Cardiac hypertrophy is defined as an increase in heart size resulting from an increase in cardiomyocyte cell volume. The hypertrophic growth of the myocardium is initiated by a wide array of endocrine, paracrine, and autocrine growth factors in response to increased workload, injury, or intrinsic defects in contractile performance. Although it is initially an adaptive response that temporarily augments or maintains cardiac output, sustained cardiac hypertrophy is a leading cause of the development of heart failure and sudden death in humans. To begin to understand the molecular mechanisms that underlie cardiac hypertrophy, an increasing number of signal transduction pathways have been identified as important regulators of the hypertrophic response, including the low–molecular weight GTPases (Ras, RhoA, and Rac), mitogen-activated protein kinases, protein kinase C, and calcineurin. This review will discuss an emerging body of evidence that implicates the calcium-calmodulin–activated protein phosphatase calcineurin as a physiological regulator of the cardiac hypertrophic response. Although the sufficiency of calcineurin to promote cardiomyocyte hypertrophy in vivo and in vitro is established, its overall necessity as a hypertrophic mediator is currently an area of ongoing debate. The use of the calcineurin-inhibitory agents cyclosporine A and FK506 have suggested a necessary role for calcineurin in many, but not all, animal models of hypertrophy or cardiomyopathy. The evidence implicating a role for calcineurin signaling in the heart will be weighed against a growing body of literature suggesting necessary roles for a diverse array of intracellular signaling pathways, highlighting the multifactorial nature of the hypertrophic program. (Circ Res. 2000;87:731–738.)

Key Words calcineurin ■ cardiac hypertrophy ■ transcription ■ heart failure ■ signaling

Cardiac hypertrophy can lead to decompensation and cardiomyopathy. Recent studies have focused on characterizing the molecular mechanisms that underlie cardiac hypertrophy. An increasing number of signal transduction pathways have been identified as important regulators of the hypertrophic response, including the low–molecular weight GTPases (Ras, RhoA, and Rac), mitogen-activated protein kinases, protein kinase C, and calcineurin. This review will discuss an emerging body of evidence that implicates the calcium-calmodulin–activated protein phosphatase calcineurin as a physiological regulator of the cardiac hypertrophic response. Although the sufficiency of calcineurin to promote cardiomyocyte hypertrophy in vivo and in vitro is established, its overall necessity as a hypertrophic mediator is currently an area of ongoing debate. The use of the calcineurin-inhibitory agents cyclosporine A and FK506 have suggested a necessary role for calcineurin in many, but not all, animal models of hypertrophy or cardiomyopathy. The evidence implicating a role for calcineurin signaling in the heart will be weighed against a growing body of literature suggesting necessary roles for a diverse array of intracellular signaling pathways, highlighting the multifactorial nature of the hypertrophic program. (Circ Res. 2000;87:731–738.)

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Calcineurin and Beyond
Cardiac Hypertrophic Signaling

Jeffery D. Molkentin

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motes T-cell activation and cytokine induction has been largely attributed to the family of transcriptional regulators referred to as nuclear factor of activated T cells (NFAT). Calcineurin directly binds to NFAT transcription factors in the cytoplasm, resulting in their dephosphorylation and subsequent translocation into the nucleus (Figure 2). Five NFAT transcription factors have been identified, of which NFATc1 through NFATc4 are regulated by calcineurin-mediated dephosphorylation, whereas NFATc5 is constitutively nuclear and not subject to calcineurin regulation.11,12

**Calcium and Calcineurin Signaling in the Heart**

Although calcium is the fundamental regulator of actin-myosin crossbridge interaction and contraction, it has also been implicated as an inducer of cardiac hypertrophy in response to neurohumoral stimulation, stretch, and pacing.13–16 Many studies have identified alterations in calcium handling in the failed myocardium such that the amplitude of the intracellular calcium transient is decreased and prolonged.17 Such studies have suggested the hypothesis that alterations in intracellular calcium handling progressively exacerbate a hypertrophic or cardiomyopathic phenotype, in part, through sustained activation of calcium-sensitive signal transduction pathways. The recent identification of calcineurin as a potential hypertrophic regulatory factor supports such a hypothesis.

