CAR might provide a survival signal for myocardial cells

We read with great interest the *Research Article* by Dorner et al. (Dorner et al., 2005) describing their coxsackievirus and adenovirus receptor (CAR)-deficient mouse, as we have previously published details of our own CAR-knockout mouse (Asher et al., 2005). Our findings of embryonic death associated with cardiac defects at day 11.5 post-conception were very similar to theirs. However, our conclusions differ from Dorner et al. in terms of the possibility that CAR-deficient myocardial cells die by an apoptotic pathway. Like Dorner et al. we agree that, despite the blood vessel abnormalities, the systemic vascular insufficiency that could account for the myocardial lesions was not evident, as the heart was the only tissue affected. Dorner et al. did investigate the possibility of an apoptotic cause of cellular death by performing TUNEL staining, but they obtained a negative result. We also attempted TUNEL staining but the results were inconclusive, and so we conducted staining for the apoptotic marker activated caspase 3. The cells in the center of the myocardial lesions stained positively for this marker. In addition, the swollen mitochondria observed by Dorner et al. are considered a hallmark of apoptosis (Dvorakova et al., 2005; Sun et al., 2005; Teranishi et al., 2000), which further suggests that these cells die by a programmed pathway.

Given these findings and the lack of any apparent extracellular cause for the observed cellular damage, the possibility that these cells die by apoptosis should not be discounted. Although the significance of these data remains to be determined, the suggestion that these cells die by a programmed pathway raises the possibility that CAR provides a positive survival signal during myocardial development. The findings of several other groups indicating that CAR is upregulated in regenerating adult cardiac tissue are consistent with the hypothesis that a CAR-mediated signal is essential for the survival of growing cardiomyocytes (Fechner et al., 2003; Ito et al., 2000; Noutsias et al., 2001). The observed cellular pathology and the gross malformation of the CAR-deficient embryonic hearts may be a consequence of the absence of this putative survival signal.

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Reply

Asher et al. have analyzed independently generated mice deficient for the coxsackievirus and adenovirus receptor (CAR) (Asher et al., 2005) and reported, in agreement with our results (Dorner et al., 2005), that embryos of such mice die during midgestation owing to malformations of the heart. In the absence of other detected defects, Asher et al. suggested that the embryos die from cardiac malformations caused by myocardial apoptosis. Although no ultrastructural analysis was conducted, evidence for apoptosis was based on immunohistochemical staining of regions of the myocardial wall for activated caspase 3. As stated in their letter above, TUNEL staining did not generate conclusive evidence for apoptosis in such regions. In agreement with this, we did not find any TUNEL-positive apoptotic cells within the myocardium of our CAR-deficient embryos. Importantly, we have analyzed the hearts of these embryos by electron microscopy and found no sign of changes in the structure of nuclei (no condensation of chromatin and no blebbing). The enlarged mitochondria that we reported cannot be taken as reliable evidence for apoptosis for two reasons. First, the examples in the literature where apoptosis has been correlated with swollen mitochondria refer to mitochondria that are increased in volume and therefore display a reduced density of cristae inside the organelle. The enlarged (not 'swollen') mitochondria that we detected were simply increased in size with normal cristae structure. Second, swelling of cardiac mitochondria can be clearly observed in the absence of apoptosis (Bushdid et al., 2003).

Staining for activated caspase 3 is a generally accepted method for detection of apoptotic cells. However, there is accumulating evidence that caspase 3 can also be activated in the context of other events that are not related to apoptosis (Kroemer and Martin, 2005; Schwerk and Schulze-Osthoff, 2003). It may not always be sufficient to rely on this staining as evidence for apoptosis.

In conclusion, on the basis of TUNEL staining and ultrastructural analysis of cellular nuclei we have found no evidence for apoptosis. Instead, we have found that

the number of myofibrils in cardiomyocytes was reduced, the myofibrils were shorter and contained smaller numbers of sarcomers and their close arrangement was distorted. Furthermore, the organization of myofibrils and the continuity of the orientation of myofibrils across the plasma membrane to the neighboring cell was disturbed. Such defects were analyzed by electron microscopy of tissue sections and by immuno-fluorescence microscopy of isolated cardiomyocytes. We suggest that these defects affect the contractile functions of the cardiomyocytes, which is likely to lead to malformations of the embryonic heart.

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