0041-1337/03/7508-1307/0
TRANSPLANTATION
Vol. 75, 1307–1312, No. 8, April 27, 2003
Printed in U.S.A.

CD103 mRNA LEVELS IN URINARY CELLS PREDICT ACUTE REJECTION OF RENAL ALLOGRAFTS[1]

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Background. **CD103 is displayed on the cell surface of alloreactive CD8 cytotoxic T lymphocytes (CTLs) and is a critical component for the intraepithelial homing of T cells. Because intratubular localization of mononuclear cells is a feature of acute cellular rejection of renal allografts, we explored the hypothesis that CD103 messenger (m)RNA levels in urinary cells predict acute rejection.**

Methods. **We collected 89 urine specimens from 79 recipients of renal allografts. RNA was isolated from the urinary cells, and we measured CD103 mRNA levels and a constitutively expressed 18S ribosomal (r)RNA with the use of real-time quantitative polymerase chain reaction assay.**

Results. **CD103 mRNA levels, but not 18S rRNA levels, were higher in urinary cells from 30 patients with an episode of acute rejection (32 biopsies and 32 urine samples) compared with the levels in 12 patients with other findings on allograft biopsy (12 biopsies and 12 urine samples), 12 patients with biopsy evidence of chronic allograft nephropathy (12 biopsies and 12 urine samples), and 25 patients with stable graft function after renal transplantation (0 biopsies and 33 urine samples) ($P = 0.001$; one-way analysis of variance). Acute rejection was predicted with a sensitivity of 59% and a specificity of 75% using natural log-transformed value 8.16 CD103 copies per microgram as the cutoff value ($P = 0.001$).**

Conclusion. **CD103 mRNA levels in urinary cells are diagnostic of acute rejection of renal allografts. Because CD103 is a cell surface marker of intratubular CD8 CTLs, a noninvasive assessment of cellular traffic into the allograft may be feasible by the measurement of CD103 mRNA levels in urinary cells.**

Renal allograft failure is the fourth common cause of end-stage renal disease in the United States, and acute rejection is a major risk factor for the failure of allografts (*1*,*2*). Acute rejection is associated with a 20% reduction in the 1-year survival rate of cadaveric renal grafts and, despite refinements in immunosuppressive regimens, almost 35% of renal transplant recipients have an episode of acute rejection in the first posttransplant year (*3*,*4*). A better comprehension of the antiallograft repertory may help reduce the toll extorted by the acute rejection process.

We have reported that measurement of messenger (m)RNA for granzyme B and perforin in urinary cells is a noninvasive means of diagnosing acute rejection (*5*). We measured mRNA for granzyme B and perforin in view of the role of CD8 cytotoxic T cells (CTLs) in acute rejection (*6*) and the contribution of granzyme B and perforin to the lytic activity of CTLs (*7*).

This investigation measured the level of CD103 mRNA in urinary cells. CD103 defines a subset of CD8 CTLs and is a component of integrin $\alpha_{E\beta7}$ responsible for binding to epithelial cells through E-cadherin (*8*,*9*). CD103-positive cells have been implicated in the acute rejection process by their association with tubulitis and by their presence in renal allografts removed for uncontrolled rejection (*10*,*11*). Because tubulitis is a histologic hallmark of acute rejection (*12*) and because intraepithelial lymphocytes display CD103 and normal renal tissue contains no intratubular CD103+ cells (*13*,*14*), we tested the hypothesis that CD103 mRNA would be present in high abundance in urinary cells obtained during an episode of acute rejection.

METHODS

Collection of Urine Samples and Kidney Biopsy Specimens

We collected 89 urine specimens from 79 kidney transplant recipients (Table 1). Among the 79 recipients, 54 underwent core needle biopsy of the allograft to determine the cause of graft dysfunction, and 56 biopsies were obtained. All biopsy specimens were fixed in formalin, embedded in paraffin, stained with hematoxylin and eosin, periodic acid-Schiff, and Masson's trichrome, and studied by a single pathologist who was unaware of the results of molecular studies. On the basis of 97 Banff classification (*12*), the 32 biopsy specimens from 30 patients (age, 40 ± 13 [mean ± SD]; 16 men and 14 women; 14 cadaver donor grafts, 11 living related donor grafts, and 5 living unrelated donor grafts) were classified as acute rejection, 12 biopsies from 12 patients (age, 49 ± 13; nine men and three women; seven cadaver donor grafts, four living related donor grafts, and one living unrelated donor graft) were classified as "other" histologic finding, and 12 biopsies from 12 patients (age, 52 ± 13; five men and seven women; nine cadaver donor grafts and three living related donor grafts) were classified as chronic allograft nephropathy. We collected 56 urine specimens from these patients, and the urine was collected within 24 hr of the core needle biopsy of the allograft. We also

[1] This study was supported, in part, by a National Institutes of Health NIAID award (ROI AI51652) and by an award from Satellite Healthcare, Inc.

