Despite successful new treatment strategies developed in the past few decades aimed at different pathomechanisms of myocardial disease,1 morbidity and mortality due to heart failure and its complications remain a clinical reality. Among the treatment strategies available, the maintenance of contractile mass that is determined by the number of myocytes and the degree of cellular hypertrophy is a major goal in the therapy of cardiac disease. However, increasing contractile mass by myocardial hypertrophy is associated with increased risks of vulnerability to ischemia, arrhythmias, and the transition to heart failure. Recent evidence that apoptosis of cardiac myocytes is a feature in several myocardial disease states, including ischemic heart disease and congestive heart failure (for review, see Reference 2), has raised hopes that inhibition of myocyte apoptosis could prevent or slow the loss of contractile cells and thus provide a new target in a multimodal therapeutic approach to cardiac disease.

**Targets for Intervention**

In apoptotic cell death, in contrast to necrotic (oncotic) cell death, a proapoptotic stimulus initiates a cell-autonomous cascade of events that finally leads to the activation of common apoptotic effector mechanisms that seem essentially uniform in most cell lineages and involve the activation of caspases, an apoptosis-specific endonuclease, and the apoptosis-inducing factor (Figure 1).2-5 Regulation of the apoptotic effector machinery is complex and involves regulatory proteins that specifically regulate apoptosis (eg, B-cell lymphoma [Bcl]-2 family proteins or inhibitor of apoptosis proteins [IAPs]) in addition to signaling pathways that mediate several other cellular responses (eg, extracellular signal–regulated kinase [ERK], stress-activated protein kinase, PI-3 kinase). Prevention of apoptotic myocyte loss may be directed at (1) the inciting proapoptotic stimulus, (2) regulatory mechanisms of apoptosis, and (3) the effector pathways of apoptosis. At any level, strategies may be targeted at inhibiting proapoptotic mechanisms or promoting cellular survival strategies (Figure 2).

**Targeting the Proapoptotic Stimulus Acting on the Myocyte**

Current therapy directed at reducing myocardial ischemia, hypertrophy due to increased afterload, and myocardial remodeling already might include an antiapoptotic effect, as these conditions are known to induce myocyte apoptosis, as evidenced by experimental and pathologic studies.6-11 The use of β-blocking agents to treat hypertension, ischemic heart disease, and chronic heart failure may suppress the apoptotic effect of excess catecholamines. In rabbit hearts, treatment with carvedilol reduced myocyte apoptosis in response to ischemia/reperfusion.12 Likewise, angiotensin-converting enzyme inhibitors might inhibit angiotensin II–mediated myocyte apoptosis,13 although studies in human and animal heart disease are missing. Other strategies directed at reducing myocyte stretch, oxidative stress during reperfusion, or improvement of myocardial perfusion by growth factor–induced angiogenesis may all reduce myocyte apoptosis by minimizing proapoptotic stimulation of myocytes.14-17 In addition, inhibition of death receptor stimulation by scavenging ligands with soluble Fas and tumor necrosis factor (TNF) receptors or by synthetic receptor antagonists constitutes another potential approach. However, although Fas and TNF receptor 1 are expressed on cardiac myocytes, the role of death receptor stimulation in myocyte apoptosis and in clinical cardiac disease needs further evaluation.6,12,18-21

**Promotion of Antiapoptotic Signaling**

Apoptosis is tightly regulated by several mechanisms to prevent inadvertent cell loss. Among these mechanisms are the antiapoptotic members of the Bcl-2 protein family (eg, Bcl-2 or Bcl-xL), which inhibit the release of proapoptotic factors from mitochondria, and apoptosis repressor with a caspase recruitment domain, Fas-associated death domain protein (FADD)–like antiapoptotic molecule (FLAME-1), and the decoy receptors (eg, TNF-related apoptosis–inducing ligand [TRAIL] receptor without an intracellular domain [TRID]), which interfere with proapoptotic signaling through death receptors.22-25 However, at present there is no effective methodology other than gene overexpression available to augment the efficacy of these regulatory mechanisms. In addition, it is not known to which degree augmentation of these antiapoptotic pathways could prevent myocyte apoptosis in clinical cardiac disease, as the role of myocardium-mediated versus death receptor–mediated induction of apoptosis is not yet well delineated.
Earlier studies in cultured cells of the neuronal and hematopoietic lineage indicated that growth factors provide a survival signal that renders cells resistant to apoptotic cell death. Recent observations support a role for growth factors also in the survival of cardiac myocytes. Among these factors are the insulin-like growth factor-1, cardiotrophin-1, and the neuregulins, which were shown to reduce myocyte apoptosis after ischemia, serum withdrawal, myocyte stretch, and treatment with the cardiotoxic chemotherapeutic drug doxorubicin.26–31 In addition, activation of the glycoprotein 130 subunit of the leukemia inhibitory factor/cardiotrophin-1 receptor confers protection against cardiac dilation induced by aortic banding in mice.32 In this study, inhibition of cardiac dilation correlated with a decrease in myocyte apoptosis. Intracellular signaling of the survival factors includes the ERK and phosphatidylinositol-3-kinase/Akt pathways,30,33 although the role of these pathways in myocyte protection still needs to be further substantiated. Initial attempts to treat chronic heart failure with recombinant human growth hormone, which increases serum concentrations of insulin-like growth factor-1, have so far created ambiguous results with regard to clinical outcome.34,35 Unfortunately, the effect of growth hormone treatment on myocyte apoptosis was not studied, and it is not known whether the treatment protocol affected apoptotic myocyte loss. Nevertheless, growth factor treatment deserves further studies, as different survival factors may differ in their protective potential. Also, cell surface receptors are more easily amenable to pharmacologic therapy.

