Rabbit trail may lead to human gene therapy

In a small hop toward a gene therapy for humans, scientists have temporarily abated an inherited, cholesterol-elevating disease in rabbits using transplants of genetically modified liver cells.

The disease, known as familial hypercholesterolemia, results from a genetic defect causing a lack of the cell receptors that normally bind to and mediate the breakdown of low-density lipoprotein (LDL) cholesterol. In the absence of these receptors, LDL cholesterol builds up in the bloodstream, leading to atherosclerosis and an increased risk of heart attack or stroke.

A severe form of the disease strikes one in every million people in the United States, most of whom die of a heart attack between age 5 and 30. At present, the only moderately successful treatment for these patients is liver transplantation. Physicians treat a milder form, affecting one in 500 Americans, with drugs that are only partially effective and have undesirable side effects.

Researchers led by James M. Wilson of the Howard Hughes Medical Institute at the University of Michigan in Ann Arbor are now testing a gene therapy on the Watanabe rabbit, which invariably suffers from familial hypercholesterolemia and provides "as good an animal model as they come," Wilson says. The scientists targeted hepatocytes — cells of the liver, the sole organ that metabolizes and excretes cholesterol — in severely affected rabbits. Using a retrovirus, they inserted the normal human LDL-receptor gene into hepatocytes removed from three rabbits, then transplanted the modified cells into seven recipient rabbits.

Although the genetically altered cells boosted LDL-receptor activity to only 1 to 3 percent of the normal level, they substantially reduced blood cholesterol levels, the researchers report in the November PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES (Vol.87, No.21). Before treatment, the recipient rabbits had an average cholesterol concentration of 600 milligrams per deciliter of blood. (Less than 90 is normal for these rabbits.) Afterward, their counts dropped to between 400 and 450 — a decrease of roughly 30 percent. A control group of six Watanabe rabbits that received hepatocytes lacking the functional gene showed no significant changes.

Within two weeks, however, cholesterol counts in the "cured" rabbits returned to pretreatment levels, probably because the immune system rejected the modified cells, Wilson says. The researchers now seek to prolong the effect by injecting the altered hepatocytes into the same rabbits from which the cells were taken. "That way we can circumvent the problem of rejection based on different cells," he says.

If the revised approach works, the next steps will be to "make the efficiency of gene transfer a whole lot better" and to perfect the transplantation technique, says study coauthor J. Roy Chowdhury of the Albert Einstein College of Medicine in New York City.

"For sure, it has a great potential [for human gene therapy]," comments Barbara Obrepalska-Bielska, a biologist at Lehigh University in Bethlehem, Pa. "But our knowledge about all the immunological [aspects of such transplants in humans] is very small, so there will be a lot of technical problems."

To get an idea of how humans would react to injections of these genetically modified hepatocytes, scientists would first have to investigate the effects of transplanting normal, unaltered liver cells into people with familial hypercholesterolemia, Wilson adds. Indeed, that approach may offer a workable alternative to gene therapy. Researchers at the University of Illinois in Chicago have already shown that transferring normal hepatocytes from healthy New Zealand White rabbits into severely afflicted Watanabe rabbits can lower cholesterol levels — but "it didn't work 100 percent," says study leader Raymond Pollak. After injecting each of four Watanabe rabbits with four doses of normal hepatocytes over a three-week period, his team observed temporary cholesterol reductions ranging from 32.8 to 82.7 percent and lasting for more than 18 weeks. The findings will appear in the February TRANSPLANT PROCEEDINGS.

While Pollak's results are encouraging, such transplants would require a continuing series of injections, whereas Chowdhury's group hopes that improvements in the gene therapy approach will eventually bring lasting results with just one or two injections of modified, self-proliferating cells. "Trying to introduce the gene would probably be the highest form of refinement [in treating the disorder]," Pollak says. "But the next best thing would be to transfer the normal hepatocyte."

When and if human gene therapy for this disease becomes reality — a prospect some scientists envision within the next decade — it would benefit only those individuals whose high cholesterol results from familial hypercholesterolemia. Many different factors can elevate cholesterol levels, Chowdhury says, and unless a patient lacks the functional LDL receptor, gene-altered liver cells "won't help."

— I. Chen

Defusing arthritis with oral collagen

Oral doses of collagen — a structural protein especially important in joints — may hold promise as a treatment for rheumatoid arthritis, according to new animal research. Indeed, the study's authors propose that ingestion of different bodily "building blocks" might combat other autoimmune diseases. Physicians might someday limit or even shut down the self-destructive cycle of autoimmunity, they suggest, by feeding patients small quantities of the substance under attack.

Howard L. Weiner, an immunologist at Brigham and Women's Hospital in Boston, says he opted for the oral route in his collagen tests with rats because people "generally don't become sensitized to proteins that go through the gut." In clinical practice, he adds, oral doses offer a simpler approach than intravenous collagen injections, which have yielded mixed results in previous trials.

To create an autoimmune disease that mimics human rheumatoid arthritis, Weiner and his colleagues injected *Mycobacterium tuberculosis* into the tails of approximately 200 rats. Although rats with this disease develop an autoimmune attack against collagen II, scientists have generally assumed that collagen reactions do not play a central role in the animals' arthritis, Weiner notes. But in the Oct. 15 JOURNAL OF IMMUNOLOGY, his team reports data indicating that an experimental treatment limiting the body's attack against collagen II also dramatically diminished symptoms of arthritis.

The researchers fed 3 to 3,000 micrograms (μ) of collagen II to groups of 20 or 40 rats three times in the week preceding the bacterial injection. Thirteen days after the injection, animals receiving no collagen pretreatment developed the classic swelling and redness of arthritic joints. The same symptoms took up to two days longer to develop, and were only 56 to 68 percent as severe, in animals pretreated with 3 or 30 μ of collagen (the most effective doses). Moreover, these rats showed little allergic response to collagen in standard skin tests. The study also suggests that white cells called suppressor T-cells are involved in the arthritis suppression.

Similar results with animals fed myelin — a nerve-sheath material under autoimmune attack in multiple sclerosis — have led Weiner to begin oral myelin trials in multiple sclerosis patients. Next year, working with David E. Trentham at Boston's Beth Israel Hospital, he plans to conduct a six-month trial of oral collagen in 10 patients with rheumatoid arthritis.

— J. Raloff