Bcl-2 overexpression corrects mitochondrial defects and ameliorates inherited desmin null cardiomyopathy

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One of the hallmarks of cardiomyopathy and heart failure is pronounced and progressive cardiomyocyte death. Understanding the mechanisms involved in cardiomyocyte cell death is a topic of great interest for treatment of cardiac disease. Mice null for desmin, the muscle-specific member of the intermediate filament gene family, develop cardiomyopathy characterized by extensive cardiomyocyte death, fibrosis, calcification, and eventual heart failure. The earliest ultrastructural defects are observed in mitochondria. In the present study, we have demonstrated that these mitochondrial abnormalities are the primary cause of the observed cardiomyopathy and that these defects can be ameliorated by overexpression of bcl-2 in desmin null heart. Overexpression of bcl-2 in the desmin null heart results in correction of mitochondrial defects, reduced occurrence of fibrotic lesions in the myocardium, prevention of cardiac hypertrophy, restoration of cardiomyocyte ultrastructure, and significant improvement of cardiac function. Furthermore, we have found that loss of desmin also diminishes the capacity of mitochondria to resist exposure to calcium, a defect that can be partially restored by bcl-2 overexpression. These results point to a unique function for desmin in protection of mitochondria from calcium exposure that can be partially rescued by overexpression of bcl-2. We show that bcl-2 cardiac overexpression has provided significant improvement of an inherited form of cardiomyopathy, revealing the potential for *bcl-2*, and perhaps other genes in the family, as therapeutic agents for heart disease of many types, including inherited forms.

Cardiomyocyte death commonly contributes to development of many cardiac disease states, including cardiomyopathy and heart failure. Recent efforts toward treatment of cardiac disease have focused on prevention of cell death (1). The antiapoptotic *bcl-2* gene (2) is of particular interest for protection against cardiomyocyte death. Although bcl-2 is expressed at low levels in cardiomyocytes, this potent modulator of cell death is a promising therapeutic agent for heart disease, because it has been shown to reduce cell death during ischemia (3, 4), block p53-mediated apoptosis in cardiomyocytes (5), and increase the ability of mitochondria to resist high levels of calcium (6). Members of the bcl-2 family are thought to function at the contact sites (CS) of the mitochondrial membrane (7–9) and influence the membrane potential ($\psi\Delta$) through mechanisms that are not completely understood.

Previous studies have shown that desmin serves an important role in the maintenance of cellular integrity of muscle tissues (10). Multiple mutations within the human desmin gene lead to myopathies of varying intensity (11). Desmin null mice ($des^{-/-}$) develop multiple defects in all muscle types (12, 13), particularly within cardiac muscle where a dilated cardiomyopathy develops with age (14). The initiating event in desmin null cardiomyopathy appears to be cell death occurring in focal areas, resulting in formation of fibrotic lesions variably associated with large calcium deposits. Mitochondrial abnormalities are the first defects visible in desmin null cardiomyocytes and include loss of shape and positioning, proliferation, aggregation, swelling, and degeneration. Desmin null mitochondria also display diminished ADP-stimulated respiratory function and loss of creatine kinase coupling (15, 16).

Although the above data strongly link desmin to mitochondrial behavior and function (16), the mechanism by which the absence of desmin leads to the observed mitochondrial abnormalities and cell death remains elusive. With the central role of bcl-2 in mitochondrial-regulated cell death and recent linkage of intermediate filaments, including desmin, to cell death events (17, 18), the $des^{-/-}$ mouse is an excellent model to assess both the protective impact of bcl-2 overexpression on cardiomyopathy and the mechanism by which desmin regulates mitochondrial behavior and cell survival. For this purpose, transgenic mice were generated with cardiac-specific overexpression of a murine bcl-2 cDNA and crossed into a desmin null background. Bcl-2 overexpression in the $des^{-/-}$ heart ameliorates cardiomyopathy and corrects the observed mitochondrial defects. All aspects of desmin null cardiomyopathy display reduced pathology, and bcl-2 overexpression significantly improves the diminished capacity of $des^{-/-}$ mitochondria to resist exposure to calcium. This finding points to a unique role for desmin in mitochondria buffering of calcium release in cardiomyocytes and presents a mechanism by which desmin deficiency would lead to cardiomyocyte cell death.

Methods

Transgenic Animal Production and Care. Desmin null mice ($Des^{tm1Cap/tm1Cap}$) were generated in the 129SV inbred background through standard methods (12). Two *bcl-2* transgenic lines [$Tg(bcl2)Acap$ and $Tg(bcl2)Bcap$] were generated by using the α-myosin heavy chain promoter (19) to drive a murine bcl-2 cDNA (20) in a C57BLK/6 background. Bcl-2 lines were assessed for expression by Western blot using a bcl-2 rabbit polyclonal antibody (N-19, Santa Cruz Biotechnology) at 1:200 dilution. Mice were maintained in a manner prescribed by National Institutes of Health regulations.

