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Abstract—Caspase-1/interleukin-converting enzyme (ICE) is a cysteine protease traditionally considered to have importance as an inflammatory mediator, but not as an apoptotic effector. Because of the dual functions of this caspase, the pathophysiological impact of its reported upregulation in hypertrophy and heart failure is not known. Here, the consequences of increased myocardial expression of procaspase-1 were examined on the normal and ischemically injured heart. In unstressed mouse hearts with a 30-fold increase in procaspase-1 content, unprocessed procaspase-1 was well tolerated, without detectable pathology. Cardiomyocyte processing and activation of caspase-1 and caspase-3 occurred after administration of endotoxin or with transient myocardial ischemia. In post-ischemic hearts, procaspase-1 overexpression was associated with strikingly increased cardiac myocyte apoptosis in the peri- and noninfarct regions and with 50% larger myocardial infarctions. Tissue culture studies revealed that procaspase-1 processing/activation is stimulated by hypoxia, and that caspase-1 acts in synergy with hypoxia to stimulate caspase-3 mediated apoptosis without activating upstream caspases. These data demonstrate that the proapoptotic effects of caspase-1 can significantly impact the myocardial response to ischemia and suggest that conditions in which procaspase-1 in the heart is increased may predispose to apoptotic myocardial injury under conditions of physiological stress. **(*Circ Res*. 2005;96:1103-1109.)**

Key Words: apoptosis ■ myocardial infarction ■ myocardial ischemia ■ caspase

Increased cardiomyocyte apoptosis in human and experimental cardiomyopathies supports a role for programmed cardiomyocyte death in ischemic and nonischemic heart disease.[1–5] Antiapoptotic therapeutic strategies have been designed that target the evolutionarily conserved caspase (calcium-activated aspartate protease) apoptosis effector pathway.[6,7] Eleven human caspases homologous to the original nematode caspase, CED-3, have been identified to date.[8] Although all caspases share the ability to cleave substrate proteins at specific aspartate residues, members of the caspase family are divided into 2 subgroups with distinct substrate preferences and biological functions; the cpp-32/caspase-3 group (caspases 2, 3, 6, 7, 8, 9, and 10) primarily involved in apoptosis, and the ICE (interleukin-converting enzyme)/caspase-1 group (caspases 1, 4, 5, and 11) responsible for cytokine maturation.[8–10]

Caspase-1/ICE was the first mammalian caspase to be described.[11] It is generally felt that the most relevant actions of capsase-1 are proinflammatory, being related to cytokine processing.[10–12] This conclusion is based largely on the phenotype obtained with caspase-1 gene ablation: caspase-$1^{-/-}$ mice had no mature interleukin-1β (IL-1β) or IL-18 and were defective in producing IL-1α, IL-6, and tumor necrosis factor-α (TNF-α) in response to lipopolysaccharide.[13,14] Notably, apoptotic processes appeared normal in the caspase-$1^{-/-}$ mice.[10] On the other hand, an absolute requirement for caspase-1 was elucidated for Fas-mediated apoptosis signaling in thymocytes,[15] and caspase-1 transfection was sufficient to produce apoptosis in some cultured cell systems.[16] Taken together, the data suggest that there is an essential cytokine processing function for caspase-1, but that there are also situational or opportunistic effects on apoptotic cell death. The relative impact of these 2 functions in the heart is unknown.

Both caspase-1 and caspase-3, ie, the prototypical proinflammatory and proapoptotic caspases, are upregulated in diseased hearts.[17–21] However, data supporting a pathophysiological effect of increased myocardial caspase expression have been inconsistent. Whereas one study of pharmacological caspase-3 inhibition reported decreased myocardial apoptosis and diminished infarct size,[7] the antithetic experiment of forced myocardial caspase-3 expression increased myocardial infarct size without clearly stimulating cardiomyocyte apoptosis.[22] Assigning a meaningful role for upregulated

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From the Department of Medicine (F.M.S., H.S.H., A.O., R.A.L., G.W.D), University of Cincinnati, Cincinnati, Ohio; the Winters Center for Heart Failure Research (D.L.M., J.G.V.), Baylor College of Medicine, Houston, Tex; and the Institute of Molecular Cardiology (R.B., Y.G.), University of Louisville, Louisville, Ken.

