An emerging role for calcineurin Aα in the development and function of the kidney

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Gooch, Jennifer L. An emerging role for calcineurin Aα in the development and function of the kidney. Am J Physiol Renal Physiol 290: F769–F776, 2006; doi:10.1152/ajprenal.00281.2005.—For many years, calcineurin has been a familiar molecule as a target of the immunosuppressive agents cyclosporin A and FK-506. Calcineurin inhibition interferes with T cell signaling by preventing activation of the transcription factor NFATc. However, calcineurin is expressed in most tissues in the body, and calcineurin inhibition undoubtedly alters many other cellular processes. As a result, serious side effects of calcineurin inhibitors regularly occur, including hypertension and renal dysfunction. Because nephrotoxicity is often a barrier to continued clinical use of calcineurin inhibitors, understanding the role of calcineurin in the kidney is of particular importance. Recent work has demonstrated that the two main isoforms of the catalytic subunit of calcineurin, Aα and Aβ, may have distinct functions, particularly in the kidney. Calcineurin isoforms may be differentially expressed, and/or the activity of each may be differentially regulated, leading to tissue-specific functions. Differences between the action of the two isoforms are most evident in knockout mice lacking each isoform. Mice lacking the β-isofrom are characterized principally by altered development and function of immune cells. α-Knockout mice, in contrast, can still be immune suppressed by cyclosporin A but display pervasive developmental defects, including renal dysfunction. Therefore, it is intriguing to consider that while the β-isofrom may be responsible for calcineurin action in T cells, the α-isofrom may be the predominant catalytic isoform in the kidney. This conclusion, if correct, may have substantial clinical implication for novel strategies to selectively target calcineurin action in T cells without associated nephrotoxicity.

nephrogenesis; p27; cyclosporin; nephrotoxicity

CALCINEURIN IS A CALCIUM-DEPENDENT serine/threonine phosphatase that is found throughout the phylogenetic tree and is distributed throughout most mammalian tissues. The conservation of the catalytic domain suggests that the phosphatase is an integral cell component. In fact, there is growing evidence that calcineurin is a major participant in development, cellular regulation of calcium homeostasis, and intracellular trafficking. The array of calcineurin functions in mammals is also evident in the branching of the catalytic subunit into three isoforms (i.e., α, β, and γ), each produced from separate genes. These three isoforms allow for more acute regulation and specificity of function. For example, the γ-isofrom is the predominant isoform in the testis, where it interacts with a testes-specific regulatory subunit, calcineurin B2. The remaining two isoforms, α and β, can be found in most mammalian tissues and bind a common regulatory subunit, calcineurin B1. Despite this common partner, recent data suggest that there are mechanisms to specifically regulate the isoforms in different tissues. For example, the β-isofrom is upregulated in the heart in response to a hypertrophic stimulus (23), whereas the α-isofrom appears to be the predominant isoform upregulated in the diabetic kidney (13). Moreover, mice which lack either the α- or β-isofrom have distinctly different phenotypes. β-Knockout mice are reported to have immature immune systems (3) and a blunted response to cardiac hypertrophy (4) but grow comparably to wild-type littermates and reproduce. In contrast, α-knockout mice produce T cells with only minor defects (6, 48) and appear to have normal cardiac development and function (33). However, α-null mice are significantly smaller than their wild-type littermates, are infertile, and on average live only a few weeks (15). Whereas it is possible that some tissues compensate for the loss of one isoform by upregulating the remaining two isoforms, it is also apparent that each isoform is likely playing distinct, tissue-specific roles.