**Sufficiency of Calcineurin and NFAT to Induce Cardiac Hypertrophy**

Calcineurin was originally proposed as a hypertrophic signaling factor based on the identification of NFATc4 as a GATA4 interacting factor in the heart.18 To characterize the potential involvement of calcineurin in cardiac hypertrophy, an activated truncation mutant of calcineurin was overexpressed in the hearts of transgenic mice.18 Eleven separate transgenic lines were generated that each demonstrated a profound

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**Figure 1.** Amino acid sequence comparison of the 3 human calcineurin catalytic subunit genes (CnAα, CnAβ, and CnAγ). The blue, green, red, and yellow shaded boxes highlight the catalytic, calcineurin B subunit binding, calmodulin binding, and autoinhibitory domains, respectively.

**Figure 2.** The mechanism of calcineurin-NFAT signaling was first established in T cells. Activation of the T-cell receptor (TCR) promotes increased [Ca2+]i, which saturates calmodulin, resulting in calcineurin activation and subsequent dephosphorylation of NFAT transcription factors. Dephosphorylated NFAT translocates to the nucleus, where it interacts with the promoters of cytokine genes. Immunosuppressive drugs CsA and FK506 complex with immunophilin proteins, which subsequently bind and inhibit calcineurin. T-cell activation also utilizes MAPK (JNK) activation to promote transcriptional activation through activator protein-1 (AP-1), which synergizes with NFAT factors to promote inducible gene expression.
hypertrophic response (2- to 3-fold increase in heart size) that rapidly progressed to dilated heart failure within 2 to 3 months (Figure 3). Such data implicated calcineurin as a sufficient inducer of the hypertrophic response and as a potential causative factor associated with the transition to decompensation and heart failure. In vitro, infection of cultured neonatal cardiomyocytes with a calcineurin-expressing adenovirus also induced a hypertrophic response, supporting the sufficiency of calcineurin as a hypertrophic mediator.19

That NFAT transcription factors act downstream of calcineurin in the heart was suggested by the observation that transgenic mice expressing a constitutively nuclear NFATc4 protein (N-terminal truncation) also developed profound hypertrophy within 2 to 3 months of age.18 However, overexpression of full-length (cytoplasmic) NFATc4 did not produce detectable hypertrophy, suggesting a critical role for calcineurin-mediated activation of NFAT proteins in the heart.18 Although these studies have demonstrated a sufficiency for calcineurin and NFAT transcription factors as mediators of the cardiac hypertrophic response, a number of questions remain. The requirement of calcineurin and NFAT as endogenous regulators of physiological stress responses in the heart remains largely unproven and an active area of investigation. In addition, the degree to which NFAT factors are required to mediate the hypertrophic effects of calcineurin in the heart remains unresolved (see Role of NFAT Transcription Factors in the Heart, below).

Calcineurin Activation in Cardiac Hypertrophy

Treatment of cultured neonatal cardiomyocytes with the calcineurin-inhibitory agent CsA was reported to attenuate agonist-induced hypertrophy in vitro.18 This initial observation suggested that calcineurin is likely activated in cultured cardiomyocytes in response to agonist stimulation. Accordingly, agonist stimulation (phenylephrine, angiotensin II, and 1% FBS) significantly increased calcineurin enzymatic activity in cultured cardiomyocytes, which was associated with an increase in both calcineurin Aβ mRNA and protein levels.10 The observed increase in calcineurin Aβ protein in association with myocyte hypertrophy suggests a secondary mechanism (apart from calcium) whereby calcineurin activity can be regulated in the heart. Endothelin-1–stimulated hypertrophy of cultured cardiomyocytes also induced a significant (3-fold) increase in calcineurin activity, although protein levels were not examined.20 In addition, electrical pacing-induced hypertrophy of cultured cardiomyocytes was inhibited by CsA, further implicating calcineurin as a regulator of cardiomyocyte hypertrophy in vitro.31 Collectively, these studies demonstrated that calcineurin enzymatic activity was upregulated in cultured cardiomyocytes in association with the hypertrophic response.