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Correspondence to G.W. Dorn II, University of Cincinnati Medical Center, 231 Albert B. Sabin Way, Cincinnati, OH 45267-0542. E-mail dorngw@ucmail.uc.edu

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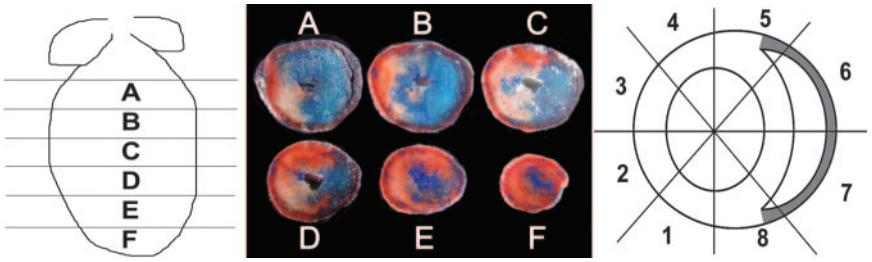

Figure 1. Regional assessments relative to infarction in mouse hearts. Left, Schematic depicting transverse sectioning of hearts. Middle, Corresponding sections of TTC-stained, phthalo blue perfused heart 24 hours after transient left anterior descending coronary artery occlusion. Right, Schematic diagram of radially divided heart section. Gray area (right ventricular free wall) was excluded from analysis.

caspase-1 in cardiomyocyte apoptosis is also controversial because its dominant function in the heart is assumed to be proinflammatory rather than proapoptotic.[12] Indeed, it has even been reported that caspase-1 is not processed to an active form in the heart.[23]

In this study, we used forced myocardial expression of inactive procaspase-1 in normal and ischemic hearts to address the following questions: (1) Can caspase-1 be activated in cardiac myocytes? (2) Does activation of myocardial caspase-1 cause cardiomyocyte apoptosis, and with what pathological effects? And (3) what other caspases are involved in caspase-1 apoptosis signaling?

Materials and Methods

In Vitro Studies of Caspase-1

The murine procaspase-1 cDNA (GenBank accession no. BC008152) cloned into pcDNA3 (Stratagene) was expressed in HEK293 cells after transfection with Lipofectamine (Gibco/BRL). Standard culture conditions were Dulbecco Minimum Essential Medium (DMEM) containing 4.5 mmol/L glucose plus 1% antibiotic/antimycotic solution (Gibco/BRL) and 10% fetal bovine serum in an atmosphere of 5% CO_2, 95% air. For hypoxia studies, standard medium was exchanged for DMEM with 1 mmol/L glucose, and cells were maintained in a hypoxic chamber gassed with 5% CO_2, 95% N_2. Oxygen content, measured continuously by O_2 probe, was always less than 1%. For serum deprivation studies, cells were washed twice and the standard culture medium was exchanged for DMEM without added serum.

Generation of Procaspase-1 Transgenic Mice

Transgenic mice (FVB/N background) were generated using the α-myosin heavy chain (MHC) promoter to express the murine procaspase-1 cDNA (Harlan, Indianapolis, IN). Five founders were identified by genomic Southern analysis of tail clip DNA. Animals were treated in accordance with approved University of Cincinnati IACUC protocols.

Immunoblot Analysis

Clarified myocardial homogenates (10 000*g* supernatant) were size-separated on 10% or 8% to 16% continuous gradient SDS-PAGE gels, transferred to polyvinylidene membranes, blocked with 5% dry milk, and incubated with primary and secondary antibodies. Anticaspase-1, p10 (M20) from Santa Cruz Biotechnology Laboratories (1:200) was used to detect pro- and processed caspase-1. The anti- processed caspase-3 was from Cell Signaling Laboratories and antibodies recognizing caspases-8 and -9 were from Santa Cruz.