Although cellular functions of calcineurin have been explored in some depth in the immune system, central nervous system, and heart, less is known about the role of calcineurin in the kidney. It is clear that calcineurin is a central regulator of calcium-mediated signals generated by numerous factors that are of particular importance in the kidney, including ANG II (20, 40), TGF-β (11), and IGF-I (14). Moreover, there is evidence that calcineurin is regulated by disease states such as diabetes (10, 13) and in the development of the kidney (15). Finally, the importance of calcineurin in the kidney is underscored by the frequent nephrotoxic side effects of pharmacological agents that inhibit calcineurin, such as cyclosporin A.
and FK-506. Although there are few clear examples of specific cellular functions of calcineurin in the kidney, little is known about the role of different catalytic isoforms. Are there functional differences between the closely related calcineurin isoforms? Is there a preferential requirement for either calcineurin isoform in renal development? Are the nephrotoxic changes associated with calcineurin inhibitors the result of inhibition of the α- or β-isoform, or both? As yet, these questions remain to be fully answered. There is evidence to suggest that calcineurin signaling in kidney cells activates signaling mechanisms that have been well characterized in other cell types. Nuclear factors of activated T cells (NFAT) proteins are transcription factors that are dephosphorylated by calcineurin (16, 24). Phosphorylated NFATc proteins are inactive in the cytosol, and removal of the phosphate group reveals a nuclear localization signal. Once in the nucleus, NFATc proteins interact with other transcription factors and modulate transcription of specific genes (Fig. 1A). NFATc proteins appear to be regulated in the kidney in vitro and in vivo. We have previously found that both IGF-I (14) and TGF-β (11) induce nuclear translocation of NFATc in mesangial cells (Fig. 1B). In vivo, ANG II induces nuclear localization of NFATc proteins in the glomerulus (40). However, it is not yet known what specific genes are regulated by NFATc in the kidney or if NFATc proteins work in conjunction with other, kidney-specific factors.

In addition to substrates of calcineurin, differential expression and/or activity of calcineurin isoforms may contribute to kidney-specific functions of calcineurin. There are differences between the isoforms that are most evident in knockout mouse models. Moreover, calcineurin is, indeed, required for normal kidney development, and the α- and β-isoforms do not contribute equally. Finally, the α-isoform is emerging as the probable mediator of nephrotoxic changes in the kidney associated with calcineurin inhibitors. As such, therapeutic implications of calcineurin inhibitors need to be evaluated in light of recent work published by our laboratory and others to consider nephrotoxic side effects not only during calcineurin inhibitor therapy but also during gestation and the possible additive effects of calcineurin inhibitions to other renal disease states such as diabetes.

Invited Review

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Fig. 1. Calcineurin (Cn) acts via activation of nuclear factors of activated T cells (NFATc) transcription factors. A: antigen/MCH complexes bind T cell receptor and activate a signaling cascade that leads to generation of myo-inositol 1,4,5-trisphosphate (IP3) and activation of the IP3 receptor (IP3R) in the endoplasmic reticulum (ER). ER calcium is released, triggering alterations in membrane calcium channels and a further increase in cellular calcium. Calcineurin, in a complex with calmodulin and calcium, becomes activated and dephosphorylates NFATc, which translocates to the nucleus. In the nucleus, NFATc couples with other transcription factors including NFATn, API-1, NF-κB, and possibly others to activate specific gene promoters including IL-2. B: NFATc was visualized by immunofluorescence using an antibody that is broadly cross-reactive with all 4 calcium-dependent NFATc proteins. NFATc distribution is cytoplasmic in serum-starved mesangial cells (a) and nuclear translocation is induced by both TGF-β (b) and IGF-I (c).
CALCINEURIN: A BRIEF OVERVIEW

Calcineurin is composed of two subunits, A and B, and binds to calmodulin in the presence of calcium, to form an active enzyme. The calcineurin A subunit contains a phosphatase domain and mediates interaction with phosphorylated substrates, whereas the B subunit binds calcium and calmodulin and facilitates the conformational change that activates the phosphatase domain of the A subunit (36). Three genes encode isoforms of the A subunit: α, β, and γ. Figure 2 demonstrates the sequence identity of the three isoforms, which is slightly greater than 80%. The greatest variance occurs at the NH2 terminus and the COOH termini, whereas the phosphatase domains are virtually identical. At the COOH terminus, the β-isofrom contains a proline-rich region not found in the α- or the γ-isofroms. All three isoforms contain essentially unique COOH termini, with the exception of a conserved region that corresponds to an inhibitory domain (residues 84–336 of the α-isofrom). It is interesting to speculate that such a motif might contribute to substrate-specific protein-protein interaction, thus differentiating potential targets of β- from those of the α-isofrom. However, there are substrates yet to be identified which are exclusively dephosphorylated by either the α- or β-isofroms.

