

"Only love can mend a broken heart."

—Dionne Warwick

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"What's love got to do with it?"

—Tina Turner

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Many of us realize, usually too late, the evanescent nature of the skeletal muscle hypertrophy that follows endurance training, weightlifting, or hard physical activity. The sedentary academic and research lifestyle seems, alas, inextricably linked to accumulation of flab and loss of muscle tone. But a remarkable feature of skeletal muscle is that it will hypertrophy again in response to exercise, a prize that many covet but few achieve. Muscle has, also, the propensity to regenerate after injury or inflammation. No such advantage befalls the injured heart. After the developmental period of cardiomyocyte proliferation ending shortly after birth, these cells withdraw permanently from the cell cycle. Myocardial growth and hypertrophy is accomplished primarily by enlargement of individual cardiomyocytes, not by hyperplasia. Injury to the myocardium, as evoked by ischemia, is compensated little if at all by cardiomyocyte regeneration; the subsequent loss of functional myocardial mass can be a major contributor to ventricular failure.

Thus it is no surprise that interest has focused on the remarkable developmental capacities of skeletal muscle as a potential means to replenish, repair, or otherwise mend an injured heart. Several heterodox approaches use skeletal muscle to assist in ventricular contraction. Cardiomyoplasty, using a warp of the latissimus dorsi muscle mobilized as a pedicled flap and still attached to its vascular and nerve supply, has had some success in improving hemodynamic function in animal models and patients with dilated or ischemic cardiomyopathy (reviewed by Hooper and Stephenson [1]). Similarly, creation of an artificial second left ventricle using the same muscle as an aortic diastolic counterpulsator also has been attempted experimentally (2). In both surgical approaches success hinges on another remarkable aspect of skeletal muscle biology, the ability of sustained low-voltage pacing to convert fast-fiber skeletal muscle to slow-fiber, indefatigable muscle capable of sustaining frequent contraction without anaerobic consequences.

In this issue of *The Journal*, Koh et al. (3) advance a new approach in this arena: engraftment of skeletal muscle myoblasts into the myocardium by direct injection of cells grown in vitro. The key element in the remarkable capacity of skeletal muscle to regenerate and grow is the satellite cell (a mononuclear cell committed to the skeletal muscle lineage) that can replicate and fuse into existing myofibers, thereby contributing to the number of myonuclei during normal growth or hypertrophy, or during regeneration after muscle injury. The cells used in the paper by Koh et al. (3) were a well-studied immortalized mouse cell line, C2, originally isolated from satellite cells of mouse thigh muscle by Yaffe and Saxel (4). In vitro,

when mitogens are withdrawn, these committed myogenic cells withdraw from the cell cycle and turn on their program of myogenic gene expression. Cell-to-cell fusion follows and the cells differentiate into striated multinucleated myotubes with a full panoply of functional contractile elements.

What Koh et al. (3) have done is to determine the fate of injected C2 cells in the mouse myocardium. In such syngeneic transplants the cells not only survive but withdraw from the cell cycle, fuse with each other, and differentiate, at least by morphological criteria. They do not appear to fuse with any cardiomyocytes, which is not surprising since the cells of cardiac origin do not normally fuse to one another but, rather, become coupled through specialized tight junctions. No such junctions or electrical coupling between the heart cells and the transplanted muscle cells were observed. Of potential importance is that the patch of muscle elicited no cellular reaction or encapsulation and, while physiologic function was not evaluated, the mice and their EKGs appeared normal for as long as 3 mo after transplantation.