Heart-to-Body Weight Ratio (HW/BW) and Northern Blotting. HW/BW was determined as described (14), and a Fischer's ANOVA test was used to establish significance. Heart weights (mean ± SD) for $des^{+/+}$ $Tg(bcl2)BCap^{-/-}$, $des^{-/-}$ $Tg(bcl2)BCap^{-/-}$, $des^{-/-}$ $Tg(bcl2)BCap^{+/-}$, and $des^{-/-}$ $Tg(bcl2)BCap^{+/+}$ mice were 94.6 ± 11.7, 113 ± 5.94, 78.3 ± 10.6, and 91.6 ± 10.1 mg at 2 months and 126 ± 17.6, 129 ± 23.1, 88.6 ± 6.87, and 102 ± 10.1 mg at 6 months.
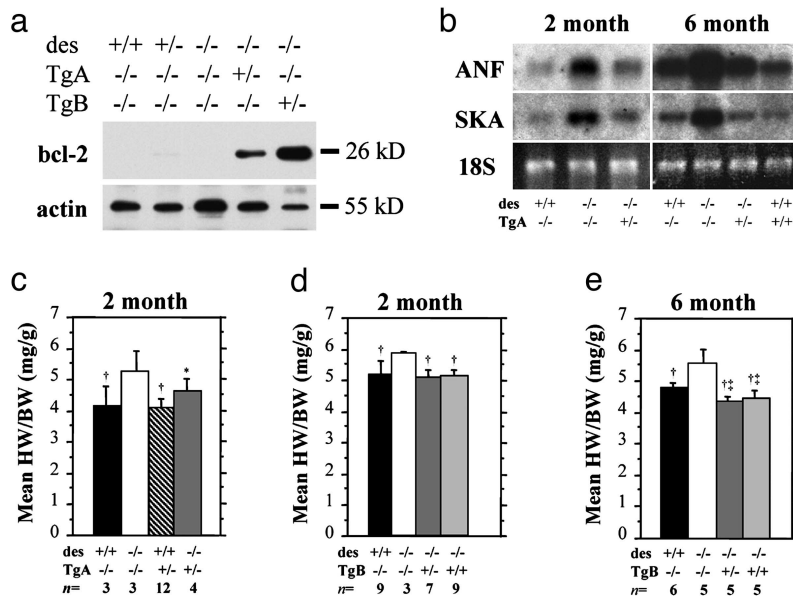
For RNA isolation, five to seven animals of each age and

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Fig. 1. Expression of a *bcl-2* transgene prevents hypertrophy in desmin null hearts. (*a*) Whole heart protein from 2-month-old animals indicates endogenous bcl-2 expression is undetectable in the WT and *des*$^{-/-}$ heart. The *Tg(bcl2)ACap* transgenic line expresses high levels of bcl-2. A second transgenic line, *Tg(bcl2)BCap*, expresses bcl-2 at an even higher level. (*b*) Levels of hypertrophy markers atrial natriuretic factor (ANF) and α-skeletal actin (SKA) indicate both are up-regulated in *des*$^{-/-}$ ventricles at 2 and 6 months of age. Expression of the *bcl-2* transgene reduces markers back to WT levels. Two alleles of the *bcl-2* transgene are capable of reducing ANF and SKA expression below WT levels in 6-month-old animals. (*c–e*) Although bcl-2 expression does not significantly alter HW/BW in a WT background (*c*), it does return the increased HW/BW ratio in *des*$^{-/-}$ animals back to WT levels at both 2 (*d*) and 6 (*e*) months of age. ∗, $P < 0.05$ vs. desmin null at the same age; †, $P < 0.005$ vs. desmin null at the same age; ‡, $P < 0.05$ vs. WT at 6 months.

genotype were killed, and dissected ventricles were frozen and ground into powder under liquid nitrogen. Total RNA was isolated from the pooled frozen tissues by using the TotallyRNA kit (Ambion, Austin, TX) per the manufacturer's instructions. Northern blotting was completed by using standard techniques with 8 μg of RNA loaded for each sample. Probes were used for α-skeletal actin and atrial natriuretic factor as described (14).

