T-Cell Receptor-Stimulated Calcineurin Activity Is Inhibited in Isolated T Cells from Transplant Patients

James A. Tumlin, Brian R. Roberts, Kenneth E. Kokko, Osama El Minshawy,¹ and Jennifer L. Gooch

Department of Internal Medicine, University of Tennessee College of Medicine, Chattanooga, Tennessee (J.A.T.); Department of Medicine, Division of Nephrology, Emory University School of Medicine, Atlanta, Georgia (B.R.R., K.E.K., O.E.M., J.L.G.); and Atlanta Veterans Administration Medical Center, Atlanta, Georgia (J.L.G.)

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ABSTRACT

The addition of calcineurin inhibitors, including cyclosporine A (CsA) and FK-506 (tacrolimus), to transplant protocols has markedly reduced acute allograft rejection and prolonged patient survival. Although monitoring of serum drug levels has been shown to be a poor indicator of efficacy, there is little data on calcineurin enzymatic activity in humans. Therefore, we measured calcineurin in isolated CD3⁺/4⁺ T cells from 81 non-transplant controls and 39 renal allograft patients by using a ³²PO₄-labeled calcineurin-specific substrate. A gender difference was observed in the control cohort, with activity in males significantly higher than that in females (1073 ± 134 versus 758 ± 75 fmol/μg/min, respectively). Activity of both groups was comparably inhibited by 5 ng/ml tacrolimus (27 ± 4 versus 30 ± 4%). Calcineurin is a downstream target of the T-cell receptor (TCR). Therefore, activity was measured in isolated T cells after incubation with anti-CD3/CD28 antibodies to stimu-

late the TCR. Calcineurin activity increased significantly from 1214 ± 111 to 1652 ± 138 fmol/μg/min; addition of either tacrolimus or CsA (500 ng/ml) blocked CD3/CD28 stimulation. Despite therapeutic levels of tacrolimus and CsA (mean 11.4 and 172 ng/ml), basal calcineurin activity was significantly higher among renal transplant recipients than controls (1776 ± 175 versus 914 ± 78 fmol/μg/min). In contrast, anti-CD3/CD28 antibodies failed to stimulate calcineurin activity in transplant subjects. Finally, we found that basal and stimulated calcineurin activities are inversely related. Consistent with this finding, basal activity in resting T cells rose over time after transplant but stimulation fell ($r^2 = 0.785$, $p < 0.05$). These data suggest that examination of TCR-stimulated calcineurin activity after renal transplantation may be useful for monitoring immunosuppression of individual patients.

Calcineurin is a heterotrimeric serine-threonine phosphatase that is composed of a catalytic subunit, a regulatory subunit, and calmodulin (Rusnak and Mertz, 2000). Calcineurin is unique among phosphatases in that its activity is calcium-dependent and is central to T-cell receptor (TCR) signaling and amplification of immune responses. The activation of the TCR complex leads to the release of intracellular calcium and calcineurin-mediated dephosphorylation of transcription factors that regulate IL-2 and other proinflammatory cytokines (Macian, 2005).

Cyclosporine A (CsA) and FK-506 (tacrolimus) are structurally unrelated compounds that form drug-receptor com-

plexes with immunophilins (cyclophilin-18 and FK506 binding protein-12, respectively) and potentially inhibit calcineurin phosphatase activity. The widespread use of CsA and tacrolimus in the past two decades has markedly reduced the frequency of acute allograft rejection and prolonged patient survival. Despite their proven benefits, therapeutic monitoring of CsA and tacrolimus levels has proven to be a poor clinical indicator of transplant outcomes. Some patients experience rejection in the presence of adequate or even high blood concentrations (Caruso et al., 2001), whereas others develop toxicity even when blood trough concentrations are low (Citterio, 2004; Kahan, 2004). However, in the absence of an alternative means of monitoring calcineurin inhibitor efficacy, current treatment protocols continue to rely upon plasma drug levels for therapeutic monitoring and optimizing immunosuppression.

