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Retrospective tissue typing of the kidney donor from recipient urine

Maithili Srikantha[1,2,6], Ruhena Sergeant[3,6], Thahminna Khan[3], Robert I. Lechler[4,5], Nick Davey[3], Maria P. Hernandez-Fuentes[4,5] and Anthony N. Warrens[1,2,3]

[1]Department of Immunology, Hammersmith Hospital, Imperial College London, London, UK; [2]Renal Section, Imperial College London, London, UK; [3]Division of Immunology, Hammersmith Hospital NHS Trust, London, UK; [4]Immunoregulation Laboratory, Department of Nephrology and Transplantation, King's College London, London, UK and [5]NIHR Biomedical Research Centre, Guy's and St Thomas' NHS Foundation Trust, Guy's Campus, London, UK

Given that it is possible to extract DNA from the urine of kidney transplant donors and recipients we studied whether the donor HLA type can be determined from recipient urine. This would be useful especially when there is limited information on donors or when the transplant was performed long ago when tissue typing was less precise. We extracted and purified DNA from fresh urine and used the standard HLA class I and class II PCR-SSP assays comparing the findings to those obtained from peripheral blood of donor and recipient HLA types. Using the urine of 31 renal transplant recipients we assayed for the 140 known mismatches, and all were detected in technically successful assays with only a single false positive. This shows that urine samples of transplant recipients can be used to generate historical HLA typing information of the donor thereby aiding post-transplant immunologic monitoring.

Kidney International (2008) **74,** 952–955; doi:10.1038/ki.2008.340; published online 16 July 2008

KEYWORDS: kidney transplantation; HLA matching; transplantation immunology; post-transplant monitoring; HLA sensitization

Correspondence: *Anthony N. Warrens, Department of Immunology, Hammersmith Hospital, Imperial College London, Commonwealth Building, Ducane Road, London W12 0NN, UK. E-mail: a.warrens@imperial.ac.uk*

[6]These authors contributed equally to the work.

Received 9 January 2008; revised 25 April 2008; accepted 6 May 2008; published online 16 July 2008

Faced with the worldwide shortage of organs,[1,2] patients are increasingly traveling abroad for a transplant.[3] Often there is limited donor information when they return which can pose management problems. One example is the lack of information about the donor human leukocyte antigen (HLA) type. In our practice, around 6% of all transplanted patients do not have adequate donor HLA information. If this kidney is subsequently lost, the lack of such information may be a concern in retransplantation. It is a common practice not to retransplant someone with a kidney that expresses mismatched antigens present on previously failed transplants, even in the absence of HLA-specific antibodies. It would not be possible to avoid such antigens if the initial donor HLA type were unknown.

Second, there is increasing interest in post-transplant immunological monitoring,[4] both donor-specific antibodies,[5] and cellular reactivity.[6] Having complete information on the donor HLA type would assist the performance and interpretation of such assays using surrogate cells or beads, if donor material is not available.

Third, many donors of current long-lived transplants were never tissue typed for class II and their class I typing was less precise.

Donor and recipient DNA are detectable in urine from kidney transplant recipients.[7] With the development of tissue typing techniques, requiring less DNA, we explored the possibility of obtaining retrospective HLA-type information on transplant donors from recipient urine. We believe this is the first demonstration that this can be done.

RESULTS

DNA was extracted from urine specimens from 34 stable renal transplant recipients, and 13 control individuals who were free of known renal pathology and who had not undergone renal transplantation. All eluates were subjected to HLA class I (Figure 1) and II (online Supplementary Information) typing. The recipient's known HLA type (as identified from HLA typing of a whole-blood sample from the recipient) was subtracted from the HLA type determined from urinary DNA. HLA specificities not present

