DIGITAL CONTENT

Table S2. Sequence and Location of Oligonucleotide Primers and TaqMan Probes for Measurement of Urinary Cell mRNA Levels by the Use of Real-Time PCR Assay[a]

Gene	Accession Number	Sequence	Position
TLR4-4	NM_138554.1	Sense: 5'-CATGGCCTTCCTCTCCTGC-3'	209-227
		Antisense: 5'-GAAATTCAGCTCCATGCATTGA-3'	302-281
		Probe: 5'-FAM-AGGAACCACCTCCACGCAGGGCT-TAMRA-3'	*269-247*
C3	NM_000064.2	Sense: 5'-CAGCACCGGAAACAGAAAAGAG-3'	4168-4189
		Antisense: 5'- CCCCGGTACCTGGTACAGATC-3'	4243-4143
		Probe: 5'-FAM-AAGAACACTATGATCCTTG-MGB -3'	*4203-4221*
C5	NM_001735.2	Sense: 5'-TTCCTTGGGAGGCCAGTAGA-3'	4027-4046
		Antisense: 5'- AGCCAAGCCACTGCCAAA-3'	4101-4084
		Probe: 5'-FAM-ACCTCATTGTCAGTACAGG-MGB -3'	*4064-4082*
CFB	NM_001710.5	Sense: 5'-TGGCGGCCCCTTGATAGT-3'	2375-2392
		Antisense: 5'- CCCAGCTGATTACACCAACTTG-3'	2436-2415
		Probe: 5'-FAM-CACAAGAGAAGTCGTTTCA-MGB -3'	*2394-2312*
Properdin	NM_002621.2	Sense: 5'-GATGTGCCGGGCAACAG-3'	1318-1334
		Antisense: 5'- CACTCTGACCATGATCCTTTCAAG-3'	1396-1378
		Probe: 5'-FAM-TATCCGGCACTGCTACA-MGB -3'	*1340-1356*
CD46	NM_002389.3	Sense: 5'-GATCGGAATCATACATGGCTACCT-3'	397-420
		Antisense: 5'- GGCCATTTAAAGGATCCCGTATA-3'	481-459
		Probe: 5'-FAM-CTCAGATGACGCCTGTTATAGAGAAACATGTCCA-TAMRA -3'	*423-456*
CD55	NM_001114752.1	Sense: 5'-CACCACCTGAATGCAGAGGAA-3'	1130-1150
		Antisense: 5'- GAACATTTACTGTGGTAGGTTTCTGAAC-3'	1207-1180
		Probe: 5'-FAM-CTAACTTCCAAGGTCCC-MGB -3'	*1156-1172*
CD4	NM_000616	Sense: 5'-TTTCATTGGGCTAGGCATCTTC-3'	1382-1403
		Antisense: 5'-CTGAGGAGTCTCTTGATCTGAGACAT-3'	1471-1446
		Probe: 5'-FAM-CGGCACCGAAGGCGCCAAG-TAMRA-3'	*1419-1437*
CD8	NM_001768.3	Sense: 5'-CACAGGAACCGAAGACGTGTT-3'	709-729
		Antisense: 5'-TAGACGTATCTCGCCGAAAGG-3'	794-774
		Probe: 5 '-FAM-CCCGGCCTGTGGTCAAATCGG-TAMRA-3'	*740-760*