Could these experiments and others recently reported (5, 6) be considered as a foundation for the development of cell transplantation therapies to patch a weakened myocardial wall in patients? A large number of questions and technical obstacles remain. While the approach could conceivably work for local areas of dysfunction or local cardiomyopathy, therapeutic approaches to diffuse cardiomyopathy would continue to rely on surgical techniques, including transplantation, or, in the not too distant future, the delivery of DNA vectors capable of transducing a majority of cells. The patch made by C2 cells was not innervated and its vascular supply was not investigated, two potentially critical issues. Furthermore, whether these patches contributed to regional and/or global left ventricular function was not studied. Since the myoblasts used were immortalized tumorigenic cells, the risk of uncontrolled hyperplasia by transformed cells, although not seen in the animals examined by Koh et al. (3), would remain a disturbing possibility if similar approaches were applied in patients. Accordingly it would be of interest to evaluate more carefully primary myoblasts as potential donor cells in myocardial cell transplantation. It is possible to obtain and expand myoblasts from human skeletal muscle biopsies *ex vivo* (7, 8). Clearly, *ex vivo* genetic manipulation of myoblasts to alter the phenotype of the cells or to deliver recombinant proteins at the site of implantation (9–11) might be of additional therapeutic interest. Such patches could prove valuable to strengthen scars and alter ventricular compliance. Delivery of such cells by a noninvasive endomyocardial route also remains a technical challenge for the future.

Adult cardiomyocytes do not proliferate in culture, with the exception of cells with a cardiomyocyte phenotype derived from tumors of the heart (5). Whether the proliferative nature of such transformed cells could be easily controlled to make their use practical is uncertain. The ability to induce endogenous cardiomyocytes to reenter the cell cycle and regenerate damaged areas in a controlled manner would, in theory, be a major therapeutic achievement. If such manipulation proved to require the local production of cytokines, however, then patches of skeletal muscle myoblasts engineered to secrete appropriate growth factors might elicit a useful response from the

surrounding, normal myocardium that would engender the proliferation of functionally normal cardiomyocytes, appropriately coupled and physiologically organized, to assist or mend a broken heart.

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References

1. Hooper, T. L., and L. W. Stephenson. 1993. Cardiomyoplasty for end-stage heart failure. *Surg. Annu.* 1:157-73.
2. Niinami, H., A. Pochettino, and L. W. Stephenson. 1991. Use of skeletal muscle grafts for cardiac assist. *Trends Cardiovasc. Med.* 1:122-126.
3. Koh, G. Y., M. G. Klug, M. H. Soonpaa, and L. J. Field. 1993. Differentiation and long-term survival of C2C12 myoblast grafts in heart. *J. Clin. Invest.* 92:1548-1554.
4. Yaffe, D., and O. Saxel. 1977. Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle. *Nature (Lond.)*. 270:725-727.
5. Koh, G. Y., M. H. Soonpaa, M. G. Klug, and L. J. Field. 1993. Long-term survival of AT-1 cardiomyocyte grafts in syngeneic myocardium. *Am. J. Physiol.* 264:1727-1733.
6. Marelli, D., F. Ma, and R. C. Chiu. 1992. Satellite cell implantation for neomyocardial regeneration. *Transplant. Proc.* 24:2995.
7. Blau, H. M., and C. Webster. 1981. Isolation and characterization of human muscle cells. *Proc. Natl. Acad. Sci. USA*. 78:5623-5627.
8. Webster, C., G. K. Pavlath, D. R. Parks, F. C. Walsh, and H. M. Blau. 1988. Isolation of human myoblasts with the fluorescence-activated cell sorter. *Exp. Cell Res.* 174:252.
9. Barr, E., and J. M. Leiden. 1991. Systemic delivery of recombinant proteins by genetically modified myoblasts. *Science (Wash. DC)*. 254:1507-9.
10. Dhawan, J., L. C. Pan, G. K. Pavlath, M. A. Travis, A. M. Lanctot, and H. M. Blau. 1991. Systemic delivery of human growth hormone by injection of genetically engineered myoblasts. *Science (Wash. DC)*. 254:1509.
11. Dai, Y., M. Roman, R. K. Naviaux, and I. M. Verma. 1992. Gene therapy via primary myoblasts: Long-term expression of factor IX protein following transplantation in vivo. *Proc. Nat. Acad. Sci. USA*. 89:10892-10895.