Activation of calcineurin in response to pathophysiological stress in vivo remains more controversial. Calcineurin enzymatic activity was upregulated by ≈2-fold in hearts from juvenile tropomodulin transgenic mice, a model of dilated heart failure.22 This increase was later shown to be associated with a 3-fold increase in total calcineurin A protein content in the heart.23 Similarly, pressure-overload hypertrophy in aortic-banded rats and exercise-induced cardiac hypertrophy in the rat were each associated with increased calcineurin enzymatic activity in the heart.24–27 In contrast, one group reported no change in cardiac calcineurin activity in response to pressure-overload stimulation,28 whereas another group reported decreases in calcineurin activity.29

The disparate reports discussed above suggest that either differences exist between experimental animal models with respect to calcineurin activation or, more likely, quantification of calcineurin phosphatase activity from tissue or cell lysates is problematic. Experimentally, calcineurin enzymatic activity is determined by monitoring the release of free phosphate (radioactively or chemically) from a phosphorylated substrate such as the RII peptide from whole-cell or tissue protein extracts. Because multiple phosphatases are present in cell extracts, parallel reactions are required in which calcineurin activity is specifically inhibited with the autoinhibitory peptide domain. The resultant activity is then calculated as the difference between the blocked and unblocked states.24 A mixture of phosphatase inhibitors is required to reduce background phosphatase activity and enhance sensitivity, a necessary strategy that also has the effect of partially inhibiting calcineurin activity (okadaic acid). An additional consideration relates to the lability of calcineurin due to oxidation of the Fe-Zn active center, which underscores the observation that calcineurin is 10 to 20 times more active in situ compared with purified protein extracts.30

Despite technical concerns surrounding the calcineurin activity assay, a more important consideration is the relevant information that such an assay gives and its inherent limitations. Even if the in vitro assay is properly performed, it is only an indirect measure of activity and, as such, is inadequate for assessing calcineurin activity in vivo. The cal-
CsA and FK506 each induced cardiomyocyte hypertrophy in vitro,\textsuperscript{18–21} its effectiveness in vivo is problematic. CsA and FK506 each prevented the phenotypic manifestations of hypertrophic and dilated cardiomyopathy in 3 separate transgenic mouse models of intrinsic heart disease.\textsuperscript{22} In the same report, CsA and dilated cardiomyopathy in 3 separate transgenic mouse prevented the phenotypic manifestations of hypertrophic tiveness in vivo is problematic. CsA and FK506 each induced cardiomyocyte hypertrophy in vitro,\textsuperscript{18–21} its effec-

Whereas most pharmacologic studies support a role for calcineurin in the hypertrophic response, the negative accounts may reflect factors such as drug dosage, differences in the surgical preparations, sex, age, or animal strain. For example, the drug dosages that have been used to date vary between 5 and 40 mg/kg of CsA injected either once or twice a day for periods of time between 6 days and 5 weeks.

Although technical details might certainly underlie some of the conflicting data discussed above, an alternative consideration is the degree to which specific animal models require/utilize a calcineurin-dependent signaling

### Use of CsA and FK506 as Calcineurin-Inhibitory Agents in the Heart

Although CsA has been reported to attenuate agonist-induced cardiomyocyte hypertrophy in vitro,\textsuperscript{18–21} its effectiveness in vivo is problematic. CsA and FK506 each prevented the phenotypic manifestations of hypertrophic and dilated cardiomyopathy in 3 separate transgenic mouse models of intrinsic heart disease.\textsuperscript{22} In the same report, CsA administration to aortic-banded rats over 6 days prevented the induction of cardiac hypertrophy (Table).\textsuperscript{22} Although these results suggested new theoretical strategies for treating certain forms of human heart disease, enthusiasm was tempered by the known side effects of CsA and FK506 in humans (see Clinical Implications section, below). Furthermore, 4 subsequent studies concluded that calcineurin inhibitors did not significantly block pressure-overload hypertrophy in either aortic-banded mice or rats, suggesting that CsA and FK506 might not be effective antihypertrophic agents (Table).\textsuperscript{29–32} However, 6 reports demonstrated a partial or complete inhibition of load-induced cardiac hypertrophy with CsA or FK506 in aortic-banded rats or mice (Table).\textsuperscript{28,29,31,32} In addition, CsA prevented exercise-induced cardiac hypertrophy in the rat,\textsuperscript{26} attenuated hypertrophy and histopathology in renin and angiotensin transgenic rats,\textsuperscript{35} attenuated myocardial infarction–induced cardiac hypertrophy in the rat,\textsuperscript{36} and reduced cardiac hypertrophy in activated Gqoq transgenic mice.\textsuperscript{37} In contrast, CsA did not prevent cardiac hypertrophy due to hypertension in the spontaneously hypertensive rat.\textsuperscript{28}