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[6] Dr. Muthukumar is a recipient of a fellowship award from the International Society of Nephrology.

[7] Dr. Dadhania is a recipient of the American Society of Transplantation Fujisawa Clinical Fellowship Award.

[8] Dr. Medeiros is supported by the Patronato del Hospital Infantil de México "Federico Gómez", and CONACYT, Mexico.

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DOI: 10.1097/01.TP.0000064210.92444.B5

TABLE 1. Study cohorts for CD103 messenger (m)RNA measurements in urine samples

Diagnostic groups[a]	Renal allograft recipients (n=79)	Renal allograft biopsies (n=56)	Urine specimens[b] (n=89)
Acute rejection	30	32	32
Chronic allograft nephropathy	12	12	12
Other findings	12	12	12
Stable graft function	25	ND[c]	33

[a] Banff '97 classification was used to categorize the biopsy specimens as showing acute rejection, chronic allograft nephropathy, and other findings. The clinical diagnosis of stable graft function was based on the finding that serum creatinine levels had decreased or had not changed by more than 0.2 mg/dL during the 7 days before and the 7 days after the urine samples were collected.

[b] Urine specimens were collected within 24 hr of allograft biopsy procedure.

[c] ND, not done.

collected 33 urine samples from 25 patients (age, 45 ± 13; 14 men and 11 women; 14 cadaver donor grafts, 7 living related donor grafts, and 4 living unrelated donor grafts) who were classified as having stable allograft function after transplantation. In the patients with stable allograft function, the serum creatinine levels either had decreased or had not increased by more than 0.2 mg/dL during the 7 days before and 7 days after urine collection. These patients did not undergo biopsy of their allografts at the time of urine collection.

Immunosuppression consisted of either a cyclosporin-based or tacrolimus-based regimen. Acute rejection episodes were initially treated with a course of intravenous methylprednisolone. Antilymphocyte antibodies (muromonab-CD3 [OKT3] or antithymocyte globulin) were given to patients who had an episode of glucocorticoid-resistant acute rejection.

Isolation of RNA

Urine was centrifuged at 10,000*g* for 30 min at 4°C. RNA was isolated from the cell pellet using RNeasy minikit (Qiagen, Chatsworth, CA), quantified, and reverse transcribed to complementary (c)DNA, as previously described (*5*).

Design of Primers and Probes for the Measurement of mRNA

We designed and synthesized oligonucleotide primers and fluorogenic probes for the measurement of CD103 mRNA levels with the use of real-time quantitative polymerase chain reaction (PCR) assay (Table 2). We also designed and developed primers and probes for the measurement of 18S ribosomal (r)RNA. We measured 18S rRNA as a measure of RNA yield from a given urine specimen. The probes were labeled with 6-carboxy-fluorescein (FAM) at the 5' end and with 6-carboxy-tetramethylrodamine (TAMRA) at the 3' end. FAM functioned as the reporter dye and TAMARA as the quencher.

TABLE 2. Sequence and location of primers and probes

Human CD103	
Sense primer	5′-CGTGCTCAGCTCCCTTCTG-3′ (Exon 2)
Antisense primer	5′-CCTGGTGTCCTCTTGGTTCTG-3′ (Exon 3)
Probe	5′-6-FAM ACCAAGACCCCAGCACCAAC-CATACCT-TAMRA-3′
Accession number	XM_008508
Human 18S rRNA	
Sense primer	5′-GCCCGAAGCGTTTACTTTGA-3′
Antisense primer	5′-TCCATTATTCCT AGCTGCGGTATC-3′
Probe	5′-6-FAM AAAGCAGGCCCGAGCCGCC-TAMRA-3′
Accession number	K03432

Gene-specific oligonucleotide primers and probes were designed using Primer Express software (PE Applied Biosystems, Forest City, CA). The probes were labeled with 6-carboxy-fluorescein (FAM) at the 5′ end and with 6-carboxy-tetramethylrodamine (TAMRA) at the 3′ end. FAM functioned as the reporter dye, and TAMRA functioned as the quencher dye.