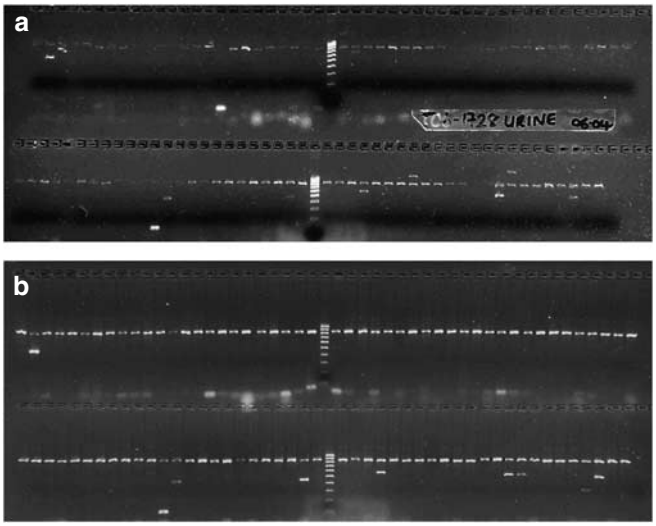

Figure 1 | Representative result of PCR-SSP for HLA class I typing of recipient urine (a) and blood (b). Each reaction is shown separately in wells numbered sequentially along the top row from left to right and then along the bottom row from left to right (not including the central molecular weight marker lanes). Those generating a band of appropriate size in each of blood and urine are marked with a cross. Each well also contains an internal control reaction yielding a band of 796 bp.

in the recipient's pretransplant tissue type were assigned to the donor and results compared with donor HLA type known from pretransplant typing, to confirm the accuracy of the urinary HLA type.

Only 3 of the 34 urine samples failed to produce adequate DNA for tissue typing from 50 ml. Of these, two had been frozen before processing and one had been left overnight before DNA was isolated. 8 samples of data, representative of the 31 patients studied, are shown in Table 1. Of these, one (patient 15) failed to generate an expected A33 and Cw16. Two expected alleles in patient 12 were not detected in the urine, but in each case this was in the context of a technical failure. Table 2 summarizes the mismatch data in all 31 patients. Patient 15 had undergone two transplants, from the first of which no donor details were available. No unexpected alleles were detected from the first kidney but the expected mismatches from the second graft were all identified.

Across all 31 patients, there were a total of 159 donor–recipient mismatches. Of these, 140 (88%) were detected from recipient's urine. Of the 19 (12%) that were not detected, the internal control reactions failed in all cases that were thus known to be technical failures. These are shaded in Table 2. This gave a 'miss (false negative) rate' of

Table 1 | Eight representative samples of deriving donor–recipient HLA mismatches by typing recipient urine

	A					B					Cw					DRB1					DRB 3, 4, 5					DQB				
Patient 3 (whole blood)	3	24				64	81				8	18				13	18				52		2	4						
Donor (whole blood)		24	68		53		81		4			18				13	18				52		2	4						
Urine (patient)	3	24	68		53	64	81		4		8	18				13	18		51		52		2	4						
Mismatches detected			68		53				4																					
Patient 4 (whole blood)	2					51	62				10	15		4		13			52		53			6	8					
Donor (whole blood)	2	3			7				7					4	7						53		2		8					
Urine (patient)	2	3			7	51	62		7		10	15		4	7	13			52		53		2	6	8					
Mismatches detected		3			7				7						7								2							
Patient 6 (whole blood)	2				51		61				12	14		4	13				52		53		6	7						
Donor (whole blood)	2				51	60			10			14			13				52				6							
Urine (patient)	2				51	60	61		10		12	14		4	13				52		53		6	7						
Mismatches detected						60			10																					
Patient 7 (whole blood)	1				8		62				7	3		3	4				52		53		2	3						
Donor (whole blood)	1	3				27	62		2			3		3	4				52		53		2	3						
Urine (patient)	1	3			8	27	62		2		7	3		3	4				52		53		2	3						
Mismatches detected		3				27			2																					
Patient 12 (whole blood)	1	2			7		44				5	7		4	15				51		53		6		8					
Donor (whole blood)		2	30			13	44		4		5			4	15				51		53		6	7						
Urine (patient)	1	2	30		7	NT	44		NT		5	7		4	15				51		53		6	7	8					
Mismatches detected			30			Y			Y															7						
Patient 15 (whole blood)	2			33			51	58	3				16	4	15				51		53		5			8				
Donor (whole blood)		3	11		8	35					4	7		4	15				51		53			6	7					
Urine (patient)	2	3	11		8	35	51	58	3		4	7		4	15				51		53		5	6	7	8				
Mismatches detected		3	11	X	8	35					4	7	X											6	7					
Patient 18 (whole blood)	1	2			8	35			4		7			17					52				2							
Donor (whole blood)	1	2			8	35			4		7			17					52				2							
Urine (patient)	1	2			8	35			4		7			17					52				2							
Mismatches detected																														
Patient 22 (whole blood)	2	24				40	44		3			16		4	7						53		2		8					
Donor (whole blood)	2		30		35		44				8	16			7	15			51		53		2	6						
Urine (patient)	2	24	30		35	40	44		3		8	16		4	7	15			51		53		2	6	8					
Mismatches detected			30		35						8					15			51					6						