Electron Microscopy. Samples from two *des*$^{+/+}$ *Tg(bcl2)BCap*$^{-/-}$, three *des*$^{-/-}$ *Tg(bcl2)BCap*$^{-/-}$, and four *des*$^{-/-}$ *Tg(bcl2)BCap*$^{+/+}$ animals were processed for transmission electron microscopy as described (15). Briefly, hearts were perfused with cold 2.5% gluteraldehyde, then dissected ventricles were sliced into 1-mm cubes and fixed for 16 h at 4°C in the same fixative followed by postfixation in 1% osmium tetroxide for 2 h at room temperature. Samples were then dehydrated through an ethanol series and passed into Embed 812 (EMS, Fort Washington, PA) by using propylene oxide as a transitional solvent. Embedded blocks were cured at 55°C for 48 h and sectioned at 80 nm.

Doppler Analysis. Aortic outflow and transmitral inflow measurements were performed on 8-month-old animals as described (14). In brief, a 10-MHz pulsed doppler probe was placed at the xiphoid of mice of each genotype, and an ECG timing signal was superimposed on the doppler display by using a R-wave trigger. Calculation of systolic and diastolic parameters required the use of DSPW software (Indus Instruments, Houston). Significance was determined by ANOVA.

Calcium-Mediated Mitochondria Swelling Assay. Mitochondria were isolated from whole hearts as described (21) with slight modification. Individual heart tissue was minced in ice-cold MSE+I (225 mM mannitol/75 mM sucrose/1 mM EGTA/1 mM Tris·HCl, pH 7.4/0.2 mM PMSF/37 μg/ml Na-p-tosyl-L-lysine chloromethylketone) and homogenized with five to seven strokes of Teflon head at 500 rpm on a Glas-Col (Terre Haute,

IN) homogenizer. Homogenized tissue was centrifuged at $700 \times g$ at 4°C for 5 min. The resulting supernatant was centrifuged at $7,700 \times g$ for 10 min at 4°C. Mitochondria were diluted to 1 μg/μl in MSE+I with 100 μM calcium or without calcium at room temperature. Spectrophotometer readings were taken at 540 nM. Significance was determined by ANOVA.

Results

Generation of Desmin Null Mice Overexpressing Bcl-2 in the Heart. To examine the effect of bcl-2 overexpression on desmin null cardiomyopathy we generated two transgenic mouse lines [*Tg(bcl2)ACap* and *Tg(bcl2)BCap*] by using the cardiac-specific α-myosin heavy chain promoter (19) to drive expression of a murine bcl-2 cDNA (20). Each line strongly expresses bcl-2 protein of appropriate size; however, *Tg(bcl2)BCap* expressed at a higher level (Fig. 1*a*). Quantitative estimation of transgenic *bcl-2* expression levels relative to the endogenous was not possible because the latter was undetectable. Both lines were crossed into the desmin null background (*des*$^{-/-}$) and assayed for the level of rescue of desmin null cardiomyopathy.