### Summary of Animal Models of Cardiac Hypertrophy Treated With CsA and FK506

<table>
<thead>
<tr>
<th>Study</th>
<th>Model</th>
<th>Drug (Dosage, Times per Day)</th>
<th>% Increase</th>
<th>% Increase + Drug</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luo et al\textsuperscript{31}</td>
<td>Rat abdominal AC</td>
<td>CsA* ?</td>
<td>42</td>
<td>27</td>
<td>No</td>
</tr>
<tr>
<td>Muller et al\textsuperscript{32}</td>
<td>Mouse transverse AC</td>
<td>CsA 25 mg/kg, 2</td>
<td>...</td>
<td>...</td>
<td>No</td>
</tr>
<tr>
<td>Ding et al\textsuperscript{29}</td>
<td>Mouse ascending AC</td>
<td>CsA 25 mg/kg, 2</td>
<td>53</td>
<td>52</td>
<td>No</td>
</tr>
<tr>
<td>Zhang et al\textsuperscript{28}</td>
<td>Rat abdominal AC</td>
<td>CsA 20 mg/kg, 1</td>
<td>47</td>
<td>21†</td>
<td>No‡</td>
</tr>
<tr>
<td>Zhang et al\textsuperscript{28}</td>
<td>SHR + high salt</td>
<td>CsA 5 mg/kg, 1</td>
<td>...</td>
<td>...</td>
<td>No</td>
</tr>
<tr>
<td>Sussman et al\textsuperscript{22}</td>
<td>TG mice with HCM</td>
<td>CsA 15 mg/kg, 2</td>
<td>120</td>
<td>0</td>
<td>Yes</td>
</tr>
<tr>
<td>Sussman et al\textsuperscript{22}</td>
<td>Rat abdominal AC</td>
<td>CsA 20 mg/kg, 1</td>
<td>27</td>
<td>0</td>
<td>Yes</td>
</tr>
<tr>
<td>Shimoyama et al\textsuperscript{35}</td>
<td>Rat abdominal AC</td>
<td>FK506 1 mg/kg, 1</td>
<td>~33</td>
<td>0</td>
<td>Yes</td>
</tr>
<tr>
<td>Lim et al\textsuperscript{34}</td>
<td>Rat abdominal AC</td>
<td>CsA 10 mg/kg, 2</td>
<td>34</td>
<td>6</td>
<td>Yes</td>
</tr>
<tr>
<td>Hill et al\textsuperscript{34}</td>
<td>Mouse transverse AC</td>
<td>CsA 25 mg/kg, 2</td>
<td>45§</td>
<td>0§</td>
<td>Yes</td>
</tr>
<tr>
<td>Meguro et al\textsuperscript{33}</td>
<td>Mouse transverse AC</td>
<td>CsA 25 mg/kg, 1</td>
<td>44</td>
<td>33</td>
<td>Yes</td>
</tr>
<tr>
<td>Et0 et al\textsuperscript{26}</td>
<td>Rat transverse AC</td>
<td>CsA 40 mg/kg, 1</td>
<td>33</td>
<td>18</td>
<td>Yes</td>
</tr>
<tr>
<td>Mervaala et al\textsuperscript{36}</td>
<td>Ang II/renin TG rat</td>
<td>CsA 5 mg/kg, 1</td>
<td>~45</td>
<td>~19</td>
<td>Yes</td>
</tr>
<tr>
<td>Qie et al\textsuperscript{37}</td>
<td>Rat infarction/failure</td>
<td>CsA 50 mg/kg</td>
<td>...</td>
<td>...</td>
<td>Yes§</td>
</tr>
<tr>
<td>Mende et al\textsuperscript{37}</td>
<td>Gyq TG mice</td>
<td>CsA 15 mg/kg, 2</td>
<td>...</td>
<td>...</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The percentage increase that is shown represents heart weight normalized to body weight with or without the indicated calcineurin inhibitory drug. ‘Significant’ refers to the statistical analysis of the data presented in each study. TG indicates transgenic; AC, aortic constriction; Ang II, angiotensin II; SHR, spontaneously hypertensive rat; and HCM, hypertrophic cardiomyopathy.