Deduction process of donor-recipient HLA mismatches by typing recipient urine. The tissue types above are a serological equivalent of a molecular type. NT: not tested due to technical failure; X: mismatches not detected in urine typing; Y: mismatches not detected in urine typing, but in context of known inadequate assay.

Table 2 | Summary of donor–recipient HLA mismatches using urine samples

Patient no.	A		B		Cw		DRB1		DRB3, 4, 5		DQB		No of detected mismatches	No of expected mismatches
1	3		27	44	2	5	4	11	52	53	7	8	11	11
2	33		58		10		14		52		5		5	6
3	68		53		4				51[a]				4	3
4	3		7		7		7				2		5	5
5													0	0
6			60		10								2	2
7	3		27		2								3	3
8	24	68	51		12	15	14		51		5	9	9	9
9	3		38		7	12	4		53		6	3	8	8
10													0	0
11	3				1	14							3	3
12	30		13		4						7		2	4
13	3	32	60		10		4		52		8		7	7
14	3		7		6	7					8		5	5
15	3	11	8	35	4	7					6	7	8	8
16	3	68	35		4		17		52		2		7	7
17	11		35		4						7		4	4
18													0	0
19		31	65	40	3		4	13	53	52	6	8	9	10
20	1												1	1
21	24		18	35	4		14	15	51		5		8	8
22	30		35		8		15		51		6		6	6
23					6		13				2	6	4	4
24	3		35		4		15		51		6		7	7
25	24	29	39	44	16		7				7		5	7
26	1	2	37		5	5	15		51		6		3	8
27	2	26	27	44	1	16	1	7	53		5		5	10
28	23		8		2								3	3
29			7				4						0	2
30	3	68			5		1				5		4	5
31			8	50							2		3	3
											Total		141	159

The tissue types above are serological equivalents of a molecular type. The number of detected mismatches includes the single false positive result observed.
Technical problem.
[a]False positive mismatch.

0% (that is, where the assay was believed to have been a technical success). A single false positive (*HLA-DRw51* on patient 3) was detected, a rate of 0.8%.

All 13 control specimens yielded expected tissue typing (data not shown).

DISCUSSION

This study demonstrates that it is possible to determine mismatched donor HLA specificities using DNA extracted from transplanted patients' urine. We missed no donor–recipient mismatched allele when the internal controls confirmed an assay was technically successful. Previous work has focused on methods for the extraction of urinary cell-free DNA[8–10] and its use to predict rejection without biopsy.[7,10] The previous focus of urinary DNA extraction has been the detection of cancer markers,[11,12] DNA polymorphisms,[13,14] and infections.[15]

Urinary DNA can be obtained from cell debris derived from the cells lining the urinary tracts, including tubules and bladder,[16] so-called 'transrenal DNA'.[17,18] Donor-derived DNA constitutes a minor proportion (8.7%) of total urinary DNA,[9] but is still readily detectable.

In a few cases, the assay failed on a fresh sample of 50 ml urine and we could not repeat the typing. However, the relative ease of obtaining fresh urine samples means that this should not be a limiting factor in practice. The test's simplicity means it is well within the capabilities of any routine histocompatibility laboratory. DNA can also be detected from frozen urine when stored appropriately.[19]

The use of this technique should help in the maintenance of an accurate patient sensitization history and may also aid post-transplant monitoring.

MATERIALS AND METHODS

Participants

Ethical approval was obtained from the Hammersmith Hospital Research Ethics Committee. Samples were collected after informed consent was granted.