In addition to possible substrate specificity due to the proline-rich region found in the β-isofrom, tissue-specific expression of the enzyme may contribute to differential action of the isoforms. The majority of organs and tissues express both the α- and β-isofroms, albeit in differing abundance, whereas the γ-isofrom is restricted to the testis and a small region of the brain. Generally speaking, α is the predominant isoform in the brain, whereas β has been described to be the primary isoform in the immune system, muscle, and heart (see Table 1). A third possible level of specificity may be due to availability of substrates, as there are tissue-specific differences in the expression of some calcineurin substrates such as NFAT proteins. There are four members of the NFAT family that are regulated in a calcium-dependent manner (NFATc1–c4). Although NFATc1 appears to be the predominant substrate of calcineurin in T cells (22), NFATc3 and c4 may be more important in the heart and muscle (26). Similarly, calcineurin dephosphorylates other transcriptional cofactors such as GATA proteins (27, 29) and myocyte enhancer factors (MEFs) (32), which may also be regulated in a tissue-specific manner.

Several groups including ours have examined expression of calcineurin A subunits in the kidney. Buttini et al. (5) have examined α-, β-, and γ-isofrom mRNA by in situ hybridization and reported that both α and β were detected primarily in the medulla but also in the cortex. The γ-isofrom, in contrast, has not been detected in any part of the kidney. Protein expression of specific isoforms was also examined in kidney microstructures in a report by Tumlin et al. (45) by Western blotting of isolated nephron segments. Calcineurin Aα is the predominant isoform expressed in the proximal tubules, whereas only a small amount of the β-isofrom has been identified. The α- and β-isofroms of the A subunit were detected in roughly equal amounts in cortical collecting ducts, whereas the β-isofrom was expressed mostly in the medulla (44, 45). Interestingly, the majority of calcineurin phosphatase activity corresponds to expression of calcineurin Aα protein in cortical tubules of regions described to bind the calcineurin B subunit (residues 248–256 and 299–306 of the α-isofrom). In addition, a region identified as a calmodulin binding domain (391–417 of the α-isofrom) is also completely conserved as is a region designated the BBH that is described to facilitate binding of the inhibitory complex FKBP12/FK-506 to both the phosphatase domain and to the calcineurin B subunit (residues 350–370 of the α-isofrom). Four residues in this region (* in Fig. 2) bind metals and are required for interaction with FKBP12/FK-506 (36). In addition to three isoforms of the calcineurin A subunit, there are two genes, B1 and B2, which encode the B subunit. B1 is ubiquitously expressed, whereas B2 is testes specific. The Aα- and Aβ-subunits have been shown to interact interchangeably with the B1 subunit, allowing for at least two functional isozymes (36).