One potential alternative to plasma drug level monitoring is direct assay of calcineurin activity. However, few studies

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ABBREVIATIONS: TCR, T-cell receptor; IL, interleukin; BMI, body mass index; CsA, cyclosporine A; FK-506, tacrolimus; ANOVA, analysis of variance.

have directly examined calcineurin activity in T cells or investigated the effects of calcineurin inhibitors on enzyme activity. Previous studies of calcineurin activity *in vivo* have focused on issues including pharmacodynamics in response to cyclosporine and tacrolimus (Koefoed-Nielsen and Jorgensen, 2002; Koefoed-Nielsen et al., 2005b, 2006; Mortensen et al., 2006) and possible effects of variables including gender and time of day (Koefoed-Nielsen et al., 2005a). In an early study using transplant patients, Batiuk et al. (1997) used a $^{32}\text{PO}_4$ -labeled calcineurin-specific substrate to measure the effects of CsA on calcineurin activity in 30 renal allograft recipients. *In vivo* measurements demonstrated that calcineurin activity was inhibited by up to 80% 1 h after an oral dose of CsA, but only 20 to 30% within 4 h. However, the degree of enzyme inhibition and effect on cytokine production varied greatly between individuals. In a similar study, Pai et al. (1994) examined the long-term effect of CsA on calcineurin activity in peripheral lymphocytes from bone marrow transplant patients. Although CsA initially inhibited calcineurin activity during the first 100 days of transplantation, enzyme activity progressively rose over time and within 6 months was similar to that of nontransplant controls.

Therefore, the aim of this study was to compare the effects of CsA and tacrolimus on calcineurin activity in $\text{CD}3^+/\text{CD}4^+$ T cells isolated from normal controls and renal transplant patients. In addition, we examined whether post-transplant immunosuppression led to changes in calcineurin activity in response to TCR stimulation.

Materials and Methods

Subject Recruitment. Subjects for this study included 81 control volunteers and 39 renal allograft recipients transplanted between 2001 and 2004. For some experiments, a subset of 30 controls was used. All participants signed informed consents and completed a brief questionnaire; additional data were obtained by review of patient charts. All data collection, storage, and analyses were carried out with the approval of the Emory University Institution Review Board. Immunosuppressive protocols in use at Emory University (Atlanta, GA) from 2001 to 2004 were based on triple drug therapy, including glucocorticoids (prednisone), calcineurin inhibitors (cyclosporine or tacrolimus), and mycophenolate mofetil.

T-Cell Isolation and Treatment. Approximately 40 ml of heparinized blood was collected, and T cells were isolated using PrepaCyte SC reagent (BioE, St. Paul, MN). Samples were treated with Vitalyse (BioE) to remove erythrocytes. T cells were pelleted by centrifugation, washed with $1\times$ phosphate-buffered saline, and resuspended in RPMI 1640 medium containing penicillin/streptomycin antibiotics, 10% fetal calf serum, 2 mM L-glutamine, 25 mM glucose, and 1 mM sodium pyruvate. Isolated cells were characterized by flow cytometry as 98 to 99% $\text{CD}3^+/\text{CD}4^+$. As indicated, isolated T cells were separated into aliquots and stimulated for 30 min with anti- $\text{CD}3/\text{CD}28$ antibodies (10 $\mu\text{g}/\text{ml}$ each) (BD Biosciences, San Jose, CA).

Calcineurin Assay. Calcineurin activity was determined using an *in vitro* assay based on the method published by Fruman et al. (1996) and as described previously (Lea et al., 2002). After treatment, isolated lymphocytes were pelleted, resuspended in reaction buffer (100 μM Tris, 250 μM KCl, 10 mg/ml bovine serum albumin, and 5 mg/ml dithiothreitol, pH 7.5), and lysed by three cycles of freeze/thawing in liquid nitrogen and a 37°C water bath. Ten micrograms of protein was used for determination of calcineurin activity. Reactions were performed in triplicate, normalized for background, and then a final mean value was determined.

Measurement of Interleukin-2. Isolated T cells were treated as described above, and the culture medium was collected at the end of the incubation period. IL-2 was then measured from the conditioned medium of control or anti- $\text{CD}3/\text{CD}28$ antibody-treated T cells by using a cytokine array according to the manufacturer's instructions (Panomics, Fremont CA). Data obtained are the -fold difference in duplicate measurements of IL-2 for each sample compared with the internal controls.