Caspase-1 and -3 Enzyme Assays

Caspase-1 and -3 enzymatic activities were measured using a fluorometric assay from Promega (CaspACE Assay System). Myocardial homogenates from control and endotoxin-treated mice, prepared as described earlier, were incubated at 37°C for 2 hours with a fluorogenic caspase substrate in the presence and absence of capsase-1 and -3 family inhibitors YVAD and DEVD, respectively. Next, 460 nm emission fluorescence (excitation with 360 nm) was quantified with a Microplate Fluorescence Reader from BioTek Instruments Inc, and related to a standard activity curve.

Functional Assessments

Two-dimensional guided M-mode echocardiography of unsedated mice measured left ventricular (LV) diastolic and systolic dimensions (LVEDD and LVESD), from which fractional shortening (FS) was derived. Pulsed wave Doppler was used to measure aortic ejection time (ET) and calculate velocity of circumferential shortening, Vcf (FS/ET). Invasive hemodynamic studies were performed on anesthetized, spontaneously breathing 8- to 12-week-old transgenic mice and their nontransgenic littermate controls.[24] Global ischemia/reperfusion studies of isolated Langendorff-perfused hearts were performed as described.[25]

In Vivo Ischemia/Reperfusion and Infarct Quantitation

These methods have been previously described.[26] Briefly, mouse hearts were exposed via a left thoracotomy, the proximal left anterior descending coronary artery reversibly occluded for 30 minutes, and then reperfused for 24 hours. This brief period of ischemia resulted in smaller infarcts, such that infarct enlargement in procaspase-1–expressing mice was detectable (data not shown). Infarct size was quantified by postmortem staining with triphenyltetrazolium chloride (TTC) and phthalo blue dye.

Cytokine Measurements

Hearts subject to the Langendorff global ischemia/reperfusion protocol[25] were flash frozen in liquid nitrogen and assayed for cytokine mRNA expression by ribonuclease protection assay as previously described.[27]

Histopathology and TUNEL Studies

Histopathological examination was performed on Masson trichrome-stained sections. Apoptosis in formalin-fixed, paraffin-embedded sections of ischemic/reperfused hearts and HEK 293 cells was measured using Promega's DeadEnd Fluorometric TUNEL System or the TACS 2TdT DAB kit from Trevigen. TUNEL-positive nuclei per HPF (40×) were counted in infarct, peri-infarct, and noninfarct zones with propidium iodide staining to label all nuclei. Regional correlation of TUNEL and caspase-3 positivity and infarction location was performed in 1-mm thick transverse cardiac sections divided into 8 radial segments (Figure 1). Infarct zones were those segments that contained white tissue after TTC and pthalo blue staining, peri-infarct zones were those segments immediately adjacent, and noninfarct zones were the remaining segments. Processed caspase-3 was visualized using Cell Signaling Technologies Signal Stain Cleaved Caspase-3 (Asp 175) IHC Detection Kit

Statistical Analysis

Results are mean±SE. Experimental groups were compared using Student *t* test or one-way ANOVA. A Bonferroni test was used for post hoc comparisons, with $P<0.05$ significant.

Results

Procaspase-1 Expression and Activation in Mouse Hearts

Caspase-1 has been reported to be upregulated in myocardial hypertrophy and heart failure.[17,19,21,28] To examine the con-