SUPPLEMENTAL DIGITAL CONTENT

CD3	NM_000733	Sense: 5'-AAGAAATGGGTGGTATTACACAGACA-3'	131-156
		Antisense: 5'-TGCCATAGTATTTCAGATCCAGGAT-3'	233-209
		Probe: 5'-FAM-CCATCTCTGGAACCACAGTAATATTGACATGCC-TAMRA-3'	*170-202*
CTLA4	BC074893	Sense: 5'-CGCCATACTACCTGGGCATAG-3'	441-461
		Antisense: 5-GATCCAGAGGAGGAAGTCAGAATC-3	*529-506*
		Probe: 5'-FAM-CAGATTTATGTAATTGATCCAGAACCGTGCCC-TAMRA-3'	473-504
CD25	NM_000417	Sense 5'-GACTGCTCACGTTCATCATGGT-3'	185-206
		Antisense 5'-AATGTGGCGTGTGGGATCTC-3'	266-247
		Probe: 5'-FAM-AGAGCTCTGTGACGATGACCCGCC-TAMRA-3'	*222-245*
Foxp3	NM_014009	Sense: 5'-GAGAAGCTGAGTGCCATGCA-3'	939-958
		Antisense: 5'-GGAGCCCTTGTCGGATGAT-3'	1025-1007
		Probe: 5'-FAM-TGCCATTTTCCCAGCCAGGTGG-TAMRA-3'	*962-983*
CD20	NM_021950	Sense: 5'-AACTCCCCATCTACCCAATACTGTT-3'	616-640
		Antisense: 5'-AGAAGGCAAAGATCAGCATCACT-3'	697-675
		Probe: 5'-FAM-CAGCATACAATCTCTGTTCTTGGGCATTTTG-TAMRA-3'	*642-672*
IP-10	NM_001565.1	Sense: 5'-TGTCCACGTGTTGAGATCATTG-3'	235-256
		Antisense: 5'-GGCCTTCGATTCTGGATTCA-3'	309-290
		Probe: 5'-FAM-TACAATGAAAAAGAAGGGTGAGAA-MGB-3'	*258-281*
Granzyme B	J04071	Sense: 5'-GCGAATCTGACTTACGCCATTATT-3'	534-557
		Antisense: 5'-CAAGAGGGCCTCCAGAGTCC-3'	638-619
		Probe: 5'-FAM-CCCACGCACAACTCAATGGTACTGTCG-TAMRA-3'	*559-585*
Perforin	M28393	Sense: 5'-GGACCAGTACAGCTTCAGCACTG-3'	492-514
		Antisense: 5'-GCCCTCTTGAAGTCAGGGTG-3'	587-568
		Probe: 5'-FAM-TGCCGCTTCTACAGTTTCCATGTGGTACAC-TAMRA-3'	*526-555*
BK virus VP1	J02038	Sense: 5'-TGCTGATATTTGTGGCCTGTTTACTA-3'	2355-2380
		Antisense: 5'-CTCAGGCGGATCTTAAAATATCTTG-3'	2438-2414
		Probe: 5-FAM-AGCTCTGGAACACAACAGTGGAGAGGCC-TAMRA 3'	2383-2410
18S rRNA	K03432	Sense: 5'-GCCCGAAGCGTTTACTTTGA-3'	929-948
		Antisense: 5'-TCC ATTATTCCTAGCTGCGGTATC-3'	1009-986
		Probe: 5'-FAM-AAAGCAGGCCCGAGCCGCC-TAMRA-3'	*965-983*

SUPPLEMENTAL DIGITAL CONTENT

[a]: We used Primer Express software (Applied Biosystems, Foster City, CA) to design gene-specific oligonucleotide primers and TaqMan probes for measurement of urinary cell mRNA by the use of real-time PCR assay. The probes were labeled with 6-carboxy-fluorescein (FAM) at the 5’ end and 6-carboxy-tetramethylrodamine (TAMRA) or dihydrocyclopyrroloindole tripeptide minor groove binder (MGB) at the 3’ end. FAM functioned as the reporter dye and TAMRA or MGB as the quencher dye.

SUPPLEMENTAL DIGITAL CONTENT

Figure S1. Pharmacokinetic Profile of Tacrolimus.