While there is remarkable identity between the α- and β-isofroms, there is one region of striking uniqueness in the β sequence. The NH2 terminus of the β-isofrom contains a proline-rich region that is not found in either the α- or γ-isofroms. This region corresponds to an inhibitory domain (residues 470–488 in the α-isofrom), which also contains an “S/T signature motif” (residues 148–153 of the α-isofrom), and two
normal rats (44). Neither group reported expression of calcineurin A isoforms in glomeruli, possibly because of low expression levels. Despite this, calcineurin activity has been reported in mesangial cells (11, 14), and NFATc nuclear localization has been observed in vivo in response to ANG II reported in mesangial cells (11, 14), and NFATc nuclear expression levels. Despite this, calcineurin activity has been observed. However, expression of the α- and β-isoforms was significantly increased in the kidney following induction of type I diabetes with streptozotocin. Interestingly, the γ-isofor m was also detected in the diabetic kidney. However, this finding lacks corroboration, and it cannot be ruled out that the anti-γ antibody is simply cross-reacting with either the α- or β-isoforms, given that the sequences of the three proteins are highly similar. The cortical think ascending limb was the site of greatest induction but increased expression of both α and β was also detected in the medullary and cortical collecting ducts. In glomeruli, diabetes was associated with an increase in primarily the α-isofor m (summarized in Table 2). Interestingly, inhibition of calcineurin with cyclosporin A in this model resulted in decreased hypertrophy and ECM accumulation in glomeruli but was additive with diabetes on regulation of TGF-β and fibronectin in the tubulointerstitium.

Substrates of calcineurin in the kidney most likely include NFATc proteins but may include other, kidney-specific substrates as well. All four of the NFATc proteins have been found to be expressed in the kidney as well as a calcium-independent NFAT, NFAT5/tocinity-responsive enhancer binding protein (49), which is activated in response to changes in medullary toxicity (43, 47). As yet, no kidney-specific substrates of calcineurin or transcriptional cofactors have been described.

CALCINEURIN AND DEVELOPMENT

Calcineurin has been demonstrated to be an important part of the developmental program of the vascular system and the heart (26). Genetic disruption of the regulatory subunit of calcineurin (calcineurin B) is embryonically lethal due to a failure of pericyte recruitment to vessel walls (26). Knockout of the calcineurin substrate NFATc1 or the double knockout of NFATc3 and NFATc4 also results in early lethality due to failures of the vasculature (26) and of heart formation (26), respectively. Recent work has shown that calcineurin is also an important part of the developmental program in the kidney. In particular, loss of calcineurin action impairs normal development of the urogenital tract in general and late nephrogenesis in particular. Moreover, there appears to be a specific requirement for the α-isofor m in renal development.

To begin, Chang et al. (7) formulated a strategy for investigating calcineurin function in development whereby the regulatory subunit of the calcineurin holoenzyme (calcineurin B) was disrupted, resulting in a loss of both α- and β-isofor m activity. They restricted their observations to the developing urogenital tract by creating tissue-specific knockout mice using cre/lox technology. First, transgenic mice were created that overexpress a construct containing the cre recombinase under the direction of the Pax3 promoter, whereby, in the developing rodent, the Pax3Cre construct is expressed primarily in mesenchymal cells in the urogenital tract and thus serves to drive cre expression in these target organs. Next, the cre transgenic animals were crossed with animals carrying a floxed calcineurin B gene, producing mice which presumably lack all calcineurin activity in mesenchymal cell derivatives of the kidneys, ureters, and bladder (7). Several defects in the development of these organs were found, including defective pykn
eloureteral peristalsis and progressive renal obstruction that ultimately led to renal failure. Chang et al. postulate that these defects stem from altered cell cycle control in cells where calcineurin B had been knocked out. Cells of mesenchymal origin that lacked calcineurin activity were not undergoing cell division, whereas adjacent cells of distinct embryonic origin retained normal proliferation.