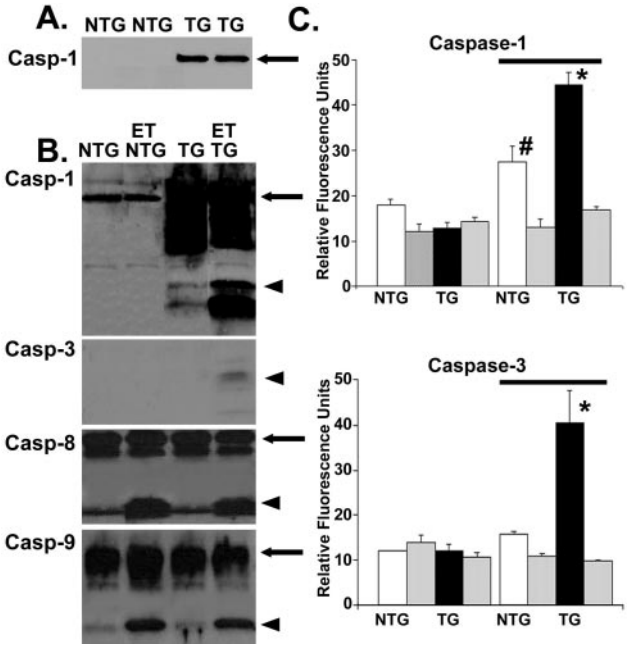

Figure 2. Myocardial procaspase-1 expression and caspase processing. A, Immunoblot analysis of transgene expression in nontransgenic (NTG) and procaspase-1 transgenic (TG) mouse hearts. B, Immunoblot analysis of endotoxin (ET)-treated hearts showing processing of caspase-1 (top, overexposed) and caspases3, 8, and 9. Arrow indicates proenzyme; arrowhead, active fragment. C, Fluorogenic assay of caspase-1 (top) and caspase-3 (bottom) proteolytic activity in hearts, with (bar) or without endotoxin treatment. Gray bars to right of NTG and TG are values in presence of respective caspase inhibitor, ie, background. n=3 or 4 per group. *$P<0.05$ vs TG without ET; #$P<0.05$ vs NTG without ET.

Baseline Characteristics of Caspase-1 Transgenic Mouse Hearts

	NTG	Caspase-1
Echo (n=16, 19)		
LVEDD	3.2±0.1	3.2±0.1
LVESD	1.62±0.07	1.6±0.06
% FS	49.6±1.2	47.5±1
Heart rate, bpm	734±8	744±5
Vcf	12.9±0.4	12.3±0.3
Langendorff (n=6, 7)		
Heart rate	254±31	303±57
+dP/dt	4074±106	4033±294
−dP/dt	−2811±122	−2713±275
CATH* (n=4, 3)		
Heart rate	476±16	476±48
+dP/dt	7585±241	7268±63
−dP/dt	−8774±641	−9151±384
Morphometry (n=16, 19)		
Body weight, g	25.4±1.3	26.7±2.6
Heart weight, mg	162±30	157±50
Heart/body weight ratio	6±0.4	5.7±0.34

There were no significant differences in any measured parameter. *CATH indicates invasive cardiac catheterization, performed according to previously published methods.[25]

sequences of increased myocardial caspase-1 content, forced expression of the native gene product, ie, the inactive proform of the enzyme, was used. Of 5 founders, 2 higher copy number lines were bred and found to be grossly normal in terms of longevity, health, and cardiac structure and function for 1 year. Procaspase-1 was expressed in hearts of both transgenic lines at ≈30 times normal endogenous levels (Figure 2A and 2B). Cardiac size and contractile function, assessed using echocardiographic, isolated perfused heart, invasive catheterization, and morphometric techniques, were normal (Table). Expression of "fetal cardiac genes," which are highly sensitive markers of cardiac hypertrophy and failure,[29] was also normal (supplemental Figure I, available in the online data supplement at http://circres.ahajournals.org).

Absence of cardiac pathology in mice with cardiomyocyte-specific overexpression of the inactive caspase-1 proenzyme was not surprising as pathological effects are expected only after proteolytic processing and activation. Indeed, although caspase-1–like activity has been reported in myocardium,[30] the preponderance of evidence was that cardiac myocytes did not process procaspase-1 to the active caspase.[18,23,31] We considered that the abundance of procaspase-1 in transgenic mouse hearts permitted a more robust read-out of caspase-1 proteolysis and examined caspase-1 processing after systemic administration of endotoxin, which is a potent stimulus for processing of myocardial caspases.[18] Mice received *E. coli* lipopolysaccharide (Sigma, *E. coli* serotype 055:B5, 5 mg/kg) or vehicle intraperitoneally and were followed for 24 hours. At this endotoxin dose, there was no mortality. As reported,[32] hemodynamic function initially declined, but returned to normal after 24 hours (not shown). A subset of hearts was assayed for procaspase-1 processing by immunoblot analysis after 6 hours, with intentional overexposure to detect proteolytic fragments. As shown in Figure 2B, processed caspase-1 was not detectable in nontransgenic hearts, with or without endotoxin, and was present only minimally in procaspase-1 overexpressors at baseline. In contrast, the processed p10 caspase-1 fragment was abundant in endotoxin-treated caspase-1–overexpressing hearts. Associated increases in myocardial caspase-1 proteolytic activity were observed by enzymatic assay (Figure 2C, top). Importantly, caspase-3 enzymatic activity and immunoreactive active 17-kDa caspase-3 were also increased in endotoxin-treated procaspase-1 overexpressors (Figure 2B and 2C), suggesting that there could be caspase-1–mediated processing of caspase-3.[15,33,34] However, associated processing of the proximal death receptor pathway caspase-8 and the mitochondrial pathway caspase-9 in endotoxin-treated nontransgenic hearts (Figure 2B) indicated that widespread endotoxin-mediated activation of the proximal caspase cascade occurred, independent of caspase-1 overexpression. Thus, cardiac myocytes processed procaspase-1 to active caspase-1, but it was not possible to determine whether caspase-1 directly activated caspase-3, or indirectly activated caspase-3 via upstream caspases of the death receptor and mitochondrial pathways. More definitive studies were therefore performed using tissue culture systems.