*CsA given at 40 mg/kg in the drinking water; all other studies performed with subcutaneous injection once or twice each day.
†Values given for 4-week study at 20 mg/kg, although rats treated with CsA at 10 mg/kg showed a 27% increase.
‡Heart weight-to-body weight ratios were back normalized to blood pressure gradient. Because CsA-treated rats showed reduced blood pressure, the decrease in heart weight-to-body weight ratio was not deemed significant.
§Mice were analyzed at 5 successive weeks; each time point demonstrated a block in hypertrophy with CsA, although only the 5-week time point is shown.
||Measurement of heart weight-to-body weight ratio was not significantly different, but heart weight normalized to tibial length was significantly reduced by CsA.

### Calculation of Calcineurin Phosphatase Assay

The simple Western blot for calcineurin A protein might suffice. Where calcineurin phosphatase assay measures peak activity in the presence of saturating levels of calmodulin and hence probably only reflects the content of calcineurin available for activation. Given these concerns, it can be argued that a simple Western blot for calcineurin A protein might suffice. Indeed, we have observed that increased enzymatic activity in the heart is often associated with a secondary increase in calcineurin A protein levels.\textsuperscript{22–24}
pathway or other hypertrophic signaling pathways. For example, 2 transgenic mouse models of hypertrophic cardiomyopathy have been reported that are resistant to CsA treatment. Transgenic mice overexpressing a mutated retinoid X receptor-α or constitutively nuclear NFATc4 in the heart develop cardiac hypertrophy that is insensitive to calcineurin inhibition.22,23 In addition, CsA did not block hypertrophy in ascending aortic-banded juvenile mice, a model of gradual onset pressure overload during postnatal development.29 Taken together, these observations underscore the multifactorial nature of the cardiac hypertrophic response and suggest calcineurin-independent pathways for regulating myocyte reactivity.

Targeted Inhibition of Calcineurin Attenuates Cardiomyocyte Hypertrophy

Another aspect of controversy surrounding CsA and FK506 studies in animal models of hypertrophy pertains to drug specificity. CsA and FK506 each affect multiple intracellular targets besides calcineurin, suggesting alternative mechanisms whereby such drugs might attenuate the hypertrophic response.38–40 To address the issue of specificity, an alternate approach using the noncompetitive calcineurin-inhibitory domains from the calcineurin-interacting proteins Cain/Cabin-1 and A kinaseanchoring protein 79 (AKAP79) were used.41–43 Adenovirus expressing the calcineurin-inhibitory domains of Cain or AKAP inhibited calcineurin activity and attenuated phenylephrine- and angiotensin II–induced hypertrophy in cultured cardiomyocytes.10 The inhibition of hypertrophy by Cain and AKAP adenoviral infection was similar to the inhibition observed with CsA and FK506, suggesting calcineurin as the determinative factor.10 Calcineurin activity is also negatively regulated by the inhibitory proteins MCIP1 and MCIP2 (DSCR1 and ZAKI-4), which are each highly expressed in the heart and skeletal muscle.44,45 It will be interesting to generate and characterize transgenic mice expressing these various calcineurin-inhibitory proteins in the heart to more specifically evaluate the importance of calcineurin as a regulator of cardiac hypertrophy in vivo. Alternatively, targeted disruption of the calcineurin Aα and/or Aβ genes might also provide important genetic evidence confirming or disputing calcineurin as a necessary regulator of cardiac hypertrophy in vivo. Indeed, both calcineurin Aα and Aβ knockout mice are viable, which should permit a final, definitive analysis of the role of calcineurin in the hypertrophic response in the near future. However, because the viability of double-null calcineurin Aα and Aβ mice is uncertain, transgenic dominant-negative–based approaches might still be of significant value.