Quantification of RNAs with the use of Real-Time Quantitative PCR Assay

CD103 mRNA levels and the levels of 18S rRNA were measured with the use of ABI Prism 7700 sequence detection system (PE Biosystems, Foster City, CA). PCR reaction for each sample was set up in duplicate as a 25 μL reaction volume using 12.5 μL of TaqMan Universal PCR Master Mix (Applied Biosystems, Forest City, CA), 2.5 μL of 1:10 diluted (1:1000 for 18S RNA measurement) template cDNA, 200 nM of primers, and 200 nM of probe. PCR amplification included an initial incubation at 50°C for 2 min and denaturation at 95°C for 10 min. This was followed by heating at 95°C for 15 sec and 60°C for 60 sec repeated for 40 cycles. The PCR amplicon for 18S rRNA was prepared, quantified, and used for developing standard curves. The standard curves were based on the principle that a plot of the log of the initial target copy number of a standard versus threshold cycle results in a straight line. mRNA levels and rRNA levels in the samples were expressed as number of copies per microgram of total RNA isolated from the urinary cells.

Statistical Analysis

We used GraphPad Prism 3.02 statistical software for Windows (GraphPad Software, San Diego, CA). Before comparison of mRNA steady-state levels among the various diagnostic groups, distributions of transcript levels were examined for non-normality. The levels of mRNA for CD103 in the acute rejection group deviated significantly from a normal distribution ($P = 0.0024$), which was substantially reduced by use of a log transformation. The natural log-transformed mRNA levels were used as the dependent variable in a one-way analysis of variance to test for any differences among the four diagnostic groups. Fisher's protected least significant difference (PLSD) test for multiple comparisons was then used to control the risk of a type I error while comparing the mRNA levels in the acute rejection group with those in the "other", chronic allograft nephropathy, and stable posttransplant groups. Conventional receiver operating characteristic curve analysis of mRNA levels was used to determine cut points that maximized the combined sensitivity and specificity for distinguishing patients with acute rejection from those without acute rejection. Area under the curve was calculated, and sensitivity and specificity at the selected cut point were determined.

RESULTS

Histological Classification of Renal Allograft Biopsies

The Banff '97 classification (*12*) was used to categorize the biopsies as showing acute rejection (32 biopsies), chronic allograft nephropathy (12 biopsies), or "other" (12 biopsies). Of the 32 biopsies showing acute rejection, 2 were graded as borderline, 7 as type IA (focal moderate tubulitis), 15 as type

IB (severe tubulitis), 4 as type IIA (mild to moderate intimal arteritis), and 4 as type III (transmural arteritis). Six of the 32 biopsies showing acute rejection features showed features of chronic allograft nephropathy (grade I changes in one and grade II changes in five). Among the 12 biopsies classified as chronic allograft nephropathy, four showed grade I changes, seven showed grade II changes, and one showed grade III changes. Among the 12 biopsies classified as "other", three were diagnosed as toxic tubulopathy, four as nonspecific changes, and five as acute tubular necrosis.

mRNA Levels in Urinary Cells

The level of CD103 mRNA was higher in urinary cells from patients with acute rejection as compared with those without acute rejection. Figure 1 and Table 3 show CD103 mRNA levels in urinary cells from 30 patients with an episode of acute rejection (32 biopsies and 32 urine samples), 12 patients with other findings on allograft biopsy (12 biopsies and 12 urine samples), 12 patients with biopsy evidence of chronic allograft nephropathy (12 biopsies and 12 urine samples), and 25 patients with stable graft function after renal transplantation (no biopsies and 33 urine samples). The natural log-transformed CD103 mRNA levels were used as the dependent variable in a one-way analysis of variance to test for any differences among the four diagnostic groups. CD103 mRNA levels, measured during an episode of acute rejection, were significantly higher compared with the three other diagnostic groups ($P = 0.0025$). Fisher's PLSD test for multiple comparisons was then used to control the risk of a type I error while comparing the mRNA levels in the acute rejection group with those in the "other", chronic allograft nephropathy, and stable posttransplant groups, and this analysis revealed that CD103 mRNA levels in urinary cells obtained during acute rejection were significantly higher than in those classified as the stable posttransplant ($P = 0.0046$), "other" ($P = 0.0038$), or chronic allograft nephropathy ($P = 0.0043$) groups.

In contrast to CD103 mRNA levels, and as expected, the levels of constitutively expressed 18S rRNA did not vary significantly among the four diagnostic categories ($P = 0.44$; Fig. 1 and Table 3).

In the Banff '97 classification, tubulitis is graded on the basis of the number of mononuclear cells per tubular cross section. CD103 mRNA levels varied directly with the inten-