Next, a more direct examination of the role of calcineurin specifically in kidney development was reported by Tendron et al. (42). In their 2003 paper, the authors report that administration of cyclosporin A to pregnant rabbits at specific time periods during gestation resulted in significant changes in the kidneys of resulting pups (42). The authors examined the effects of cyclosporin A administration from embryonic days 13–17, which coincide with the onset of nephrogenesis, and days 18–21, which coincide with early renal function in the developing offspring. Pups born following administration of cyclosporin A in utero, whether during the earlier target period of the onset of nephrogenesis or the later time point coinciding with early renal function, demonstrated reduced nephron number and alterations in postnatal maturation of the kidney. Interestingly, the most striking impairments were localized to the nephrogenic zone (NZ) of the postnatal cortex, which was markedly attenuated compared with control pups. This suggests that terminal differentiation of the kidney is under the control of a specific genetic program that requires early activity of calcineurin to set the necessary sequence in motion. Despite histological evidence of impaired development, serological evidence of renal function was normal. In the three decades since cyclosporin A was introduced as a posttransplantation immune suppressor, a small but growing number of children have been born to mothers receiving calcineurin inhibitors throughout pregnancy. While early functional studies have not shown signs of renal impairment in children exposed to cyclosporin in utero, the data from Tendron et al. suggest that long-term follow-up of these children, some of whom are now adults, is particularly important as there could be changes in renal structure that may affect kidney function in years to come.

Finally, a third model of calcineurin inhibition during development comes from mice that lack either the α- or β-isoform of the catalytic subunit. While several reports have been published detailing alterations with the loss of each isoform (3, 4, 48, 50), nothing was known about kidney development or function in the two knockout models until recently. Our laboratory became interested in the role of calcineurin isoforms as a potential mechanism of specificity of calcineurin action in the kidney. As a result, we obtained α- and β-null animals and investigated the effect of loss of either isoform on the kidneys. We found several significant alterations in the kidneys of α-null mice that were consistent with previous reports of calcineurin function in maturation including changes in the cell cycle, developmental abnormalities in the NZ, and a reduction in functional nephrons. However, interestingly, these changes were specific to the loss of only the α-isoform and not found in mice lacking calcineurin Aβ.

As previously reported (15), we found that differences in growth and development of the two knockout mice were readily apparent. While the body mass and individual organ weights of calcineurin Aβ-knockout mice were not different from their wild-type littermates at 18 days of age, α-knockout mice were significantly smaller, and examination of organ weights indicated that the brain, heart, liver, and kidney were all significantly reduced. Interestingly, while the reduction in the size of the heart was proportional to the overall diminished size of the animals, kidney and liver weights were significantly reduced independently of body weight. Kidney growth of calcineurin Aα −/− mice was particularly impaired, as evidenced by a significantly decreased kidney/body weight ratio of not only homozygous null animals but also heterozygous animals compared with their wild-type littermates. Further examination of the four main regions of the kidney, i.e., the inner medulla, inner stripe of the outer medulla, outer stripe of the outer medulla, and cortex, revealed that the outer stripe of the outer medulla and cortex are particularly affected. This finding mirrors that from the rabbits that were exposed to cyclosporin during brief periods of gestation, as reported by Tendron et al. (42). Also similar to that study, the defect in cortical development in α-null mice appears to be specific to the NZ. However, whereas Tendron et al. described a reduction in nephron number as a key feature of gestational calcineurin inhibition, glomeruli number were not significantly different in our study of calcineurin Aα-null kidneys. One possibility for this difference may be due to the postnatal period when counts were completed. The requirement for calcineurin appears to be specific for final postnatal maturation, as the defects are considerably less pronounced in kidneys shortly after birth. In the following weeks, when normal rodent kidneys undergo final maturation, the loss of calcineurin becomes most apparent in α-null animals and ultimately limits their mortality. For this reason, our studies were limited to the time period around 18 days postnatal. At this point, a statistically comparable number of glomeruli were present in the null animals compared with wild-type. In the study by Tendron et al., the pups were examined at an older age (1 mo postnatal) and were not as severely affected as α-null mice. As a result, it is probable that the immature glomeruli had deteriorated to such an extent that they were no longer identifiable.