In Vitro Studies of Caspase-1–Mediated Apoptosis in Hypoxia and Serum Deprivation

Early studies of caspase-1/ICE utilized pharmacological inhibitors and activity assays to suggest that caspase-1 caused apoptosis by activating caspase-3 because caspase-1 was not sufficient to induce apoptosis in the absence of caspase-3.[15,34] However, direct proteolytic processing of caspase-3 by caspase-1 has not been demonstrated in intact cells. Accordingly, the murine procaspase-1 cDNA was expressed in cultured cells and the effects of hypoxia or serum withdrawal were examined on caspase processing and TUNEL labeling. As initial studies using neonatal rat cardiomyocytes were confounded by substantial apoptosis independent of procaspase-1 expression after serum-deprivation or hypoxia[35,36] (data not shown), more apoptosis-resistant HEK293 cells were used.

Transfection of procaspase-1 by itself was not sufficient to cause apoptosis of HEK293 cells (Figure 3A and 3B). Likewise, neither hypoxia nor serum withdrawal for 24 hours significantly increased apoptosis, measured as percentage TUNEL positivity (Figure 3A and 3B). However, the combination of procaspase-1 transfection and hypoxia or serum deprivation strikingly increased apoptosis compared with hypoxia or serum deprivation alone. Thus, expression of otherwise innocuous procaspase-1 synergized with physiologic stress to cause apoptosis.

In the in vivo procaspase-1 overexpressing mouse heart exposed to endotoxin, we observed processing of caspases 1, 3, 8, and 9. However, processing of upstream caspases 8 and 9 was also seen in endotoxin-treated nontransgenic hearts (Figure 2B), indicating that widespread activation of the myocardial caspase cascade occurs in response to endotoxin. We therefore used immunoblot and immunofluorescence analyses to examine proteolytic processing of caspase cascade components in the HEK293 hypoxia model. As expected, procaspase-1 was processed to caspase-1 after hypoxia, as was caspase-3 (Figure 3C and 3D). In contrast, there was no increase in proteolytic cleavage of caspases 8 or 9 in procaspase-1–transfected cells (Figure 3C). These results indicate that activation of upstream caspases in the death receptor or mitochondrial pathways is not essential in HEK 293 cells for caspase-1–mediated activation of caspase-3 and are consistent with direct caspase-3 activation by caspase-1.