Statistics. All statistical calculations were carried out using Prism scientific graphing and analysis software (GraphPad Software Inc., San Diego, CA). Paired *t* tests and repeated measure analysis of variance (ANOVA) were used as indicated to compare multiple treatments of individual samples. For comparison of three or more groups, ANOVA (or repeated measures ANOVA, as appropriate) was used in conjunction with Tukey's post-test. Comparison of two sets of variables was completed by two-way ANOVA as indicated. All results were considered significant if $p < 0.05$.

Results

To compare calcineurin activity in circulating T cells, blood was obtained from 81 normal controls and 39 patients with functioning renal allografts. Patients undergoing bone marrow transplantation or other forms of solid organ transplantation were excluded. All normal controls were reported as healthy and free of hypertension, diabetes, or chronic renal disease. The demographics of renal transplant patients and normal controls, including age, height, weight, gender, and racial identification, are shown in Table 1. Both control and transplanted cohorts were approximately 50% male and 50% female. The average age of controls was 35.3 years, and the average body mass index (BMI) was 27.5. The average age and BMI of the post-transplant patients were slightly higher than those of the control group, 47.5 years and 28.6, respectively. Both groups were racially diverse, with approximately half Caucasians and half African-Americans.

The duration of renal transplantation ranged from 1 month to 14 years (mean = 22 months). Twenty (51%) patients were transplanted within 1 year of study enrollment, and the remaining 19 (49%) patients were greater than 1 year after transplant. Two patients had received a prior transplant. All transplant patients were currently taking calcineurin inhibitors, with 31 taking tacrolimus and eight receiving CsA. Plasma levels of tacrolimus and CsA were

TABLE 1

Demographic characteristics of study participants

Study participants completed a questionnaire, and self-reported gender, race, age, and body mass index are reported for the control and transplant cohorts.

Group	n	Gender		Race			Age	BMI
		Male	Female	Caucasian	African American	Asian		
					%			
Control	81	49.0	51.0	49.4	45.7	4.9	34.5	27.4
Control (subset)	30	50.0	50.0	50.8	41.0	8.2	34.5	27.5
Transplant	39	56.4	43.6	48.7	46.2	5.1	47.5	28.6

within therapeutic ranges and averaged 11.4 and 172 ng/ml, respectively.

Calcineurin Activity in the Control Cohort. To ensure that calcineurin activity was measured in a uniform population of cells, experiments were performed in isolated lymphocytes that were enriched for CD3⁺/4⁺ T cells by using erythrocyte lysis, gradient centrifugation, and Prepacyte SC reagent (BioE). Flow cytometric analyses confirmed that more than 98% of isolated lymphocytes were viable CD3⁺/4⁺ T cells (data not shown).

First, T cells were isolated from control subjects and were then treated in vitro with vehicle (dimethyl sulfoxide) or 5 ng/ml tacrolimus for 15 min. Figure 1 shows that tacrolimus significantly reduced calcineurin activity from 913 ± 6 to 662 ± 6 fmol/ μ g protein/min ($p < 0.001$). Although previous studies have reported no effect of gender on calcineurin activity (Koefoed-Nielsen et al., 2005a), females in our control cohort had statistically lower levels of activity compared with those of males (758 ± 75 versus 1073 ± 133 fmol/ μ g protein/min, respectively; $p < 0.05$). Despite differences in basal activity, both male and female subjects exhibited equal sensitivity to tacrolimus (27 ± 4 versus $30 \pm 4\%$ inhibition, respectively).

Next, the effect of stimulating the TCR with anti-CD3/CD28 antibodies on calcineurin activity was examined. Isolated T cells were treated with a variety of stimuli, including calcium ionophore, anti-CD3 antibody, phorbol 12-myristate 13-acetate, and anti-CD3/CD28 antibodies. Anti-CD3/CD28 treatment elicited the maximal response (data not shown) and was chosen for further study. As shown in Fig. 2A, calcineurin activity increased significantly from 1214 ± 111 to 1652 ± 138 fmol/ μ g/min ($p < 0.001$) in the control group. Pretreatment with either tacrolimus or CsA (500 μ g/ml) completely blocked the rise in calcineurin activity. As a functional marker for TCR activation, IL-2 production was measured in the conditioned medium from control and anti-CD3/CD28-treated cells. Figure 2B shows that there was a statistically significant increase in IL-2 release. There were no gender differences in the activation of calcineurin or the inhibitory effect of tacrolimus or CsA.

