adjuncts to surgical procedures for atherosclerosis—balloon angioplasty and bypass surgery—remain on the distant horizon.

Gene therapy approaches presented by both Elizabeth Nabel (University of Michigan, Ann Arbor) and Jeff Leiden favor intervention with cell cycle proteins p21 and Rb—which arrest the cell cycle in smooth muscle cells during the two to three weeks that the adenovirus vector is transiently expressed. Victor Dzau's (Stanford University, Palo Alto, CA) work attempts to accomplish the same goal of cell cycle arrest—specifically in vein grafts—by using oligonucleotide decoys for E2F—an important cell cycle regulatory transcription.

While the results look promising, these speakers agreed that widespread clinical application of any of these techniques should not be expected in the near future. "We still have a long ways to go in the field," says Nabel, "While we have proved in principle that genes can be introduced and gene expression can be induced, it will take quite some time to move this through the development phase and into the clinic."

Gene transfer to the mothers of all cells

James Kling

At a technical level, gene therapy depends fundamentally on researchers' ability to get genes into cells. Strategies for doing just that with hematopoietic stem cells, including one that increases the efficiency of adeno-associated virus (AAV) 100 to 1,000 fold, were among the techniques discussed at a Keystone meeting held in Taos, New Mexico on February 4-10.

Clinicians want to introduce drug resistance genes into a cancer patient's immune cells in order to combat one of the major side-effects of chemotherapy—suppression of rapidly dividing immune cells. Stem cells, with their unlimited reproductive potential and ability to divide and differentiate to give rise to all types of blood cells, are an attractive target for gene therapy. They offer the prospect of treatments that might last a patient's lifetime. But the inefficient transfer into the stem cells is remains one of gene therapy's greatest hurdles.

The retrovirus vectors most commonly used for gene transfer work best in cells that are dividing. This is a problem, since only a small percentage of stem cells (probably less than 5 percent) are growing and dividing at any given time. A number of cytokines do encourage stem cells to divide and are often used in combination with retrovirus vectors to effect gene transfer. However, the composition of the cytokine "cocktail" has usually been poorly defined.

Makio Ogawa at the Medical University of South Carolina (Charleston, SC) reported at Taos that his group now has useful insights into the cytokine mix. They categorized the most commonly used cytokines by function into three groups. In one group are Steel Factor, the ligand for kit, and the ligand for FLT3/FLK-2; in the second, IL-3, GMCSF, and IL-4; and in the third are IL-6, GCSF, IL-11, LIF, and IL-12. The combinations that appear most effective at stimulating dormant stem cells in vitro are those that include at least one cytokine from each of the groups. There are also, apparently, cytokine combinations to avoid: when IL-1 or IL-3 is added to an effective cytokine cocktail, they seem to eliminate B-cell or T-cell lineages from the descendant blood cells.

Other work has focused on identifying viral vectors that can insert genes into nondividing cells. Adeno-associated virus (AAV), a nonretroviral vector with widely acknowledged promise, has proved to be an effective vector. But, oddly, preparations of high purity have a reduced ability to transfer DNA. R. Jude Samulski of the University of North Carolina (Chapel Hill, NC) has now explained why. The apparently effective AAV samples used in earlier work contained residual adenovirus that was critical in aiding gene transfer. It was not the adenovirus per se that was important, however. Samulski mapped the gene transfer function to a particular region of the adenovirus genome (E4 ORF6) and has now constructed AAV vectors that express the sequence. Impressively, these new constructs exhibit 100 to 1,000 times better gene transfer.

One other piece of the stem cell gene therapy puzzle was put into place at the Taos meeting. The stem cells, once transplanted into a host whose immune cells have been incapacitated, will regenerate all the immune cell types. Transplanted genes have been observed in these daughter cells, but proof that the marked cells descend from a true stem cell—rather than some other early progenitor cell—has until now eluded researchers.

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