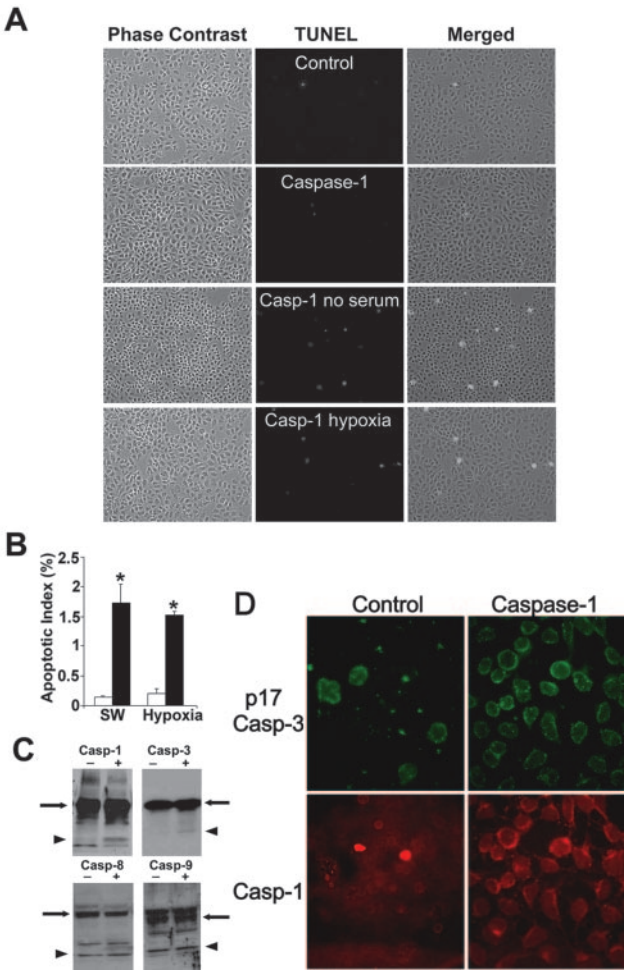

Figure 3. Caspase crosstalk and apoptotic synergy with stress in cultured cells. A, TUNEL staining of HEK293 cells as a function of procaspase-1 transfection and serum deprivation or hypoxia, as labeled. B, Quantitative TUNEL results of nontransfected cells (white bars) or procaspase-1 transfected cells (black bars) 24 hours after withdrawal (SW) or hypoxia. *$P<0.05$ vs transfected controls. C, Immunoblot analysis of caspase processing in absence (−) or presence (+) of hypoxic stress. Arrows indicate proenzymes, arrowheads indicated active proteolytic fragments. D, Immunohistochemical analysis of caspase-3 activation (green) as a function of procaspase-1 expression (red) in hypoxic HEK293 cells. All fields were 70% confluent and gain for anticaspase-1 in nontransfected cells (bottom left) was increased to see background. In nontransfected cells, cells with caspase-3 activation were comparatively rare (top left).

In Vivo Proapoptotic Effect of Caspase-1 After Myocardial Infarction

We considered that proapoptotic synergy such as was observed between caspase-1 and hypoxia in cultured HEK293 cells could alter the myocardial response to ischemia/reperfusion in procaspase-1 overexpressors. To test this notion, procaspase-1 overexpressing and nontransgenic mice underwent reversible left anterior descending coronary artery occlusion. Twenty-four hours later they were assessed for infarct size, regional myocardial apoptosis, and caspase-1 and -3 processing. Periprocedural mortality was the same for both groups (4.7% in procaspase-1 and 5.3% in nontransgenic mice). Likewise, the area at risk for infarction (Figure 4B, right panel), myocardial work (heart rate×developed pressure), and cardiomyocyte sensitivity to ischemia (measured as immediate CPK release) were all similar between procaspase-1 transgenic and nontransgenic controls (online Figure 2). Thus, conventional determinants of infarction size were not affected by procaspase-1 overexpression. However, at 24 hours, procaspase-1 transgenic hearts developed ≈50% larger infarcts than their nontransgenic littermates (Figure 4A), measured either as a percentage of total left ventricular area or as a percentage of area at risk (Figure 4B). As the immediate myocardial ischemic damage (CPK release) was identical between nontransgenic and procaspase-1