In addition to gender, the influence of other demographic variables on basal and TCR-stimulated calcineurin activity was examined by multivariate analysis. There was no signif-

Fig. 1. Basal and tacrolimus-inhibited calcineurin activity in normal controls. T cells were isolated from healthy control subjects ($n = 81$). Samples were then lysed, and calcineurin activity was measured at baseline and after the in vitro addition of tacrolimus (Tac) (5.0 ng/ml). Data shown in each column are the mean $\pm$ S.E. calcineurin activity for each group. Tacrolimus significantly decreased calcineurin activity in the whole group as well as in female and male subsets ($p < 0.001$, two-way ANOVA). Calcineurin activity in T cells isolated from female controls was significantly lower than that of males ($p < 0.05$, ANOVA, Tukey's post-test).

Fig. 2. T-cell receptor stimulation increases calcineurin activity in normal controls. A, T cells isolated from a subset of control subjects ($n = 30$) were separated into four aliquots that were then incubated with dimethyl sulfoxide (control), anti-CD3/CD28 antibodies (10 μ g/ml each), tacrolimus (Tac) + CD3/CD28, or CsA + CD3/CD28. Samples were then lysed, and calcineurin activity was determined. Data shown are the mean $\pm$ S.E. calcineurin activity after each treatment for the whole group as well as female and male subpopulations. Anti-CD3/CD28 treatment resulted in a significant increase in calcineurin activity, and pretreatment with either tacrolimus or CsA was effective at blocking the induction (*, $p < 0.05$; **, $p < 0.01$, two-way ANOVA). There was no gender difference in stimulated calcineurin activity or inhibition by tacrolimus or CsA. B, IL-2 release into the conditioned medium of control and anti-CD3/CD28 cells was measured using a cytokine array (Panomics). Anti-CD3/CD28 treatment resulted in a significant increase in IL-2 in the control group (**, $p < 0.01$). There were similar changes in both females and males, although the data did not reach significance.

icant correlation between calcineurin activity and age, BMI, or race in the control cohort (Table 2).

Calcineurin Activity in Post-Transplant Subjects. Calcineurin activity was measured in isolated T cells from 39 postrenal transplant subjects. Compared with normal controls, calcineurin activity was significantly higher in renal transplant patients (1776 ± 175 versus 914 ± 78 fmol/ μ g/min) ($p < 0.001$) (Fig. 3). In contrast with control subjects, calcineurin activity in male transplant recipients was lower compared with that of females ($p < 0.01$). As a result, a significant increase was observed when female control and transplant patients were compared, whereas the difference between male control and transplant subjects did not reach significance.

Next, isolated T cells from the transplant cohort were stimulated with anti-CD3/CD28 antibodies. In contrast to TCR stimulation in controls, anti-CD3/CD28 treatment failed to increase calcineurin activity in the transplant cohort (Fig. 4, A and B). Moreover, the mean increase in calcineurin activity for the control group was significantly higher at 36% compared with only 3% for the transplant group (Fig. 4C). Consistent with a decrease in TCR activity, IL-2 production in treated T cells from transplant patients was significantly reduced compared with that of controls (Fig. 4D).

TABLE 2

Calcineurin activity and patient demographics

Basal and TCR-stimulated calcineurin activity and study participant characteristics were analyzed by Pearson correlation. Data shown are the Pearson r/p value (if less than 0.05).

	Control		Transplant	
	Basal	TCR-Stimulated	Basal	TCR-Stimulated
Age	0.037/N.S.	0.108/N.S.	-0.268/N.S.	-0.025/N.S.
BMI	-0.220/N.S.	0.067/N.S.	-0.397/ $P < 0.05$	0.078/N.S.
Race	0.292/N.S.	0.128/N.S.	0.314/N.S.	0.212/N.S.
Tacrolimus (trough)	N.A.	N.A.	0.091/N.S.	-0.208/N.S.