Decreased Appearance of Fibrotic Lesions and Calcification in Desmin Null Animals Overexpressing Bcl-2. Initial morphological observations of *des*$^{-/-}$ *Tg(bcl2)ACap*$^{+/-}$ hearts showed little difference from *des*$^{-/-}$ hearts. A similar degree of calcification on the surface of the heart appeared in both genotypes (data not shown). However, hearts with higher levels of bcl-2 expression, such as *des*$^{-/-}$ *Tg(bcl2)BCap*$^{+/+}$ (Fig. 2*d*), display either no or significantly less calcification than that seen in the *des*$^{-/-}$ mice (Fig. 2*b*). These results were mirrored by histological analysis, with *des*$^{-/-}$ *Tg(bcl2)BCap*$^{+/+}$ mice showing fewer fibrotic lesions that usually have a smaller surface area than that seen in *des*$^{-/-}$ or *des*$^{-/-}$ *Tg(bcl2)ACap*$^{+/+}$ hearts (Fig. 2 *e–j*). Fewer lesions contain calcification in *des*$^{-/-}$ *Tg(bcl2)ACap*$^{+/+}$ (Fig. 2 *g, k,* and *o*) and even fewer in *des*$^{-/-}$ *Tg(bcl2)BCap*$^{+/+}$ hearts (Fig. 2 *h, l,* and *p*) when compared to *des*$^{-/-}$ hearts (Fig. 2*n*). These results
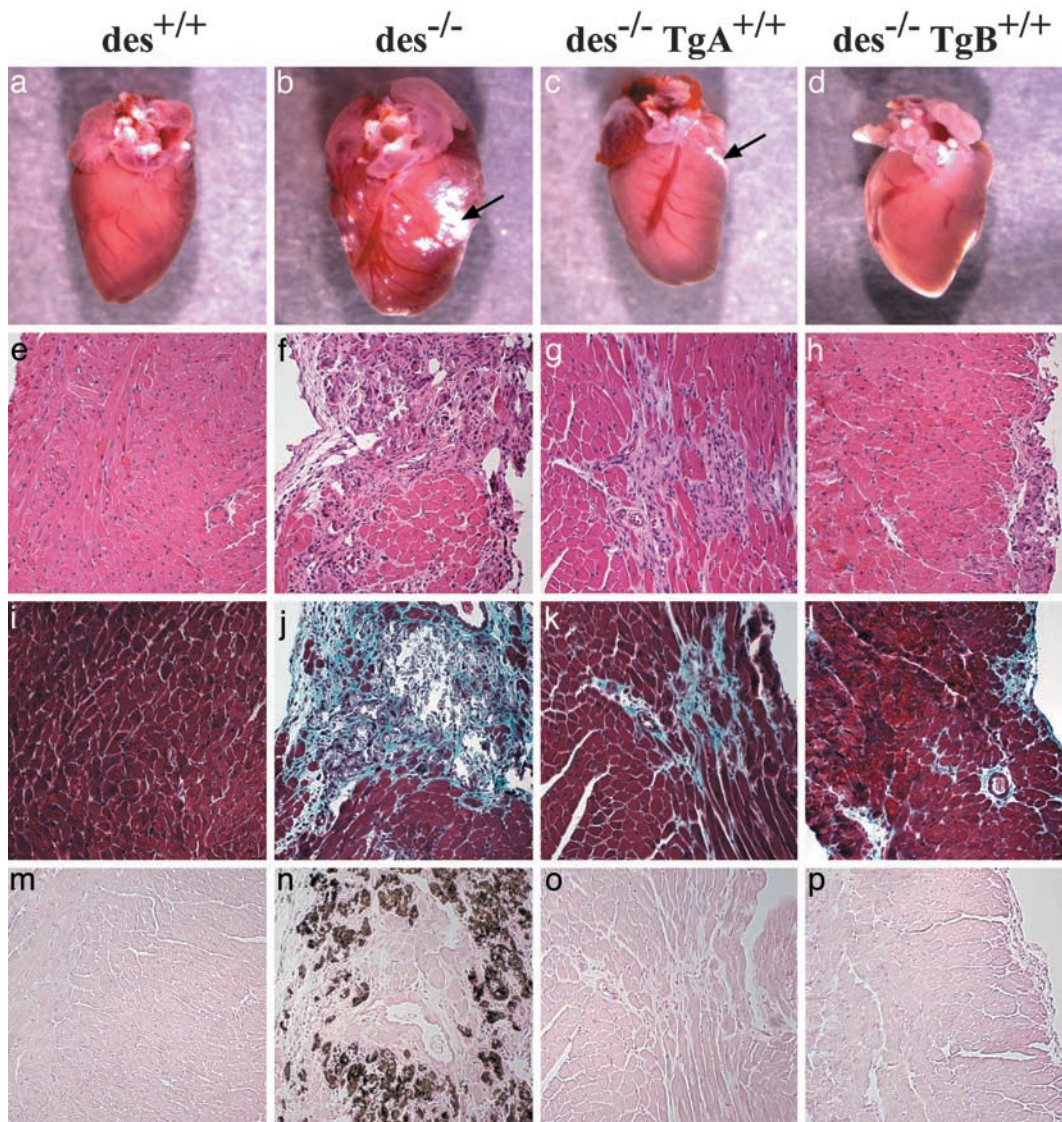
Fig. 2. Decreased fibrotic lesions and calcification in desmin null animals overexpressing bcl-2. Whole-mount heart from 2-month-old WT (*a*), *des*$^{-/-}$ (*b*), *des*$^{-/-}$ *Tg(bcl2)ACap*$^{+/+}$ (*c*), and *des*$^{-/-}$ *Tg(bcl2)BCap*$^{+/+}$ (*d*) animals. (*e–h*) Hemotoxylin and eosin-stained sections from the same genotype animals. (*i–p*) Fibrosis associated with calcium deposits is found throughout the left ventricle of *des*$^{-/-}$ hearts, as revealed by Mason's trichrome staining (*j* and *n*). Compared to *des*$^{-/-}$, fibrotic lesions are less frequent and smaller in *des*$^{-/-}$ *Tg(bcl2)ACap*$^{+/+}$ (*k*) and *des*$^{-/-}$ *Tg(bcl2)BCap*$^{+/+}$ myocardium (*l*). Although calcification is obvious in Von Kossa staining of *des*$^{-/-}$ myocardium (*n*), there is less calcification of lesions in *des*$^{-/-}$ *Tg(bcl2)BCap*$^{+/+}$ hearts (*p*). (Magnification: ×200.)

demonstrate that overexpression of bcl-2 in the *des*$^{-/-}$ heart can prevent lesion formation in a dose-dependent manner.