Targeting Proapoptotic Signaling
Apoptosis mediated either by mitochondria or death receptors involves proapoptotic signaling pathways or apoptosis-specific regulatory proteins. Best known are the proapoptotic members of the Bcl-2 protein family such as Bax, Bad, and Bid, which induce a transition of mitochondria to a proapoptotic state, and the adaptor protein FADD, which is involved in the recruitment of upstream caspases in response to death receptor stimulation.22,36 Although currently not available, it may be possible to develop synthetic drugs that are membrane permeable and interfere with the proapoptotic activity of these proteins. In addition, pharmacologic inhibition of the permeability transition pore may represent another target for antiapoptotic treatment.37,38 Furthermore, experimental studies in isolated cardiac myocytes and transgenic mice have provided evidence for a role of “classical” intracellular signaling pathways in promoting apoptosis. Stimulation of the MKK3/p38α pathway caused increased myocyte death.39,40 Likewise, overexpression of Gsα or Gqα induced myocyte apoptosis in transgenic mice and cultured cardiac myocytes, respectively.41,42 However, the pleiotropic effect of these signaling proteins in both cardiac myocytes and other cell lineages may limit an approach that targets these signaling pathways due to an increased risk of side effects.

Targeting the Downstream Execution Phase of Apoptosis
In most cellular models, apoptosis relies on the activation of downstream executioner caspases (caspase-3, -6, or -7) that cleave a plethora of cellular target proteins.3,43 Inhibition of downstream caspases constitutes a cellular regulatory mechanism exerted by IAPs (eg, IAP-1, IAP-2, or XIAP).44 In addition, potent pharmacologic agents have been developed that efficiently inhibit caspase activity (eg, benzoylvaline-alanine-aspartate-fluoromethylketone). We currently do not know how to augment the endogenous antiapoptotic activity mediated by the IAPs. On the other hand, treatment with synthetic caspase inhibitors was shown to be effective in a model of short-term myocardial ischemia and reperfusion.45 In addition, some of these agents permeate the cell membrane and might therefore permit systemic administration of the inhibitor. However, caspase activation may not be effective in inhibiting apoptosis mediated by apoptosis-inducing factor (AIF).5

Unresolved Questions
Although several lines of evidence suggest that antiapoptotic treatment might become a new therapeutic option, there are still several major open issues that need to be addressed.

What Are the Potentials of an Antiapoptotic Treatment Strategy?
The benefit that can be gained from antiapoptotic treatment of cardiac disease is clearly related to the extent and pathophys-
The biologic significance of myocyte apoptosis in different disease states. Obviously, as cardiac myocytes have no or at most a very limited proliferative capacity, it would be desirable to prevent myocyte apoptosis in any cardiac disease with detectable myocyte apoptosis. Unfortunately, at present the potentials of an antiapoptotic approach in cardiac disease in terms of amount of myocardium that can be salvaged and the impact on cardiac function and survival are largely unknown. In heart failure, for example, pathophysiology is multifactorial, and impaired excitation-contraction coupling, alterations in intracellular filaments, interstitial fibrosis, unfavorable chamber geometry, and neurohumoral deregulation have all been implicated in the disease process. The relative importance of these factors with regard to initiation and progression of heart failure is still a matter of debate. Interestingly, direct ultrastructural evidence (eg, by microscopy) for myocyte loss in nonischemic heart failure has long been scant. With the advent of terminal deoxynucleotidyl transferase deoxy-UTP nick-end labeling (TUNEL) staining in heart failure specimens, surprisingly high levels of apoptotic myocyte loss have been reported. Extrapolation of apoptotic rates based on TUNEL staining indicates that in some cases published figures certainly overestimate the true incidence of apoptotic myocyte loss. Additional research is necessary to estimate the maximal treatment benefit that can be achieved in heart failure. In addition, it remains to be determined whether inhibition of myocyte apoptosis will prevent the initiation of clinical cardiac disease or merely reduce disease progression.