N.A., not applicable.

Fig. 3. Increased basal calcineurin activity in transplant subjects. T cells were isolated from 39 postrenal transplant subjects and basal activity was compared with controls. Data shown are the mean $\pm$ S.E. calcineurin activity of controls and transplants as well as female and male subsets of each cohort. Basal calcineurin activity was significantly higher in transplant subjects as a whole and in the female subset (**, $p < 0.01$, two-way ANOVA). Male allograft recipients had lower calcineurin activity compared with females (*, $p < 0.05$, ANOVA, Tukey's post-test).

Increased basal calcineurin but decreased TCR-stimulated calcineurin activity in transplant subjects suggests that basal and stimulated calcineurin activities may be inversely related. To examine this, basal and stimulated calcineurin were compared by linear regression. Figure 5, A and B, shows that there is a significant inverse association between basal and TCR-stimulated calcineurin in both the control and transplant cohorts. In addition, we examined the correlation between basal and stimulated calcineurin activity and changes over time after transplantation. Baseline calcineurin activity rose over time after transplantation and TCR stimulation decreased (Fig. 6). Basal calcineurin activity at 1 month was 1491 ± 316 fmol/ μ g/min, rising to 2117 ± 150 fmol/ μ g/min at 1 year and 3834 ± 987 fmol/ μ g/min by year 3. TCR stimulation decreased over the same period. There was no difference in trough tacrolimus levels between any of the groups.

Finally, basal and TCR-stimulated calcineurin activities were compared with various parameters, including age, race, BMI, and tacrolimus plasma level. There was an inverse correlation between basal calcineurin and BMI. No other parameter reached statistical significance.

Discussion

Addition of the calcineurin inhibitors CsA and tacrolimus to immunosuppression protocols has reduced acute rejection rates and prolonged renal allograft survival. However, despite the contribution of these agents to transplant outcomes, it remains to be determined why plasma drug levels fail to predict either rejection or toxicity. Moreover, little is understood about the effects of chronic immunosuppressive thera-

Fig. 4. Calcineurin activity in T cells isolated from transplant subjects is not increased in response to T-cell receptor stimulation. Calcineurin activity in control and anti-CD3/CD28-treated T cells isolated from control ($n = 30$) (A) and post-transplant ($n = 39$) (B) subjects was compared. T-cell receptor stimulation resulted in a significant increase in controls ($p < 0.001$) but not transplants (paired t test). C, percentage of increase in calcineurin activity was determined for control subjects and transplant patients. Data shown are the mean $\pm$ S.E. percentage of change in stimulated calcineurin activity for the control and transplant groups. The mean percentage of stimulated calcineurin activity was significantly less in transplant subjects compared with controls (**, $p < 0.001$; Student's t test). D, IL-2 release into conditioned medium of treated cells was compared for control and transplant cohorts. There was a statistically significant decrease in the amount of IL-2 released by T cells isolated from transplant patients (**, $p < 0.01$).

pies on calcineurin activity in lymphocytes from allograft recipients.

To better use calcineurin activity as a means of monitoring the efficacy of immunosuppressant drugs, it is essential to identify reproducible changes in activity between control and transplant populations. Reports in the literature to date have not identified an aspect of calcineurin activity that is consistently altered with CsA or tacrolimus therapy. The current study is therefore the first, to our knowledge, to identify a novel activity that is significantly inhibited in transplant patients. In addition to this important observation, data from this study offer several new insights into calcineurin activity in humans.