Expression of Bcl-2 Prevents Hypertrophy in Desmin Null Hearts. A hallmark of cardiomyopathy and heart failure is compensatory hypertrophy during the early stages of pathology. In desmin null cardiomyopathy, hypertrophy occurs in response to cardiac damage, with significant increase in heart size and distortion of the ventricles (Fig. 2*b*). Introduction of bcl-2 into the *des*$^{-/-}$ heart was able to prevent structural dilation of the heart (Fig. 2 *c* and *d*) and rescue the elevated HW/BW of *des*$^{-/-}$ animals (Fig. 1 *d* and *e*). Bcl-2 overexpression in the WT background does not appear to alter the HW/BW (Fig. 1*c*). We also find that the levels of molecular markers of hypertrophy such as α-skeletal actin and atrial natriuretic factor are reduced in all *des*$^{-/-}$ animals carrying a *bcl-2* transgene (Fig. 1*b*). Again, we see a dose-dependent response as increased bcl-2 levels produce reduced HW/BW and diminished expression of hypertrophy markers that approach WT levels.

Restoration of Ultrastructural Abnormalities in *des*$^{-/-}$ Cardiomyocytes. Examination of the ultrastructure of *des*$^{-/-}$ *Tg(bcl2)BCap*$^{+/+}$ cardiomyocytes shows a significant recovery from the damage seen in *des*$^{-/-}$ hearts. Electron microscopy of *des*$^{-/-}$ cardiomyocytes reveals multiple defects ranging from disrupted contractile apparatus to numerous mitochondrial deficiencies (Fig. 3*b*). These mitochondrial abnormalities include loss of positioning, proliferation, clumping, and formation of cleared areas of cytoplasm around mitochondria. In addition, individual mitochondria appear swollen and misshapen. Although such defects can be occasionally detected in *des*$^{-/-}$ *Tg(bcl2)BCap*$^{+/+}$ cardiomyocytes, they are not as common and appear less intense when compared to *des*$^{-/-}$ animals. The most frequent mitochondrial defect observed in *des*$^{-/-}$ *Tg(bcl2)BCap*$^{+/+}$ cardiomyocytes involves a loss of mitochondria alignment. These mitochondria fail to form the well-defined rows in between myofibrils that are seen in WT cardiomyocytes (Fig. 3*c*), illustrating the importance of desmin

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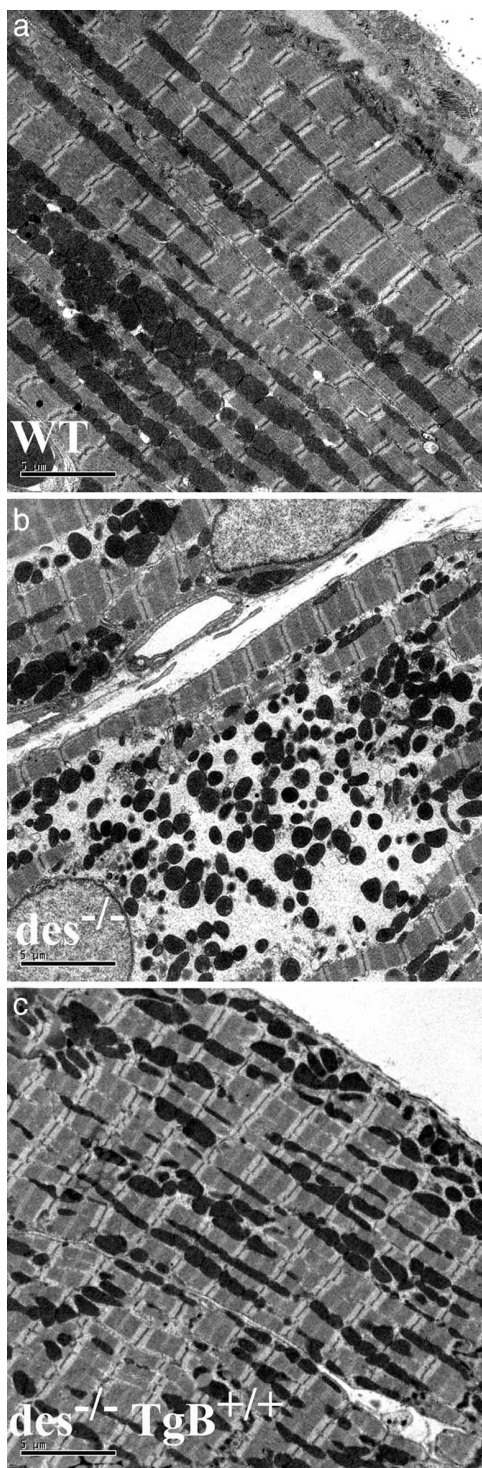
Fig. 3. Reduction of damage to mitochondria and contractile apparatus. Transmission electron microscopy was performed in hearts from WT (*a*), *des*$^{-/-}$ (*b*), and *des*$^{-/-}$ *Tg(bcl2)BCap*$^{+/+}$ (*c*) mice. Most sections from *des*$^{-/-}$ *Tg(bcl2)BCap*$^{+/+}$ hearts (*c*) appear very similar to WT sections (*a*), in contrast to *des*$^{-/-}$ sections that display typical mitochondrial swelling and rupture and broken contractile apparatus (*b*). Occasionally, some areas within *des*$^{-/-}$ *Tg(bcl2)BCap*$^{+/+}$ hearts display some degree of pathology, but usually not to the extent seen commonly in *des*$^{-/-}$ tissue. (Scale bar: 5 μm.)