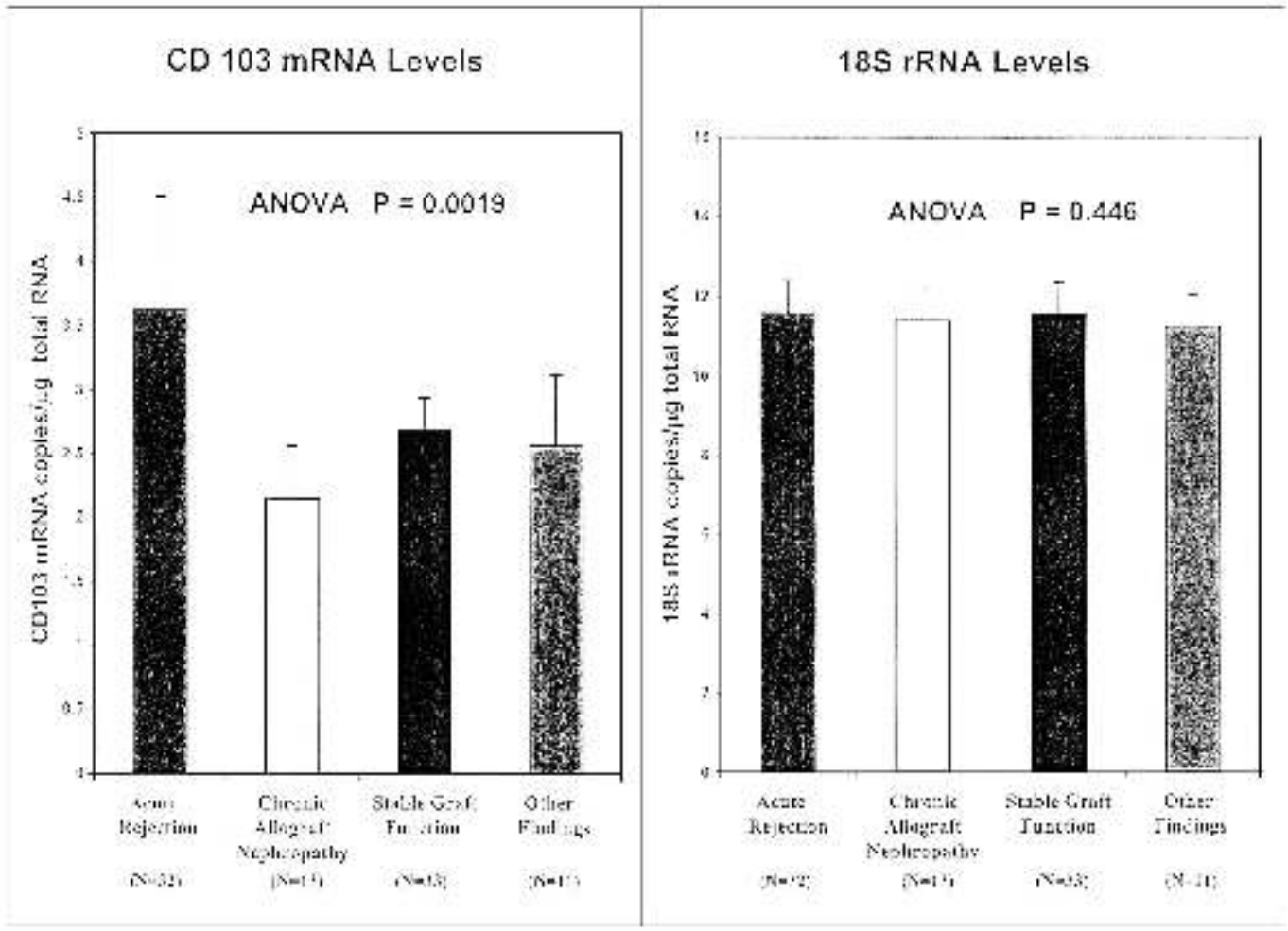

FIGURE 1. CD103 mRNA levels (A) and 18S rRNA (B) were measured with the use of gene-specific primers and probes by real-time quantitative PCR assay. *P* values were calculated with the use of natural log-transformed RNA copy numbers as the dependent variable in one-way analysis of variance. Values in parenthesis are the numbers of urine samples.

TABLE 3. Levels of CD103 mRNA and 18S rRNA in urine specimens[a]

Type of mRNA	Acute rejection (n=32)[b]	Other findings (n=12)	Chronic allograft nephropathy (n=12)	Stable graft function (n=33)	*P* value[c]
	Log copy of mRNA/μg total RNA (mean ± SE)				
CD103 mRNA	8.37±0.20	5.41±1.16	5.36±1.15	6.21±0.59	0.0025
18S rRNA	24.41±0.32	23.82±0.46	23.82±0.40	24.37±0.17	0.447

[a] Copies of mRNA and rRNA were measured with the use of gene-specific primers and probes by real-time polymerase chain reaction (PCR) assay. Plus-minus values are mean (±SE) of the natural logarithm of mRNA and rRNA copy number. The diagnoses of acute rejection, other findings, and chronic allograft nephropathy were made by histologic evaluation of renal-allograft-biopsy specimens. The clinical diagnosis of stable graft function was based on the finding that serum creatinine levels either had decreased or had not changed by more than 0.2 mg/dL during the 7 days before and the 7 days after the urine samples were collected.