First, we found that gender influenced baseline calcineurin activity. Among normal controls, basal calcineurin activity was significantly lower in T cells from females compared with males. The mechanism for reduced calcineurin activity among females is unknown. One possibility may be related to estrogen, because previous studies have shown that estro-

Fig. 6. Correlation between basal calcineurin activity and time since renal transplantation. Basal calcineurin activity and response to TCR stimulation were compared in renal allograft recipients and grouped by time since transplantation. Calcineurin activity in patients 1 to 2 months after transplant was compared with patients of 3-, 12-, 24-, and 36-month transplant duration. There was a significant positive correlation between basal calcineurin activity and duration of transplantation ($p < 0.05$) and a significant inverse correlation between response to T-cell receptor stimulation and duration of transplantation ($p < 0.05$).

gens can alter protein expression and calcineurin activity in cultured T cells (Rider et al., 2000). Contrary to our study, however, estrogen stimulated expression and activity of calcineurin *in vitro*. It is interesting that there are no studies to our knowledge that investigate the effect of testosterone on calcineurin expression in human T cells. It is also possible that additional cofactors may influence gender differences in calcineurin activity. For example, our control and transplant cohorts are racially diverse, with roughly 50% African-Americans in each group. Racial influences on gender could also explain why our study identified differences, whereas data from Koeford-Nielsen et al. (2005a) taken from a Danish population did not.

Next, somewhat paradoxically, we report that basal calcineurin activity is increased rather than decreased in transplant patients compared with controls. Although there is overlap between the two groups, the finding was highly significant and suggests that the primary change in calcineurin activity with chronic CsA or tacrolimus treatment is not a sustained decrease in basal levels of the enzyme. In support of this finding, Pai et al. (1994) reported that, although calcineurin activity was lower in the first 100 days after bone marrow transplant, after 6 months there was no difference in basal calcineurin activity between CsA-treated patients and age-matched controls.

Despite the importance of stimulated calcineurin activity in the dephosphorylation of nuclear factor of activated T cells and the amplification of an immune response, few studies have examined calcineurin activity in activated lymphocytes, and none, to our knowledge, have compared activated cal-

Fig. 5. Basal and T-cell receptor-stimulated calcineurin activity are inversely related. Basal calcineurin activity and anti-CD3/CD28-stimulated activity were compared. There is a statistically significant inverse relationship between basal calcineurin activity and the percentage of stimulation in response to T-cell receptor stimulation in both the control (A) and transplant (B) groups ($r^2 = 0.136$, $p < 0.01$ and $r^2 = 0.154$, $p < 0.05$, respectively; linear regression).

calcineurin between control and transplant populations. To address this question, we measured calcineurin activity in CD3⁺/4⁺ T cells isolated from normal controls and renal transplant patients stimulated with CD3/CD28 costimulatory antibodies. Stimulation of the TCR in transplant patients with therapeutic blood levels of tacrolimus or CsA did not increase calcineurin activity above basal levels, whereas enzyme activity in normal controls increased by roughly 40%. These data suggest that TCR-stimulated calcineurin may be a more sensitive indicator of calcineurin inhibitor efficacy than basal levels of enzyme activity.

Our data identified a decrease in TCR-stimulated calcineurin in conjunction with an increase in basal activity in transplant subjects, suggesting that there may be a relationship between basal enzyme activity and stimulation. In control and transplant cohorts, a significant, inverse relationship was identified. One explanation for this finding could be that increased basal activity is a compensatory response to loss of TCR stimulation and, in a chronic setting, drugs such as CsA and tacrolimus act preferentially to inhibit acute activation of calcineurin rather than reduce baseline levels. Future studies examining protein levels of calcineurin could be useful in addressing this possibility. An alternative explanation for the relationship between basal and stimulated calcineurin is that immune challenges such as organ transplant may provoke a chronic elevation in T-cell activation and, consequently, in calcineurin activity. Acute challenge of the TCR may then produce no additional increase. In support of this, Fig. 5 shows that only 10% of controls had basal calcineurin levels greater than 1700 fmol/μg protein/min. In contrast, almost half of transplant subjects had calcineurin activities >1700. Although further studies are needed to understand this possibility, it is clear that monitoring of TCR-stimulated calcineurin may offer a novel alternative to plasma drug levels for therapeutic monitoring of calcineurin inhibitor efficacy.

In support of the possibility that TCR stimulation of calcineurin may be physiologically relevant in transplant populations, we found significant correlations between the duration of transplantation and calcineurin activity. Basal calcineurin activity gradually increased and TCR-stimulation declined in subsets of allograft recipients who were 1, 2, and 3 years after transplant. This would be consistent with a model of gradually declining immune activation and increased graft stability. A similar observation was reported by Mortensen et al. (2006), who noted that basal calcineurin activity rose by 40% over a period of 5 years in 20 renal transplant patients.