Conserved Role of Calcineurin and NFAT in Skeletal Muscle Hypertrophy

A number of recent reports have implicated calcineurin and NFAT in the differentiation and hypertrophy of skeletal muscle, suggesting a conservation in the regulatory program that controls striated muscle cell growth. Specifically, calcineurin promoted skeletal muscle myoblast hypertrophy downstream of an IGF-1–dependent signaling pathway.46,47 CsA treatment also blocked the growth response of cultured human myoblasts, attenuated muscle regeneration in response to acute injury in vivo,48 and prevented skeletal muscle hypertrophy in response to muscle overloading.49 Adenovirus-mediated gene transfer of activated calcineurin in cultured C2C12 and Sol8 myoblasts potentiated their growth, whereas inhibition of calcineurin with Cain or AKAP inhibitory domains attenuated myocyte growth.50 NFATc1, NFATc2, and NFATc3 have all been implicated as downstream effectors of calcineurin in the regulation of myoblast differentiation or subsequent hypertrophy in cultured skeletal muscle cells.47,48,50 Collectively, these studies suggest a conservation in the regulatory program that controls striated muscle cell hypertrophy through a calcineurin- and NFAT-dependent pathway.

Role of NFAT Transcription Factors in the Heart

Whereas NFAT transcription factors are important downstream effectors of calcineurin in T cells, neurons, and skeletal muscle myoblasts, we must also consider the alternative hypothesis that calcineurin regulates the cardiac hypertrophic response independently of NFAT factors. Indeed, calcineurin also regulates activity of the transcriptional regulatory factors nuclear factor-κB, Elk-1, and myocyte enhancer factor-2 (MEF-2).40,51–55 That MEF-2 might be an important hypertrophic transcription factor was recently suggested by the observation that transgenic mice expressing a dominant-negative MEF-2 factor in the heart presented with diminished developmental hypertrophic growth.56 As a final consideration, it has been difficult to directly demonstrate endogenous NFAT nuclear translocation in response to hypertrophic agonists in cardiac myocytes. Such a description in the literature may be lacking for technical reasons related to low NFAT protein concentration and/or the lack of reliable antibodies. To ultimately resolve the contribution of NFAT transcription factors as downstream mediators of calcineurin in the heart, it will be necessary to evaluate the ability of NFAT knockout mice to mount a hypertrophic response.

However, analysis of mRNA distribution in mammals has demonstrated that all 5 NFAT genes are expressed in the heart, suggesting that gene-targeting approaches in the mouse will be complicated by redundancy issues.12,57–59 Despite this concern, single or combinatorial knockout strategies might still implicate NFAT factors as calcineurin effectors in the heart. Accordingly, NFATc4- and NFATc3-null mice are viable and should permit such an evaluation in the near future. Alternatively, NFAT-specific dominant-negative approaches in transgenic mice might also provide significant insights, similar to strategies used in T cells.60

Calcineurin may also regulate the cardiac hypertrophic response in coordination with other intracellular signal transduction pathways. Indeed, calcineurin promotes c-Jun N-terminal kinase (JNK), extracellular signal–regulated kinase (ERK), and protein kinase C (PKC) α and θ activation in cardiac myocytes.61 This observation is consistent with reports in T cells in which calcineurin interconnects (cross talks) with PKC and MAPK signaling pathways in the regulation of cytokine gene expression.62,63 However, the mechanisms whereby a phosphatase (calcineurin) might pro-
mote the activation of kinases such as JNK and PKC in either T cells or cardiac myocytes is uncertain. A working hypothesis is that calcineurin initiates a primary transcriptional response through NFAT, Elk-1, nuclear factor-κB, or MEF-2 factors to set in motion autocrine regulatory mechanisms (angiotensin II or endothelin-1 release) that promote re-entrant signaling through G protein–coupled receptors and receptor tyrosine kinases leading to the secondary activation of PKC and MAPK factors. However, we cannot rule out the possibility that calcineurin might regulate one or more cytoplasmic regulatory factors to indirectly promote PKC and MAPK activation. For example, calcineurin, PKC, and PKA all share a common cytoplasmic docking protein, AKAP79, which suggests a mechanism whereby multiple signaling pathways are coordinately regulated by localization to common cofactors.64

