

# Systemic Delivery of Human Growth Hormone by Injection of Genetically Engineered Myoblasts

The ability to deliver recombinant proteins into the systemic circulation could facilitate the treatment of a variety of acquired and inherited diseases. The ideal recombinant protein delivery system requires a cell that is easily isolated from the recipient, reproduced in vitro, transduced with recombinant genes, and conveniently reimplanted into the host. The secreted recombinant protein from these cells needs to gain ready access to the circulation. Such cells need to survive for long periods while secreting the transduced protein product without adversely interfering with body function. Until the possible use of myoblasts was considered and tried in the experiments reported here, the production of stable and physiologic levels of circulating recombinant proteins in normal animals has been relatively unsuccessful. The use of myoblasts may open new therapeutic horizons, as discussed in the 3 presentations from a recent December 1991 issue of *Science*. These articles are abstracted here.

Myoblasts can easily be obtained from muscle tissue, and genetically engineered myoblast cells can easily be returned to muscle without causing damage. This technique was used by Dhawan and colleagues<sup>1</sup> at Stanford University, who introduced a recombinant gene that encoded human growth hormone (hGH) into cultured myoblasts from mice. A modified gelatin (MFG) retrovirus vector was utilized. These cells secreted hGH at levels ranging between 1,400 to 4,600 ng/10<sup>6</sup> cells per day in vitro. These cells were injected into muscle and hGH was demonstrated to be secreted into the serum at increasing levels over an 85-day period. It was demonstrated that hGH can be continuously produced and secreted by myoblasts that are implanted into muscle tissue. The authors state that "this type of delivery system may be useful in the treatment of children with GH deficiency." They also state that "these findings suggest that somatic cell therapy using myoblasts may have application in delivering to the circulation a number of recombinant proteins."

Barr and Leiden,<sup>2</sup> in the same issue of *Science*, published an article entitled "Delivery of Recombinant Proteins by Genetically Modified Myoblasts." They used a plasmid carrying the hGH gene as the vector to insert these genes into the murine C2C12 myoblast cell line. The cultured transfected myoblasts were then placed into the muscles of mice and circulating hGH was measured over a 3-week period. Thus, the results were corroborated in the 2 experiments. Histologic examination of muscle tissue injected with myoblasts demonstrated that many of the injected cells had fused to form multinucleate myotubules.

A concern regarding such injections with a continuous cell line, such as the C2C12 line, is the possibility that these cells have malignant potential. Long-term studies will be needed to evaluate this possibility. In addition, it remains to be determined if this system can be used to produce physiologic levels of circulating proteins in large animals.

In the same issue of *Science*, Michelle Hoffman discussed in an editorial entitled "Putting New Muscle Into Gene Therapy"<sup>3</sup> the potential applications of these techniques, including the possibility of treating the genetic defects that cause muscular dystrophy and other diseases. Myoblasts do better than the cells used in previous systems because they eventually differentiate and fuse into existing muscle tissue. Hoffman cautions against excessive and premature enthusiasm, however. The results achieved could be different using primary cell lines obtained from the individual receiving therapy, in contrast to the cells used in the mouse experiments, which were from cell lines perpetuated in culture over many years. A remaining unanswered question is: Does one get sustained expression in primary cells? Also, cell lines are probably unacceptable for use in humans because they are too frequently tumorigenic.

In spite of these difficulties, some investigators such as Barr and Leiden are optimistic about the future of myoblasts for gene therapy. Some researchers are projecting their ideas even further into the future, such as the potential to inject DNA directly into muscle cells, a technique pioneered recently by Wolff et al at the University of Wisconsin and by investigators in San Diego.

## References

1. Dhawan J, et al. *Science* 1991;254:1509.
2. Barr E, Leiden JM. *Science* 1991;254:1507.
3. Hoffman M. *Science* 1991;254: 1455.

**Editor's comment:** My editorial comment is simply this: *Wondrous innovations in science never cease. The use of myoblasts as protein carriers is only one of many recent scientific innovations, but one that will undoubtedly receive much attention in the future from the readers of GROWTH, Genetics, & Hormones. Congratulations to Dhawan, Barr and colleagues for these stimulating ideas and studies.*

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