A total of 34 transplant patients with stable creatinine levels (<10% increase) for >1-year following transplantation were recruited from the Hammersmith Hospital clinic in 2006. The control group comprised 13 individuals with no history of renal pathology or renal transplantation. We excluded infection in that none had positive urine culture or elevated serum C-reactive protein at the time of sample collection.

DNA extraction

DNA extraction from fresh urine sample. Fresh, early morning urine samples (50 ml) were centrifuged (4000 r.p.m., 30 min). DNA

was extracted from the pellet using QIAamp Viral Mini Kit (Qiagen, Crawley, UK). (The constituents of buffers AVL, AW1, AW2 and AVE have not been disclosed by the manufacturer.) Briefly, the pellet and 250 µl supernatant were pulse-vortexed with four volumes of AVL/RNA carrier buffer (1 ml of buffer AVL to one tube lyophilized carrier RNA). Following incubation (room temperature, 10 min), an equal volume of 100% ethanol was added and the sample pulse-vortexed. The mixture was applied to the QIAamp Spin Column and centrifuged. The eluate was discarded. Buffer AW1 (500 µl) was added to the column and centrifuged at 8000 r.p.m. (1 min). Next, 500 µl buffer AW2 was added and centrifuged (14,000 r.p.m., 3 min). DNA was eluted using five aliquots of 40 µl AVE buffer by spinning (8000 r.p.m., 1 min), after incubation at room temperature (1 min).

DNA extraction from whole blood. Genomic DNA was isolated from whole blood using the Nucleon Genomic DNA Extraction Kit (Nucleon Biosciences, Manchester, UK) according to manufacturer's protocol. EDTA-whole blood (3 ml) was mixed with Reagent A (17 ml) (10 mM Tris-HCl, 320 mM sucrose, 5 mM $MgCl_2$, 1%Triton X-100, pH 8.0) and centrifuged (1300 *g*, 5 min). The supernatant was discarded and 2 ml reagent B (400 mM Tris-HCl, pH 8.0, 60 mM EDTA, 150 mM NaCl, 1% SDS) added. Afterward 500 µl 5 M sodium perchlorate and 2 ml chloroform were added sequentially and the suspension was centrifuged (1300 *g*, 1 min). After centrifugation, 200 µl Nucleon Silica Suspension (Nucleon Biosciences) was added to the aqueous layer and the sample centrifuged (1300 *g*, 1 min). Finally, the DNA was precipitated in ethanol, dissolved in water and adjusted to 30–100 µg/ml.

PCR-SSP HLA class I and II typing

HLA class I typing was performed using 0.15 U Taq polymerase (Bioline, London, UK), 0.19 mM each dNTP, 5.4 mM $MgCl_2$, and ~25 ng DNA with 6% w/v glycerol and cresol red 0.3 mg. Class II typing was performed using 0.5 U Taq polymerase (Invitrogen, Paisley, UK), 0.21 mM dNTP, 2.1 mM $MgCl_2$, and ~60 ng DNA with 5% w/v glycerol and cresol red 5.2 mg.

PTC 200 or Perkin Elmer 9600 machines were used. Cycling conditions for HLA class I and *HLA-DQ* amplification were: initial denaturation at 96 °C for 90 s; 6 cycles: 96 °C for 25 s, 70 °C for 50 s, and 72 °C for 45 s; 22 cycles: 96 °C for 25 s, 65 °C for 50 s, and 72 °C for 45 s; 5 cycles: 96 °C for 25 s, 55 °C for 60 s, and 72 °C for 120 s. For *HLA-DR* typing, the cyclic conditions were: initial denaturation at 94.0 °C for 25 s; 10 cycles: 94 °C for 25 s, 65 °C for 60 s; 20 cycles: 94 °C for 25 s, 61 °C for 50 s, and 72 °C for 30 s.

If a reaction failed to amplify a PCR product from its internal control, the sample was repeated if enough DNA was still available. Otherwise, it was classified as a 'failed' reaction.

ACKNOWLEDGMENTS

This study was funded by Hammersmith Hospital NHS Trust, EU grant QLRT-2002-02127 and Immune Tolerance Network 503ST.

SUPPLEMENTARY MATERIAL

Supplementary material is available online.

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