Next, we found that not only are NZ glomeruli from calcineurin Aα −/− mice smaller, but there appears to be a defect in cellular development (15). Further examination of α-null kidneys revealed an attenuation of mesangial cells in late-developing nephrons but a persistence of endothelial and epithelial cells. Consistent with changes in cell content in the glomerulus, null mice demonstrated changes in proliferation and cell death in the NZ. Specifically, we found that loss of calcineurin Aα resulted in decreased proliferation and increased cell death. Chang et al. (7) suggest that calcineurin directly mediates proliferation, as opposed to functioning to generate a signal that regulates the cell cycle of adjacent cells, for example. Thus the lack of mesangial cells in calcineurin Aα −/− mice would suggest a direct role for calcineurin in this cell type. Consistent with this idea, we previously reported that calcineurin is a component of both IGF-I and TGF-β signaling in mesangial cells. Chief among its roles, IGF-I is a potent survival factor in many cells, and this signal may involve calcineurin (14, 28). Thus it is possible that the loss of calcineurin α prevents mesangial cell response to a survival signal and thereby increases cell death.

Interestingly, despite apparent consistencies with the effect of gestational administration of cyclosporin (which presumably inhibited both α- and β-isoform activities), changes in NZ
development, proliferation, and mesangial cell survival were observed only in α-null mice and not in β-knockout mice. However, when we examined calcineurin activity in both α- and β-null mice, we found that each isoform contributed to the total level of activity in wild-type kidneys. Loss of α or β each resulted in only a partial reduction of calcineurin activity. Thus some activity remains even in α-null mice despite phenotypic similarities with animals that have been completely suppressed via cyclosporin A. Similarly, partial activity that remains in β-null mice (attributable to the α-isoform) appears to be sufficient for renal development. One possibility is that the β-isoform contributes to an as yet unidentified process in the kidney that is distinct from that of α. Conversely, it is also possible the α-isoform is able to compensate for loss of β activity in some cell processes but that the β-isoform cannot substitute for the loss of α activity.

Finally, the combination of poor maturation and aberrant cell proliferation and cell death contributes to a steady decline in α-null mice. Within 2 wk of birth, calcineurin Aα −/− mice demonstrate significant elevations of serum creatinine and significant decreases in urine creatinine concentration compared with wild-type animals. In contrast, calcineurin Aβ mice show no difference in serum and urine creatinine levels compared with wild-type littermates. Consistent with impaired renal function (and, perhaps, contributing to the same), kidneys of calcineurin Aα −/− mice show increased deposition of extracellular matrix (15).

Targeted loss of calcineurin B resulted in changes in the cell cycle and a resulting decrease in proliferation of cells of mesenchymal origin, suggesting that calcineurin is important

Fig. 3. Differences in proliferation and cell death in calcineurin Aα- vs. Aβ-null kidneys. A–C: proliferation within the nephrogenic zone was examined in +/+ (A), α−/− (B), and β−/− (C) kidneys by immunohistochemistry using a specific antibody to proliferating cell nuclear antigen (PCNA). Sections were counterstained with hematoxylin and viewed by light microscopy. Typical results are shown for each genotype. Arrowheads in B designate persistent immature glomeruli. D–F: cell death was examined by terminal transferase-mediated dUTP nick end-labeling stain in +/+ (D), α−/− (E), and β−/− (F) kidney sections. Areas of dark brown staining denote cells that have fragmented DNA and are therefore undergoing cell death. Typical results for each genotype are shown.

Fig. 4. Putative role of calcineurin in regulation of p27 during nephrogenesis. Calcineurin activity is required at the onset of nephrogenesis. p27 Expression is normally induced coincidently with terminal differentiation and must be turned off for normal organ size and development. Calcineurin Aα−/− mice have persistent expression of p27 in late nephrogenesis and attenuated organ size and development. While juxtamedullary (JM) glomeruli, which complete maturation the earliest, are normal, final development of cortical glomeruli is impaired, suggesting a specific role for inhibition of p27 in terminal steps of nephron maturation. Thus calcineurin, through an unknown mechanism, sets in motion events that are required for inhibition of developmental expression of p27.
for normal control of the cell cycle in the developing kidney (7). This function of calcineurin was also observed in isoform-specific null animals and, once again, the changes were specific to the α-isoform and not the β. Abnormal postnatal maturation of the NZ was associated with decreased proliferation of epithelial cells and an increase in cell death with loss of calcineurin Aα but were essentially normal despite loss of the β-isoform (Fig. 3). A possible mechanism of altered cell cycle control was also identified. Loss of calcineurin Aα appears to result in increased levels of the cyclin-dependent kinase inhibitor p27, also specifically in the NZ and in epithelial cells of NZ glomeruli, which is consistent with inhibition of proliferation in these structures and increased cell death (15). Thus, in addition to a role in mesangial cells, this study implicates calcineurin in cell cycle regulation of epithelial cells in the developing kidney.