[b] Values in parenthesis are the numbers of urine samples.

[c] *P* values were calculated with the use of log-transformed mRNA copy numbers as the dependent variable in one-way analysis of variance. Fisher's protected least significant difference (PLSD) test was then used to compare the mRNA copy numbers in samples showing acute rejection with the mRNA copy numbers in each of the three other group of samples. It showed that copy number of CD103 mRNA in urinary cells obtained during an episode of acute rejection was significantly higher than those in specimens with other findings ($P = 0.0038$), chronic allograft nephropathy ($P = 0.0043$), or stable function ($P = 0.0046$). None of the pair wise comparisons copy numbers of 18S rRNA in urinary cells was significant.

sity of tubulitis, and the mean ± SE copy number was 2,578 ± 386 (non–log-transformed value) in the urine specimens obtained from the 2 patients with borderline changes in their biopsies, 5,262 ± 2,069 in the urine specimens obtained from the 7 patients with focal moderate tubulitis (type IA changes), and 11,584 ± 5,314 in the urine specimens obtained from the 15 patients with severe tubulitis (type IB changes).

Receiver Operating Characteristic Curve Analysis of mRNA Levels

The receiver operating characteristic curves (Fig. 2A and B) show the true positive fractions (sensitivity) and false-positive fractions (1-specificity) for various cut points for mRNA levels of CD103 and 18S rRNA. The natural log-

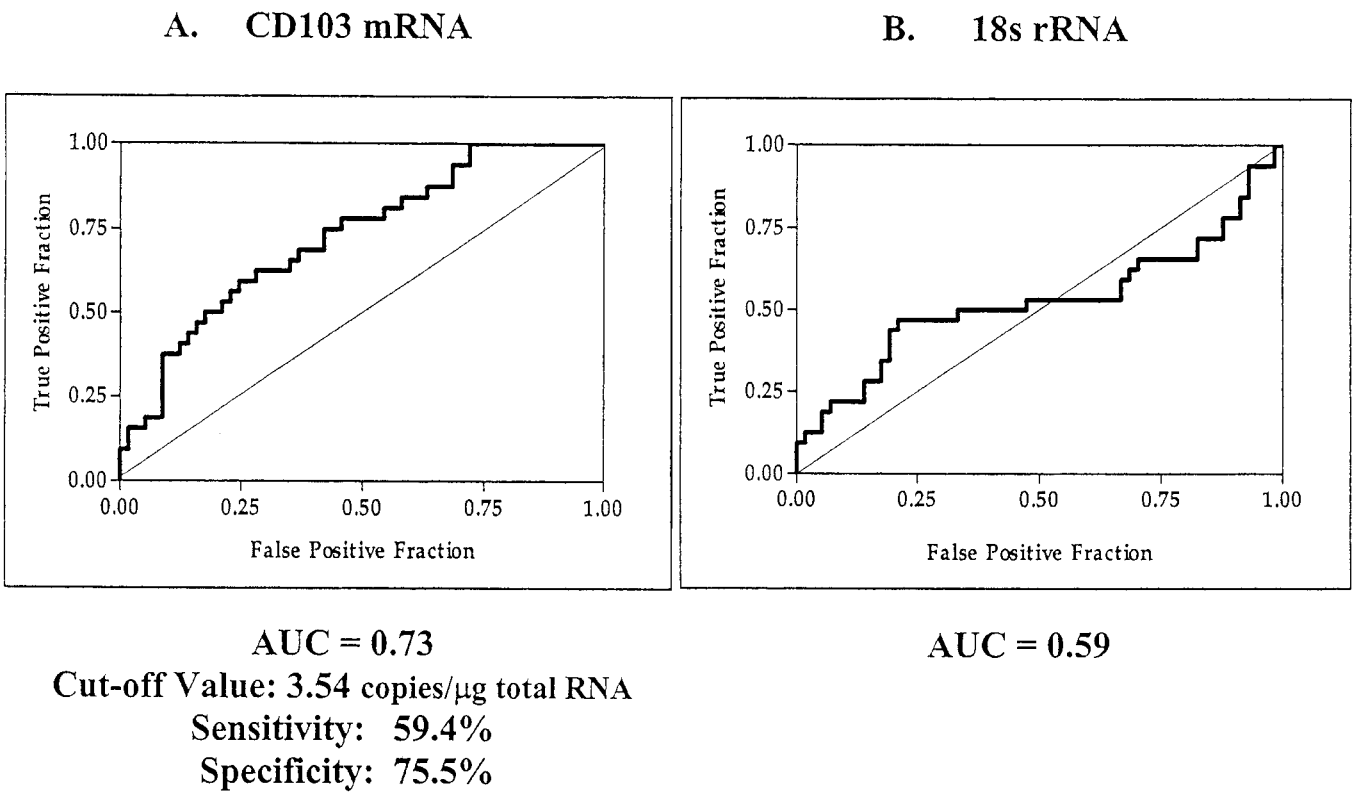

FIGURE 2. View of the fraction of true-positive results (sensitivity) and false-positive results (1-specificity) for CD103 mRNA copy numbers (A) and for 18S rRNA copy numbers (B) as markers of acute rejection. The calculated area under the curve was 0.73 for CD103 mRNA levels and 0.59 for 18S rRNA levels. A value of 0.5 is no better than expected by chance, and a value of 1.0 reflects a perfect indicator.