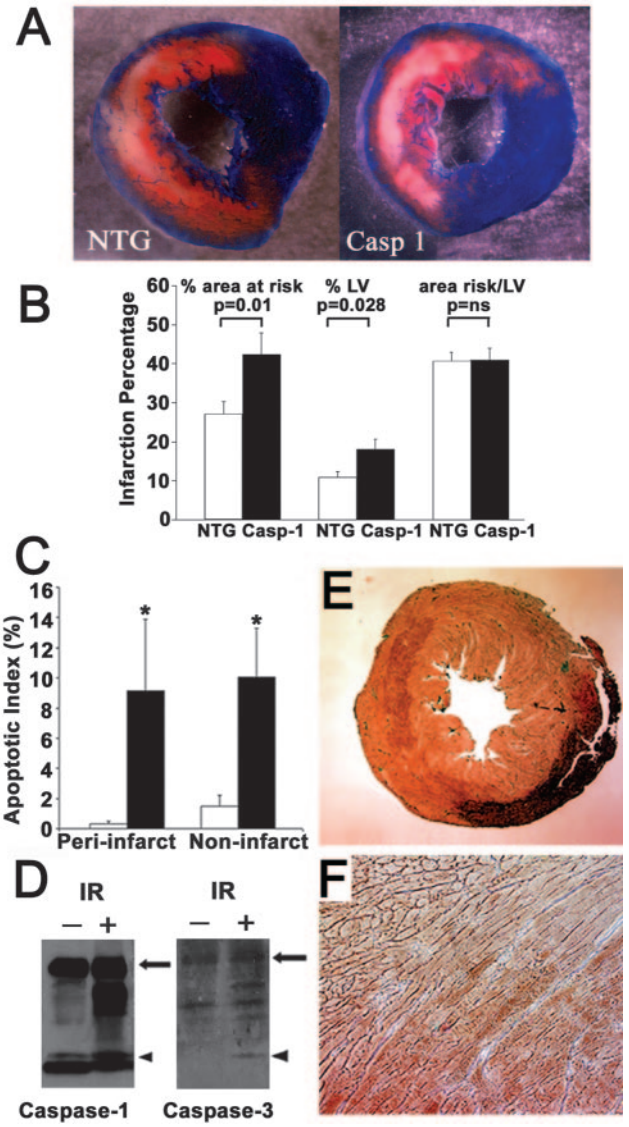

Figure 4. Infarction size in procaspase-1 transgenic hearts. A, Representative hearts showing increased infarct size (white) compared with area at risk (red) in procaspase-1 overexpressors, relative to nontransgenic littermate controls. B, Quantitative analysis of pooled infarct data. Casp-1, n=9; NTG, n=8. Apoptosis in infarcted procaspase-1 overexpressors. C, Quantitative analysis of TUNEL results in 24-hour ischemia/reperfused mouse hearts. White is nontransgenic; black is procaspase-1 transgenic. n=10 each. *$P<0.05$ vs nontransgenic. D, Caspase-1 and -3 activation 24 hours after ischemia/reperfusion. Arrow indicates procaspase; arrowhead, processed fragment. E and F, Caspase-3 activation (red staining) in infarcted procaspase-1 transgenic heart. E=10× magnification, F=200× magnification at margin of ischemic zone.

overexpressing mice, larger infarcts at 24 hours suggested involvement of a secondary process, such as programmed cell death. Indeed, associated with larger infarcts was a striking increase in apoptotic index in the peri-infarct and noninfarct regions of the heart (Figure 4C) and processing of caspases-1 and -3 (Figure 4D). Immunohistochemical studies localized the highest levels of activated caspase-3 to the peri-infarct segments (Figure 4C, 4E, and 4F). As previously reported, TUNEL positivity was low in frankly necrotic myocardium.[7,37,38] Collectively, these results show that increased cardiomyocyte procaspase-1 expression exaggerates myocardial injury after reversible ischemia through caspase-1 cross-talk with caspase-3, resulting in cardiomyocyte apoptosis and infarct enlargement. It is formally possible that other caspases were also involved in this activation sequence, although this was not suggested by our in vitro experiments.

Discussion

Caspase-1, originally called ICE, was the first mammalian analogue of the *Caenorhabditis elegans* death genes to be identified.[11] Like all caspases, it is expressed as a proform, which is activated through proteolytic cleavage of an amino-terminal 11-kDa prodomain to release p20 and p10 subunits. Active caspase-1/ICE consists of a (p20/p10)2 tetramer, which is sufficient to process precursor IL-1β[9,12–14] and, in at least some cell types, to induce apoptosis.[16] Additionally, the prodomain has been postulated to have independent proapoptotic activity by enhancing death receptor–mediated caspase-8 activation.[39] Although caspase-1 has conventionally been regarded as a proinflammatory, not a proapoptotic, caspase,[10–12] it has been observed to induce or amplify apoptosis in tissue culture models.[15,16]