In conclusion, our data provide a novel paradigm for calcineurin response in T cells of transplant patients. Both basal levels of activity as well as response to TCR stimulation are

altered in allograft recipients compared with normal controls. Moreover, these factors are probably related, although the mechanism has yet to be defined. Additional prospective studies are needed to determine whether routine measurements of T-cell calcineurin activity will enhance monitoring of calcineurin inhibitor efficacy and facilitate the development of individual immunosuppressive protocols.

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References

- Batiuk TD, Kung L, and Halloran PF (1997) Evidence that calcineurin is rate-limiting for primary human lymphocyte activation. *J Clin Invest* **100**:1894–1901.
- Caruso R, Perico N, Cattaneo D, Piccinini G, Bonazzola S, Remuzzi G, and Gaspari F (2001) Whole-blood calcineurin activity is not predicted by cyclosporine blood concentration in renal transplant recipients. *Clin Chem* **47**:1679–1687.
- Citterio F (2004) Evolution of the therapeutic drug monitoring of cyclosporine. *Transplant Proc* **36**:420S–425S.
- Fruman DA, Pai SY, Klee CB, Burakoff SJ, and Bierer BE (1996) Measurement of calcineurin phosphatase activity in cell extracts. *Methods* **9**:146–154.
- Kahan BD (2004) Therapeutic drug monitoring of cyclosporine: 20 years of progress. *Transplant Proc* **36** (2 Suppl):378S–391S.
- Koefoed-Nielsen PB and Jørgensen KA (2002) Alterations in calcineurin phosphatase activity in tacrolimus-treated renal transplant patients. *Transplant Proc* **34**:1743–1744.
- Koefoed-Nielsen PB, Karamperis N, Højskov C, Poulsen JH, and Jørgensen KA (2006) The calcineurin activity profiles of cyclosporin and tacrolimus are different in stable renal transplant patients. *Transpl Int* **19**:821–827.
- Koefoed-Nielsen PB, Karamperis N, and Jørgensen KA (2005a) 24-h monitoring of calcineurin phosphatase activity in healthy subjects. *Scand J Immunol* **62**:309–311.
- Koefoed-Nielsen PB, Karamperis N, and Jørgensen KA (2005b) Comparison of the temporal profile of calcineurin inhibition by cyclosporine and tacrolimus in renal transplant patients. *Transplant Proc* **37**:1736–1738.
- Lea JP, Jin SG, Roberts BR, Shuler MS, Marrero MB, and Tumlin JA (2002) Angiotensin II stimulates calcineurin activity in proximal tubule epithelia through AT-1 receptor-mediated tyrosine phosphorylation of the PLC-gamma1 isoform. *J Am Soc Nephrol* **13**:1750–1756.
- Macian F (2005) NFAT proteins: key regulators of T-cell development and function. *Nat Rev Immunol* **5**:472–484.
- Mortensen DM, Koefoed-Nielsen PB, and Jørgensen KA (2006) Calcineurin activity in tacrolimus-treated renal transplant patients early after and 5 years after transplantation. *Transplant Proc* **38**:2651–2653.
- Pai SY, Fruman DA, Leong T, Neuberger D, Rosano TG, McGarigle C, Antin JH, and Bierer BE (1994) Inhibition of calcineurin phosphatase activity in adult bone marrow transplant patients treated with cyclosporine A. *Blood* **84**:3974–3979.
- Rider V, Jones SR, Evans M, and Abdou NI (2000) Molecular mechanisms involved in the estrogen-dependent regulation of calcineurin in systemic lupus erythematosus T cells. *Clin Immunol* **95**:124–134.
- Rusnak F and Mertz P (2000) Calcineurin: form and function. *Physiol Rev* **80**:1483–1521.

Address correspondence to: Dr. Jennifer L. Gooch, Medicine/Nephrology, Emory University School of Medicine, 101 Woodruff Circle, WMB 338, Atlanta, GA 30322. E-mail: jgooch@emory.edu