transformed cut point (threshold) that maximized the combined sensitivity and specificity for CD103 mRNA was 8.16 copies per micrograms of total RNA (Fig. 2A). At this threshold, the sensitivity was 59.4%, and the specificity was 75.5% ($P = 0.001$) (Table 4). The calculated area under the curve was 0.73 (95% confidence intervals, 0.62–0.82).

Figure 2B shows the receiver operator characteristic curve for 18S rRNA with respect to presence or absence of acute rejection. As expected, 18S rRNA levels did not discriminate against acute rejection from other renal diagnoses, and the calculated area under the curve was 0.59 (95% confidence intervals, 0.43–0.75) for 18S rRNA levels.

DISCUSSION

We have identified for the first time that the level of CD103 mRNA in urinary cells predicts acute rejection of human renal allografts. CD103 protein is displayed on the surface of CD8 CTL, and studies have demonstrated that CD103 is an integral component of T-cell surface ligand for the epithelial cell-specific adhesion molecule E-cadherin (*8*,*9*). A compelling rationale for measuring CD103 levels in urinary cells was provided by the observations that CD103 positive cells are found in the intratubular location in allograft biopsies showing features of tubulitis and in renal allograft specimens removed after irreversible rejection (*10*,*11*).

Mechanisms of acute rejection have been explored by investigating cellular traffic into the allografts and by phenotyping and functional analysis of cells infiltrating the graft. It seems that not all cells found in the renal allograft engender proximate graft dysfunction or tissue damage. Burdick et al. (*14*) have observed that long-term normal renal allograft function, despite a biopsy showing dense cellular infiltration. D'Ardenne et al. (*15*) have noted that 80% of stable allografts show some degree of interstitial infiltration, and 42% show diffuse infiltration. On the other hand, Rush et al. (*16*) have demonstrated that allograft biopsies that show tubulitis should be treated, even when not accompanied by allograft dysfunction. Taken together, these observations support the idea that allografts biopsies showing tubulitis, defined histologically as mononuclear cells penetrating the tubular basement membrane and lying beneath or between tubular cells and not allograft biopsies displaying infiltration confined to the interstitial space without breaching of the tubular basement membrane, calls for antirejection therapy.

The obligatory physical contact between alloreactive T cells and target cells occurs at several levels. Antigen specificity is assured by the contact between the clonotypic T-cell receptor and antigenic peptide displayed in the context of major histocompatibility proteins. Full signaling of T cells is contingent upon costimulatory signals generated by the physical contact between lineage specific T-cell surface proteins and their counter receptors on the surface of antigen-presenting cells such as dendritic cells. In the case of T-cell and epithelial-cell contact, the best-described ligand-receptor interaction occurs between T-cell integrin $\alpha_{E\beta7}$ and E-cadherin expressed on epithelial cells (*9*). Thus, T cells contributing to the histologic hallmarks of acute cellular rejection (tubulitis) would be expected to express CD103 because this protein is required for the physical contact between T cells and epithelial cells. Genetic support for this hypothesis is provided by the observation that mice lacking CD103 exhibit a deficiency of intraepithelial lymphocytes (*17*).

Mononuclear cell trafficking into the tubules (tubulitis) can only be identified by the invasive procedure of core needle allograft biopsy of the allograft. Refinements in the biopsy procedure have reduced but not eliminated biopsy-associated complications such as shock because of blood loss and even allograft loss (*18*,*19*). There is also the problem of sampling error when one rather than two cores are obtained (*20–22*). All of these concerns gain greater significance in pediatric recipients with intraabdominal renal allografts (*23*). Our strategy of measuring, in the urinary cells, the level of CD103 mRNA encoding the critical protein for T-cell and epithelial interaction advances the possibility that noninvasive diagnosis of tubulitis is feasible.

CD103 mRNA levels in urinary cells had a positive correlation with the intensity of tubulitis. Thus, in addition to being a reliable biomarker of acute rejection, CD103 mRNA levels seem to provide quantitative information regarding the intensity of tubulitis in the allograft biopsy. The ability to distinguish the various grades of acute rejection by measurement of CD103 mRNA levels in urinary cells may be of significance because it has been reported that biopsy grades are a correlate of allograft outcome (*24*).