**Beyond Calcineurin: Integrated Signaling Networks**

In T cells, calcineurin functions in concert with MAPK and PKC signaling pathways to regulate cytokine gene expression and ultimately the immune response itself. Accordingly, gene knockout approaches in the mouse have demonstrated that calcineurin Aα, PKCθ, and JNK are each required for productive T-cell reactivity in vivo, suggesting that multiple intracellular signaling pathways are necessary for orchestrating the immune response.65–67 This paradigm likely extends to cardiac myocytes given the emerging literature that demonstrates a necessary role for diverse intracellular regulatory factors in the hypertrophic response. For example, transgenic mice expressing a dominant-negative Go protein in the heart failed to undergo hypertrophy in response to pressure overload.68 Similarly, expression of RGS4, a GTPase-activating protein, in the hearts of transgenic mice attenuated pressure-overload hypertrophy, supporting a critical role for G proteins as transducers of hypertrophic stimuli.69 Adenovirus-mediated gene transfer of a dominant-negative SEK1 factor (MAPK-kinase-4) blocked the hypertrophic response of aortic-banded rats, suggesting a critical role for MAPK factors in vivo.70 Cardiac-restricted deletion of the gp130 receptor in mice profoundly affected the pressure-overload response, implicating an important role for gp130 in cardiac homeostasis.71 Lastly, fibroblast growth factor 2 knockout mice and AT2 knockout mice each demonstrated a dramatic reduction in cardiac hypertrophy induced by acute aortic banding.72,73 In vitro, several studies have demonstrated necessary roles for signaling factors such as Ras, Raf-1, rac-1, p38, MAP/ERK kinase 1, focal adhesion kinase, and calcineurin in mediating aspects of cardiomyocyte hypertrophy.74–81

The above-mentioned studies underscore the multifactorial nature of the cardiac hypertrophic response and suggest a great deal of molecular heterogeneity in the process referred to as cardiac hypertrophy. These studies suggest a model of reactive signaling in the heart whereby certain central pathways are absolutely necessary for the initiation and maintenance of a balanced hypertrophy response, similar to the complexity of signaling involved in T-cell reactivity and cytokine production. In addition, many central regulatory pathways likely interconnect or cross talk with one another in the orchestration of the hypertrophic response. For example, pharmacological inhibition of calcineurin is associated with inhibition of PKCα, PKCθ, and JNK p54 in the heart.82 Such cross talk might occur through direct molecular interconnections or indirectly through autocrine release of peptide growth factors leading to re-entrant signaling at the cell membrane.

**Clinical Implications**

Although calcineurin-inhibitory drugs can attenuate cardiac hypertrophy in some rodent models of pressure overload, their usefulness in humans is doubtful. Both CsA and FK506 have a number of side effects in humans, including nephrogenic and neurogenic toxicity as well as immunosuppression.83 Indeed, chronic CsA therapy in human transplant patients induces renal toxicity leading to hypertension and secondary cardiac hypertrophy.84 This observation suggests that CsA is actually associated with cardiac hypertrophy in humans (albeit secondary) when given at immunosuppressive doses. To inhibit cardiac hypertrophy in animals, a 10-fold higher dose of CsA is used than is commonly administered in human immunosuppressive regimens.84 Such dosing could be related to higher calcineurin protein content in cardiomyocytes relative to T and B cells or to differences in tissue accessibility. In any event, because a large number of humans develop renal failure and hypertension on the lower dosage, CsA is effectively eliminated as a treatment for cardiac hypertrophy in humans. It is possible, however, that future cardiac-specific methods of inhibiting calcineurin activity will be developed and of potential benefit.

**Acknowledgments**

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