in maintenance of mitochondrial positioning. However, many *des*$^{-/-}$ *Tg(bcl2)BCap*$^{+/+}$ cardiomyocytes appear identical to WT cardiomyocytes. When compared to WT, the most striking similarities are in myofibril integrity and alignment, which in *des*$^{-/-}$ *Tg(bcl2)BCap*$^{+/+}$ cardiomyocytes appears quite normal, suggesting a minimal role for desmin in myofibril alignment.

Significant Improvement of Systolic Cardiac Function in *des*$^{-/-}$ Hearts with Bcl-2 Overexpression. Diminished cardiac systolic function with age is characteristic of desmin null cardiomyopathy (14). Although cardiac bcl-2 overexpression does not cause significant changes in cardiac function itself, it has prevented decreased cardiac function caused by ischemia (3, 4). *Des*$^{-/-}$ animals display decreased cardiac systolic function with age as measured by doppler echocardiography (14). Similar experiments with 8-month-old animals show that diastolic function was unchanged in all genotypes and no animals display arrhythmia (Table 1). Assessment of systolic function reveals that *des*$^{-/-}$ *Tg(bcl2)BCap*$^{+/+}$ hearts do not experience decreased peak aortic velocity like that seen in *des*$^{-/-}$ animals (Table 1). Bcl-2-expressing *des*$^{-/-}$ animals do not completely maintain a WT level of function. This observation mirrors the trend that bcl-2 overexpression in the *des*$^{-/-}$ heart can diminish the degree of pathology but not completely rescue cardiac function. Consistent observation of this trend implies that multiple mechanisms of cardiac damage may be at work in the desmin null heart. Thus, both cell death and contractile dysfunction caused by altered mechanical force transduction in the absence of desmin could contribute to development of compromised cardiac function, even if cardiomyocyte death is the dominant mechanism of damage.

Increased Sensitivity of *des*$^{-/-}$ Mitochondria to Calcium Improved by Bcl-2 Overexpression. Mitochondria influence intracellular Ca^{2+} regulation within myocytes by sequestration of Ca^{2+} released from the endoplasmic reticulum. Isolated mitochondria from *des*$^{-/-}$ cardiomyocytes were treated with a high Ca^{2+} buffer and assessed for their degree of swelling by measurement of absorbance at 540 nm. Isolated *des*$^{-/-}$ mitochondria swell more than WT mitochondria at the same Ca^{2+} concentration, revealing *des*$^{-/-}$ mitochondria are more sensitive than WT mitochondria to Ca^{2+} (Table 2). Expression of bcl-2 in cardiomyocytes decreases this mitochondrial swelling, indicating that bcl-2 can improve the compromised ability of mitochondria to resist Ca^{2+} in *des*$^{-/-}$ cardiomyocytes. This finding suggests that one role of bcl-2 in the prevention of cardiomyocyte death in the *des*$^{-/-}$ heart is improvement of mitochondria ability to resist Ca^{2+} within the cell.