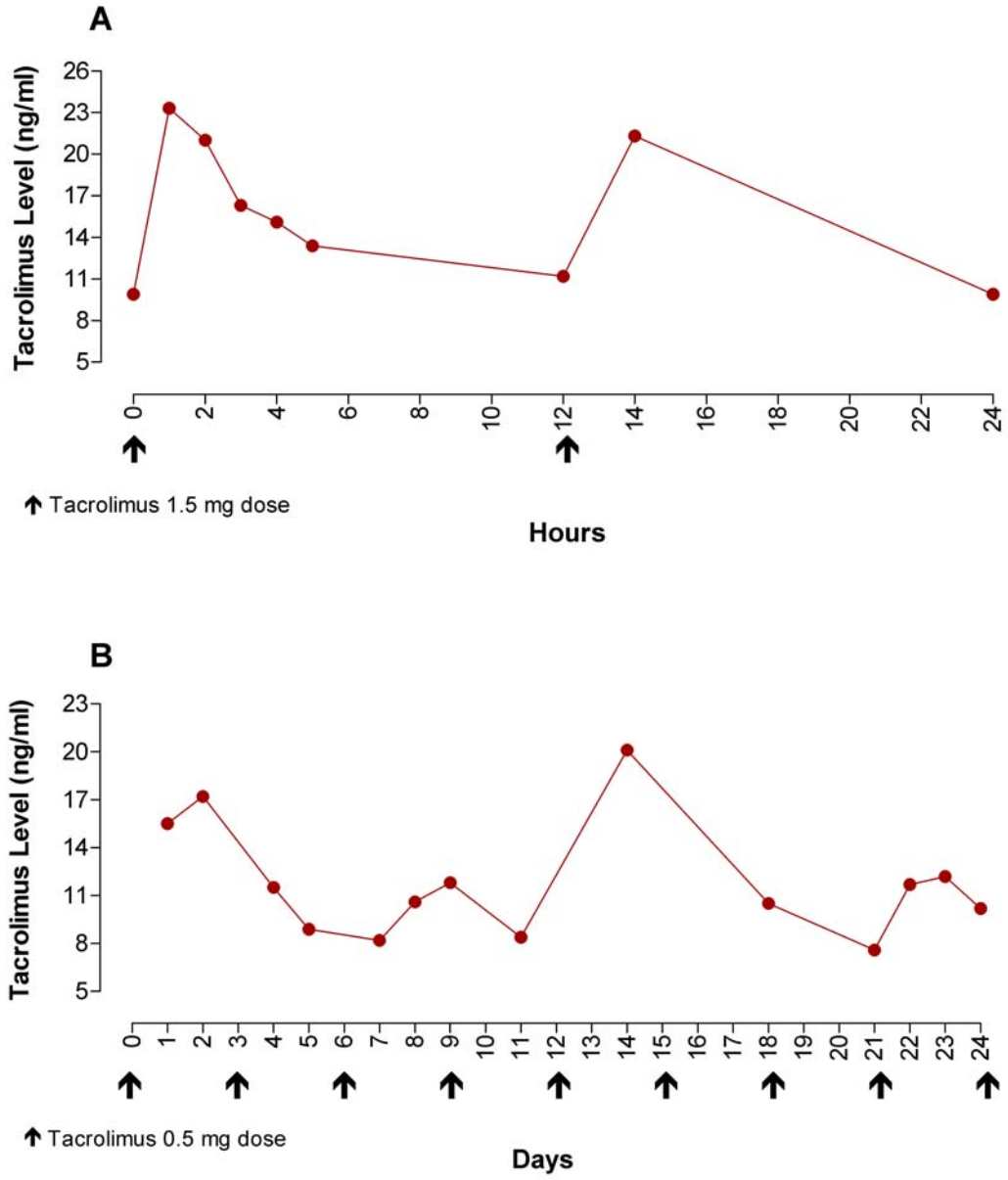

Figure S1. Tacrolimus was administered to 2 patients who were infected with HIV and received a kidney transplant. Both patient-1 (panel A) and patient-2 (panel B) were on a protease-inhibitor based HAART regimen. Patient-1 was on atazanavir. After several attempts to determine the correct dose and dosing interval of tacrolimus to achieve therapeutic drug level, pharmacokinetic profiling was done on the third post-transplant week. Patient-2 was on tipranavir and ritonavir and the profiling study was done on the first post-transplant week. X-axis represents the time from the first dose of tacrolimus. The time when the doses were given are shown by arrows. Y-axis represents the serum level of tacrolimus.