Regulated expression of caspase-1 in cardiac hypertrophy,[17] the dilated cardiomyopathy of TNF-α overexpression,[19] ischemia/infarction,[21] and endotoxin-induced myocardial dysfunction[18] prompted our evaluation of the consequences of its upregulation and activation in myocardium. Whereas a 30-fold increase in myocardial content of the inactive proform of caspase-1 resulted in no detectable pathology, cardiomyocyte processing of procaspase-1 to its proteolytically active p10 form under conditions of endotoxic or hypoxic stress permitted caspase-1 to act synergistically with these physiological stressors and induce apoptosis via crosstalk with caspase-3. In the case of regional cardiac ischemia, the consequence of increased apoptosis in procaspase-1 overexpressors was an ≈50% increase in myocardial infarct area. Importantly, because the procaspase-1 transgene had no detectable consequences on the unstressed heart, its pathology appears to be entirely opportunistic; the proform accumulates innocuously and waits for an activating stimulus after which it stimulates apoptosis. These findings reinforce the notion that otherwise silent abnormalities of inactive proenzymes in the heart can have pathogenic significance under defined conditions.[40,41]

Our data support the notion that signaling cross-talk can occur between proinflammatory ICE subgroup and proapoptotic CPP-32 subgroup caspases, thereby stimulating or facilitating cardiac apoptosis.[16,33] To our knowledge, the only prior evidence that caspase-1 can directly contribute to myocardial injury consists of a single study demonstrating infarct size reduction with pharmacological caspase-1 inhibition, but the mechanism was not well defined.[42] In another study, rescue of postischemic dysfunction of human atrial tissue by pharmacological caspase-1 inhibition was attributed to inhibition of IL-18 and IL-1β

formation, rather than an effect on apoptosis.[12] Our results using the procaspase-1 overexpressor are also consistent with an effect of myocardial caspase-1 on cytokine formation (online Figure 3), but suggest that the greatest impact of myocardial caspase-1 activity is infarct extension resulting from amplification of postischemic apoptosis signaling.

It is instructive to compare cardiac-specific overexpression of procaspases-1 and -3. Cardiac function of both procaspase overexpressors was normal at 12 weeks, both procaspases could be processed to their active forms in cardiac myocytes, and both caspase overexpressors developed comparable (≈50%) increases in infarct size, compared with nontransgenic controls.[22] However, an intriguing difference between the caspase-1 and -3 models is the uncertain role for apoptosis in the caspase-3 phenotype. Although nuclear ultrastructure of caspase-3 mice was abnormal, conventional measures of apoptosis were negative. In contrast, in the current study, cardiomyocyte apoptosis measured as TUNEL positivity and caspase-3 activation was increased in both the peri-infarct and remote myocardium of procaspase-1 overexpressors, likely reflecting both the local and global stresses of transient coronary occlusion. The link between caspase-1–induced caspase-3 activation, infarct extension, and increased apoptotic cardiomyocyte burden strongly implicates myocardial apoptosis in the deleterious effects of procaspase-1 overexpression. Together, the phenotypes of the procaspase-1 and caspase-3 overexpression models indicate that high-level myocardial expression of the precursors for both caspase-1 and caspase-3 is well tolerated in the absence of physiological stress, but that activation of these caspases, such as by ischemia/reperfusion, potentiates myocardial injury.

Finally, the current results provide an antithetic model to pharmacological caspase inhibition in support of a critical role for cardiomyocyte apoptosis in myocardial infarct expansion. Previously, inhibition of caspase-3 in rat hearts with ZVAD-fmk reduced infarct size and numbers of apoptotic cardiomyocytes in the peri-infarct region, showing that apoptosis contributes to "normal" infarct size.[7] In this study, amplification of apoptosis with procaspase-1 overexpression was sufficient to increase infarct size. Collectively, these data suggest that directly or indirectly targeting caspase-mediated apoptosis in the ischemic heart is likely to be therapeutically beneficial.

Acknowledgments

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