Discussion

We have found that bcl-2 overexpression in the *des*$^{-/-}$ heart corrects the mitochondrial defects and ameliorates cardiomyopathy caused by the absence of desmin. These data suggest that lack of desmin leads to mitochondrial dysfunction of a nature that can be corrected by high, but not lower, levels of bcl-2. The mechanism by which high levels bcl-2 can compensate for the absence of desmin in the present case can only be speculated. Several different proposed functions for bcl-2 in mitochondria could lead to prevention of cell death. It is clear that the ratio of antiapoptotic to proapoptotic members of the bcl-2 family alters the cell fate either toward or away from death (22). With the low endogenous level of bcl-2 in the heart it is possible that an increase in bcl-2 could provide additional protection over what would be seen from overexpression of other members of the bcl-2 family. Observation of a dose-dependent protective effect in *des*$^{-/-}$ mice indicates that these notions apply in the present case. Increase in bcl-2 expression in *des*$^{-/-}$ cardiomyocytes has little effect until the level reaches a threshold where the ratio of bcl-2 family members is sufficient to favor cell survival. However, it is unclear what function this additional bcl-2 plays in the cell. Although it is established that bcl-2 family members interact at

Table 1. Bcl-2 overexpression prevents decline in cardiac function in desmin null animals

Measurement	$des^{+/+}$ ($n = 8$)	$des^{-/-}$ ($n = 10$)	$des^{-/-}$ $TgB^{+/+}$ ($n = 6$)
Body weight, g	23.7 ± 0.4	25.2 ± 0.7	21.7 ± 0.9
Heart rate, min-1	458 ± 14	431 ± 13	441 ± 15
Peak aortic velocity, cm/s	88.3 ± 3.1	78.3 ± 1.5*	83.0 ± 1.4
Mean acceleration, cm/s^2	6,800 ± 300	5,200 ± 200*	6,400 ± 300
Peak E velocity, cm/s	65.2 ± 3.6	65.8 ± 2.7	60.7 ± 2.9
Peak A velocity, cm/s	46.7 ± 4.2	35.1 ± 2.8	38.9 ± 4.4
E/A ratio, peak velocities	1.41 ± 0.06	2.00 ± 0.22	1.68 ± 0.20
IVRT, ms	17.0 ± 0.9	19.8 ± 0.6	21.9 ± 1.2
SD of RR intervals, cm	2.65 ± 0.13	3.07 ± 1.43	1.87 ± 0.6

At 8 months of age $des^{-/-}$ $Tg(bcl2)BCap^{+/+}$ peak aortic velocity (PAV) was not significantly different from WT. In contrast, PAV was depressed in the desmin null heart. Data are expressed as means ± SE. Each n represents an individual animal. *, $P < 0.05$ vs. $des^{+/+}$. E/A, peak E velocity over peak A velocity; IVRT, isovolumic relaxation time; RR, interval between two consecutive R waves.

the mitochondrial membrane and influence $\psi\Delta$, the mechanism by which this effect takes place has yet to be determined. Whether the cytoskeleton in general or desmin intermediate filaments in particular facilitate such function directly cannot be defined, given the fact that desmin associates with mitochondria either directly or indirectly (15, 16) and this association can stabilize mitochondrial membrane structure and function.

One mechanism through which bcl-2 could influence mitochondria function and cell survival is by altering the calcium handling dynamics of the cell. Bcl-2 and family members Bax and Bak have been shown to alter cellular response to Ca^{2+}, by affecting either mitochondria or the endoplasmic reticulum (23–25). Mitochondria buffer the cytosolic Ca^{2+} level by sequestration of Ca^{2+} released from the endoplasmic reticulum. Destabilization of mitochondrial membranes in $des^{-/-}$ cardiomyocytes could result in compromised tolerance to normal Ca^{2+} levels, resulting in mitochondrial dysfunction and cell death. Bcl-2 can increase the Ca^{2+} buffering capacity of mitochondria from neurons (26) and cardiomyocytes (6) and prevent cell death induced by Ca^{2+} in cultured neurons (27). To test this hypothesis, we treated isolated mitochondria from $des^{-/-}$ cardiomyocytes with a high Ca^{2+} buffer and measured the degree of mitochondria swelling. $Des^{-/-}$ mitochondria have reduced ability to resist Ca^{2+}, which can be improved by overexpression of bcl-2, suggesting that a mechanism by which bcl-2 prevents cardiomyocyte death in the $des^{-/-}$ heart is by improving of mitochondria ability to resist Ca^{2+} levels within the cell.