Hadley et al. (*11*) have reported that CD8+CD103+ cells were a prominent component of graft infiltrating cells isolated from transplant nephrectomy specimens. However, Leteurtre et al. (*25*) did not detect CD103+ cells in any of the 10 renal allograft biopsy specimens showing features of acute rejection. Our analysis involving the receiver operating characteristic analysis demonstrated that acute rejection could be predicted with a sensitivity of 59%. The time for occurrence of acute rejection was not responsible for the false-negative rate of 41% observed in our investigation because there was no significant difference between the time of occurrence of acute rejections that were accompanied by CD103 mRNA levels that were above the cutoff value of 8.16 compared with the time for occurrence of acute rejection episodes that were accompanied by CD103 mRNA levels that were below the cutoff value of 8.16 (time of occurrence of acute rejection, 3 ± 1 month posttransplantation vs. 4 ± 1 month posttransplantation; $P < 0.05$). Clearly, further studies are required to

TABLE 4. CD103 mRNA levels in urinary cells predict acute rejection of renal allografts

CD103 mRNA Levels	Acute rejection		*P* value[b]
Copy per microgram of total RNA	present n=32	absent n=57	
>8.16[a]	19	14	
<8.16[a]	13	43	0.001

[a] A receiver-operating-characteristic curve was used to select the best cutoff point.
[b] *P* value is based on Fisher's exact test using dichotomized measures of CD103 mRNA levels.

resolve the mechanisms responsible for acute rejections that occur in the absence of an increased CD103 mRNA levels in urinary cells.

Robertson et al. (*10*) have observed that intragraft CD103+ cells are found in renal allograft biopsies showing acute rejection and in biopsies showing chronic rejection. In our study, CD103 mRNA levels were significantly lower in the urine specimens from patients with chronic allograft nephropathy compared with those from patients with acute rejection. However, we found that those patients with chronic allograft nephropathy who had CD103 mRNA levels above the cutoff point also had high granzyme B mRNA levels (a biomarker of acute rejection (*5*)), and those patients with chronic allograft nephropathy who had CD103 mRNA levels below the cutoff point had low granzyme B mRNA levels (84,968 ± 31,838 vs. 24,087 ± 8,785; $P = 0.04$). It is interesting to speculate that chronic allograft nephropathy accompanied by high levels of CD103 mRNA and granzyme B mRNA in urinary cells has an immune origin, whereas chronic allograft nephropathy associated with low levels of CD103 mRNA and granzyme B mRNA has a nonimmune pathogenetic mechanism.

In our investigation, analysis of CD103 mRNA levels in urinary cells with the use of receiver operating characteristic analysis demonstrated that acute rejection could be predicted with a specificity of 75%. One contributory factor to the false positive rate of 25% is the heightened expression of CD103 mRNA in urinary cells obtained from a subset of patients classified as chronic allograft nephropathy (vide supra). Multiple cell types including dendritic cells are known to express CD103, and Lehmann et al. (*26*) have made the intriguing observation that CD103 is expressed on a subpopulation of highly active regulatory CD25+ cells. An interesting hypothesis is that the high CD103 levels in the urine specimens from patients without acute rejection is caused by CD103+ regulatory cells being present in the urine rather than CD103+CD8 CTL.

CONCLUSION

We have identified for the first time that CD103 mRNA levels in urinary cells are diagnostic of acute rejection of renal allografts. Because CD103 is expressed on CD8 CTL and by subsets of highly active CD4+CD25+ or CD4+CD25- regulatory cells, a noninvasive assessment of trafficking of cells with unique functional capabilities may be feasible by the measurement of CD103 levels in conjunction with other appropriate markers in urinary cells. Also, whether CD103 mRNA levels predict long-term graft function and whether they predict response to antirejection therapy require careful scrutiny.

Acknowledgment. Our studies were stimulated, in part, by the discussion the senior author (M.S.) had with Dr. Gregg A. Hadley, University of Maryland Medical School, Baltimore, Maryland. The authors thank Linda Stackhouse for meticulous preparation of the manuscript.