Discrepancies may at least partly be due to current methodological shortcomings. For example, different protocols for tissue pretreatment before addition of the TUNEL reagents are in use by different laboratories. Direct comparison of different pretreatment protocols in myocardial tissue can result in >10-fold different TUNEL positive rates (unpublished observation, 1999). In addition, despite initial hopes it has become increasingly evident that positive TUNEL staining is not specific for apoptotic cell loss, but may also reflect other physiological processes. In a recent study of ischemia and reperfusion, myocytes staining positive for TUNEL in light and electron microscopy actually did not show ultrastructural features typical for apoptotic cell death, but they showed alterations suggestive for necrotic cell deterioration. It is not known whether dying cells that exhibit necrotic morphology with positive staining for TUNEL will be amenable to therapeutic interventions targeted at apoptosis regulation. In addition, positive staining for TUNEL has been associated with DNA repair in myocardial specimens from patients with dilated cardiomyopathy, thus calling the specificity of TUNEL staining for apoptosis in cardiac myocytes further into question. Assessment of myocyte apoptosis is further complicated, as myocardium contains a large number of nonmyocyte cells that, although adding comparatively little to myocardial mass, contribute significantly (up to 50%) to the number of nuclei in myocardium. Indeed, cell type-specific analysis showed that nonmyocyte cell populations can significantly contribute to the total number of apoptotic cells in the ischemic myocardium. As has recently been discussed by Soonpaa and Field, attribution of a positively stained nucleus to a cell lineage may not always be reliable by light microscopic methodology, on which, so far, most observations have been based.

**Will Myocyte Survival Translate Into Improved Myocyte Function?**

Another concern relates to the fact that prevention of myocyte apoptosis might not necessarily be equivalent with full functional recovery. In fact, stimulation of the death receptor Fas on cardiac myocytes reduced the membrane potential and
induced afterdepolarizations, indicating a potentially increased propensity for proarrhythmia of myocytes damaged by, but surviving stimulation of the proapoptotic Fas receptor protein.\textsuperscript{52} Likewise, the immediate benefits of rescuing myocytes from TNF-induced apoptotic cell death may be mitigated by the negative inotropic effect of TNF that seems to be independent from its proapoptotic activity.\textsuperscript{19–21} In addition, the effect of inhibition of apoptosis might only be short-lived, as only the mode of cell death (apoptotic versus nonapoptotic) might be altered, whereas eventual cell demise might not be prevented. Given these considerations, only little evidence has so far been provided to support the notion that treatment specifically targeted at apoptosis affects outcome in myocardial disease. In a short-term model of myocardial ischemia (30 minutes) and reperfusion (24 hours), Yaoita et al\textsuperscript{45} showed that caspase inhibition led to a decrease in infarct size from 67\% to 52\% of the myocardial area at risk. Myocardial salvage was associated with an improvement in ventricular contractility and left ventricular end diastolic pressures. However, it is not known whether this beneficial effect is persistent. Definitely, further interventional studies are required that allow for a better estimation of the therapeutic potentials of an antiapoptotic approach in myocardial disease.

Is Antiapoptotic Treatment for Heart Disease Safe?

Whereas apoptosis of cardiac myocytes in the adult myocardium is considered to have a detrimental effect, apoptosis of other cell lineages is physiologic, such as the elimination of autoreactive lymphocytes in the thymus or the deterioration and shedding of epithelial cells in the intestine and the skin. In addition, deficiency of the proapoptotic regulator Bax affects male fertility, suggesting an important role for apoptosis in spermatogenesis and male fertility.\textsuperscript{53} The potential implications of inhibiting physiological apoptosis is exemplified by the history of the antiapoptotic regulatory protein Bcl-2 (B-cell lymphoma 2), which was initially characterized as an oncogene in human B-cell lymphomas. Only later it became obvious that Bcl-2 represents a mammalian homologue of the antiapoptotic regulator \textit{ced-9} of \textit{Caenorhabditis elegans} and primarily inhibits cell death instead of promoting cellular replication.\textsuperscript{54,55} Furthermore, animal studies and the analysis of Fas mutations in the hereditary Canale-Smith syndrome (autoimmune lymphoproliferative syndrome, type Ia) showed that inhibition of Fas signaling may be associated with lymphadenopathy, neoplastic disease, and autoimmune disease such as hemolytic anemia and thrombocytopenia.\textsuperscript{56–59} Inhibition of apoptosis may also bear a considerable risk of teratogenicity. Loss of function of caspase-3, caspase-9, apaf-1, and Bcl-x\textsubscript{L} causes embryonic lethality in mice associated with severe brain malformation.\textsuperscript{60–64} Likewise, mice deficient in FADD and caspase-8 die in utero because of impaired myocardial development and cardiac dilation.\textsuperscript{65,66} These observations raise the possibility that drugs directed at apoptotic mechanisms may be associated with significant side effects limiting their clinical use.