There are several potential mechanisms through which desmin could improve the ability of mitochondria to resist intracellular Ca^{2+} and prevent cell death. These include an indirect mechanism, perhaps by aligning the connection of mitochondria to the endoplasmic reticulum, thus allowing the creation of Ca^{2+}

microdomains (28). Our results in an isolated mitochondria system suggest that desmin is directly involved in maintaining mitochondrial membrane integrity, particularly in the presence of Ca^{2+}, perhaps by interacting with and stabilizing protein complexes associated with the CS of mitochondria (29). CS are dynamic structures in which the inner and outer mitochondrial membranes fuse. They are key participants in protein transport (29), energy coupling via formation of creatine phosphate (30), and fatty acids uptake for oxidative metabolism (31). Porin (voltage-dependent anion channel, VDAC) channels, which control mitochondrial permeability for ADP, and adenine nucleotide translocator, which in turn controls entry of ADP into the matrix, function at the CS (32). These molecules also function in pore formation in cell death events. CS may also participate in mitochondrial Ca^{2+} handling as VDAC is thought to function in import of calcium into mitochondria (33, 34). Thus, desmin interaction with CS could contribute to the efficiency of these varied mitochondrial functions, many of which are altered in the $des^{-/-}$ mouse.

Desmin may interact with CS by binding voltage-dependent anion channel (VDAC) either directly or through a desmin-associated protein. The latter possibility is supported by the demonstration that the microtubule binding protein MAP2 binds to VDAC (35). Interestingly, intermediate filaments seem to share common binding sites with mitogen-activated proteins on the brain mitochondrial membrane (36). Determination of the molecular interaction between desmin and mitochondria will reveal a mechanism by which the intermediate filament cytoskeleton can regulate various mitochondrial functions.

Bcl-2 family members are thought to function at mitochondria CS (7–9). A potential mechanism by which elevated bcl-2 could improve pathology in $des^{-/-}$ heart would involve additional bcl-2

Table 2. Desmin null mitochondria display increased sensitivity to calcium induced swelling that can be reversed by bcl-2 expression

Mice	n	OD$_{540}$ decrease	Minimum	Maximum
$des^{+/+}$	7	0.993 ± 0.024*	0.972	1.033
$des^{-/-}$	9	1.008 ± 0.021*	0.979	1.038
$des^{-/-}$ $TgB^{+/-}$	2	0.980 ± 0.002*	0.978	0.981
$des^{-/-}$ $TgB^{+/+}$	14	0.999 ± 0.090*	0.801	1.211
$des^{+/+}$ + Ca^{2+}	8	0.856 ± 0.050†	0.758	0.910
$des^{-/-}$ + Ca^{2+}	9	0.780 ± 0.032	0.738	0.821
$des^{-/-}$ $TgB^{+/-}$ + Ca^{2+}	2	0.831 ± 0.007	0.826	0.836
$des^{-/-}$ $TgB^{+/+}$ + Ca^{2+}	14	0.836 ± 0.050†	0.742	0.931

Both $des^{+/+}$ and $des^{-/-}$ $Tg(bcl2)BCap^{+/+}$ mice display significantly improved ability to resist Ca^{2+} compared with $des^{-/-}$ $Tg(bcl2)Bcap^{-/-}$ animals. Data are expressed as mean percentage decrease in OD$_{540}$ absorbance from time 0 to 30 min ± SE. Each n represents a mitochondria fraction isolated from a single 2-month-old heart. *, $P < 0.005$ vs. with Ca^{2+}; †, $P < 0.05$ vs. $des^{-/-}$ $Tg(bcl2)BCap^{-/-}$ +Ca^{2+}.

at CS restoring a degree of the diminished CS functions in $des^{-/-}$ mitochondria, perhaps by providing additional stability to the protein complexes of CS. Although the specific defects that arise within CS of $des^{-/-}$ mitochondria have not yet been determined, it is probable that these alterations resemble mitochondrial defects that result from changes in bcl-2 family member levels within the cell. Thus, a direct link between desmin and bcl-2 member function is possible. Our studies did not identify any difference in bcl-2 levels between WT and $des^{-/-}$ heart (data not shown), indicating that the $des^{-/-}$ phenotype is not caused by altered bcl-2 expression levels. However, potential changes in bcl-2 mitochondrial localization caused by the absence of desmin have not been excluded. On the contrary, an independent study suggested, although not strongly, that such change might take place in desmin null mice (37). It will be important to verify these data, a prospect complicated because of the low endogenous level of bcl-2 in the heart. The use of bcl-2-overexpressing mice will help achieve this goal.

Although in the past bcl-2 has been shown to reduce cardiomyocyte death in cardiac ischemia models (4), we present an inherited model of cardiomyopathy that was significantly improved by overexpression of bcl-2. The role of bcl-2 members in human heart failure has not been thoroughly evaluated, but it is known that both bcl-2 and bax are expressed at high levels in the failing human heart (38). Because the bcl-2 family has the potential to affect multiple mechanisms of cardiac damage, including ischemia, calcium dysregulation, and increased oxidative stress, it is clear the bcl-2 family members are very attractive therapeutics that can benefit a broad range of cardiac diseases.

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