Calcineurin-deficient mice also reinforce the phenotypic outcomes of gestational calcineurin inhibition on late nephrogenesis. One of the more interesting aspects of the study by Tendron et al. (42) was the apparent requirement for calcineurin activity in early renal development in order for the completion of late, postnatal nephrogenesis. Consistent with this idea, kidneys of calcineurin α-null mice are grossly normal at birth, demonstrating that the early developmental program is independent of calcineurin. However, development appears to halt in the period shortly after birth and fails to progress to maturity. However, the timing of calcineurin activity that is responsible for this outcome, as suggested by the study by Tendron et al., is intriguing. It is possible that the postnatal program is dependent on activation of a component downstream of calcineurin. In addition, lacking the earlier, upstream signal, the downstream factor fails to enter the genetic stream. Again, altered regulation of p27 provides a possible mechanism for this phenomenon. Developmentally, p27 expression is normally slightly increased during final postnatal maturation of glomeruli and tubules, presumably to slow the cell cycle and bring about the final differentiated state. In animals with genetic loss of p27, organ growth including that of the kidney far exceeds normal parameters (30), demonstrating that a crucial regulator of differentiation and organ size has been removed. Conversely, in α-null kidneys, p27 is overexpressed and is associated with a smaller kidney and the disruption of normal maturation (15). Thus it seems likely that calcineurin is an upstream regulator of p27 expression in the kidney, and the timing of this action may be important for critical developmental regulation via cell cycle control (Fig. 4).

CONCLUSIONS

It is interesting to note that several clinical features of calcineurin inhibitor nephrotoxicity, i.e., elevated serum creatinine, increased matrix deposition, and increased cell death, are present in mice lacking the α-isoform but not the β. The implication seems to be that selective inhibition of the α-isoform may be functionally analogous in the kidney to calcineurin inhibition with pharmacological agents such as cyclosporin. However, other features including mesangial cell survival and changes in p27 expression suggest additional mechanisms that are also altered and that may not be readily apparent in a clinical setting but could significantly alter long-term renal function.

Also of note is the finding that many features of calcineurin inhibitor nephrotoxicity, i.e., ECM accumulation, TGF-β production, increased apoptosis, and elevated serum creatinine levels, are also present in disease states such as diabetic nephropathy. While we found increased levels of calcineurin in diabetic rat kidneys, levels of ECM accumulation, TGF-β message, and cell death were further elevated by inhibition of calcineurin in the renal cortex (10). Thus it is likely that calcineurin inhibition, perhaps by mechanisms including alteration of the cell cycle, could act in an additive manner in the presence of an additional nephotoxic process.

In conclusion, there is growing evidence detailing the importance of calcineurin as a developmental component in the kidney. Data suggest that the α-isoform has a particular role in postnatal nephrogenesis. Similarly, genetic disruption of calcineurin Aα recapitulates many features of calcineurin inhibitor toxicity and highlights additional possible consequences of loss of calcineurin function in the kidney. How α- and β-isoforms are preferentially regulated (or inhibited?) remains an important area for future investigation as does the mechanism of specific action that distinguishes the two very similar enzymes. Insights into these issues are certain to be significant for renal development and may offer an opportunity to improve the therapeutic efficacy of calcineurin inhibitors.

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