Given these considerations, is the prevention of myocyte apoptosis still a sound and viable goal? To answer this question, disease dynamics and timing need to be taken into account. On one hand, in chronic congestive heart failure, slowly progressive aortic stenosis and chronic valvular regurgitation, the apoptotic stimulus is present more or less continuously, probably requiring permanent antiapoptotic treatment to reduce myocyte loss. On the other hand, in acute cardiac events such as myocardial infarction, unstable angina, or reperfusion injury, apoptosis seems to occur in a limited time window. In the latter situation, short-term treatment targeted at common downstream events of apoptosis might be an effective approach and promalignancy and autoimmune side effects will be of minor concern, whereas salvage of a substantial number of cardiac myocytes is possible.\textsuperscript{45} In addition, interference with the apoptotic cascade may offer the unique opportunity to prevent cell death after the lethal stimulus has reached the cell, thus increasing the time window for effective treatment such as the restoration of myocardial blood flow after myocardial infarction. In this situation, inhibition of downstream caspases despite their widespread distribution might be an attractive molecular target. In contrast, chronic treatment will clearly entail the risks indicated by the physiological role of apoptosis in different tissues and by phenotype analysis of mice deficient in apoptosis-associated genes. To circumvent this problem, it would be of importance to define targets that are unique to the regulation of apoptosis in cardiac myocytes. Potential candidates will include upstream regulatory steps during the initiation of myocyte apoptosis. However, upstream regulation of myocyte apoptosis is not yet well characterized and may include signaling cascades that exert pleiotropic effects, once again entailing the risks of multiple side effects. Defining the target for chronic treatment of myocyte apoptosis will therefore become a more challenging approach.

Conclusion

Prevention of myocyte apoptosis has emerged as a potential new treatment approach for cardiac disease. Although the molecular biology of apoptosis in general and myocyte apoptosis in particular allow for the definition of potential antiapoptotic targets, there are still several open questions that need to be addressed. Among the most important issues is to determine the degree to which apoptosis contributes to the initiation and progression of heart disease. As outlined above, methodological considerations limit current assessment of potential therapeutic benefits of an antiapoptotic treatment strategy. Ways to clarify this issue in the future will be to pursue an interventional antiapoptotic rather than a descriptive experimental approach in animal models of cardiac disease. Descriptive studies aimed at evaluating the extent of myocyte apoptosis in myocyte apoptosis should extend beyond TUNEL staining and provide additional evidence for myocyte apoptosis on the basis of apoptotic ultrastructure (eg, electron microscopy), evidence for the activation of apoptotic mechanisms (eg, cytochrome c release or caspase activation), or downstream events of apoptosis (eg, DNA laddering on agarose gels or evidence for phosphatidylinerse exposure by annexin V staining). Another issue that needs to be addressed is related to the fact that proapoptotic stimuli may not only impair myocyte survival, but also affect myocyte function (eg, TNF-\(\alpha\)), thus reducing the treatment
benefit of a strategy merely aimed at myocyte survival. In addition, safety and side effects of chronic antiapoptotic treatment remain a major concern. The identification of apoptotic regulatory pathways that are specific for cardiac myocytes or the better characterization of the time course of myocyte apoptosis in cardiac disease might reduce or even prevent the risks of antiapoptotic treatment. Keeping the concerns associated with chronic inhibition of apoptosis in mind, an antiapoptotic approach for cardiac disease still figures among the most attractive future therapeutic options for cardiac disease.

Acknowledgment
A.H. was supported by the Deutsche Forschungsgemeinschaft (Grant Ha 2606/1-1) and S.I. by the NIH (Grant AG17008).

References
Toward Antiapoptosis as a New Treatment Modality
Armin Haunstetter and Seigo Izumo

Circ Res. 2000;86:371-376
doi: 10.1161/01.RES.86.4.371

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/86/4/371

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/