REFERENCES

1. Harper AM, McBride MA, Ellison MD. The USOS OPTN waiting list, 1988–1988. In: Cecka JM, Terasaki PI, eds. Clinical transplants 1999. Los Angeles, UCLA Immunogenetics Center 2000, p 71.
2. Agodoa L, Eknoyan G, Ingelfinger J, et al. Assessment of structure and function in progressive renal disease. Kidney Int Suppl 1997; 63: S144.
3. Cecka JM. The UNOS scientific renal transplant registry. In: Cecka JM, Terasaki PI, eds. Clinical transplants 1998. Los Angeles, UCLA Tissue Typing Laboratories 1999, pp 1–16.
4. Hariharin S, Johnson CP, Bresnahan BA, et al. Improved graft survival after renal transplantation in the United States, 1988 to 1996. N Engl J Med 2000; 342: 605.
5. Li B, Hartono C, Ding R, et al. Noninvasive diagnosis of renal-allograft rejection by measurement of messenger RNA for perforin and granzyme B in urine. N Engl J Med 2001; 344: 947.
6. Strom TB, Tilney NL, Carpenter CB, et al. Identity and cytotoxic capacity of cells infiltrating renal allografts. N Engl J Med 1975; 292: 1257.
7. Barry M, Bleackley RC. Cytotoxic T lymphocytes: All roads lead to death. Nat Rev Immunol 2002; 2: 401.
8. Hadley GA, Bartlett ST, Via CS, et al. The epithelial cell-specific integrin, CD103 (alpha E integrin), defines a novel subset of alloreactive CD8+ CTL. J Immunol 1997; 159: 3748.
9. Cepek KL, Parker CM, Madara JL, et al. Integrin $\alpha_{E\beta7}$ mediates adhesion of T lymphocytes to epithelial cells. J Immunol 1993; 150: 3459.
10. Robertson H, Wong WK, Talbot D, et al. Tubulitis after renal transplantation: Demonstration of an association between CD103+ T cells, transforming growth factor β1 expression and rejection grade. Transplantation 2001; 71: 306.
11. Hadley GE, Rostapshova EA, Gomolka DM, et al. Regulation of the epithelial cell-specific integrin, CD103, by human CD8 cytolytic T lymphocytes. Transplantation 1999; 67: 1418.
12. Racusen LC, Solez K, Colvin RB, et al. The Banff '97 working classification of renal allograft pathology. Kidney Int 1999; 55: 713.
13. Butcher EC, Picker LJ. Lymphocyte homing and homeostasis. Science 1996; 272: 60.
14. Burdick JF, Beschorner WE, Smith WJ, et al. Characteristics of early routine renal allograft biopsies. Transplantation 1984; 38: 679.
15. D'Ardenne AJ, Dunnill MS, Thompson JF, et al. Cyclosporine and renal graft histology. J Clin Pathol 1986; 39: 145.
16. Rush D, Nickerson P, Gough J, et al. Beneficial effects of treatment of early subclinical rejection: A randomized study. J Am Soc Nephrol 1998; 9: 2129.
17. Schoen MP, Arya A, Murphy EA, et al. Mucosal T lymphocyte numbers are selectively reduced in integrin αE (CD103)-deficient mice. J Immunol 1999; 162: 6641.
18. Huraib S, Goldberg H, Katz A, et al. Percutaneous needle biopsy of the transplanted kidney: Technique and complications. Am J Kidney Dis 1989; 14: 13.
19. Beckingham IJ, Nicholson ML, Bell PR. Analysis of factors associated with complications following renal transplant needle-core biopsy. Br J Urol 1994; 73: 13.
20. Sorof JM, Vartanian RK, Olson JL, et al. Histopathological concordance of paired renal allograft biopsy cores: Effect on the diagnosis and management of acute rejection. Transplantation 1995; 60: 1215.
21. Colvin RB, Cohen AH, Saiontz C, et al. Evaluation of pathologic criteria for acute renal allograft rejection: Reproducibility, sensitivity, and clinical correlation. J Am Soc Nephrol 1997; 8: 1930.
22. Nicholson ML, Wheatley TJ, Doughman TM, et al. A prospective randomized trial of three different sizes of core-cutting needle for renal transplant biopsy. Kidney Int 2000; 58: 390.
23. Benfield MR, Herrin J, Feld L, et al. Safety of kidney biopsy in pediatric transplantation: A report of the Controlled Clinical Trials in Pediatric Transplantation Trial of Induction Therapy Study Group. Transplantation 1999; 67: 544.
24. Nueller A, Schnuelle P, Waldherr R, et al. Impact of the Banff '97 classification for histological diagnosis of rejection on clinical outcome and renal function parameters after kidney transplantation. Transplantation 2000; 69: 1123.
25. Leteurtre E, Copin MC, Labalette M, et al. Negative immunohistochemical detection of CD103 ($\alpha E\beta7$ integrin) in the infiltrates of acute rejection in liver and kidney transplantation. Transplantation 2000; 70: 227.
26. Lehmann J, Huehn J, de la Rosa M, et al. Expression of the integrin $\alpha E\beta7$ identifies unique subsets of $CD25^+$ as well as CD25- regulatory T cells. Proc Natl Acad Sci USA 2002; 99: 13031.