GENE THERAPY

Animal Models Point the Way to Human Clinical Trials

During the past few years, the idea of using gene therapy to treat human genetic diseases has begun to move from dream to reality (also see p. 808). And that's given a new sense of urgency to researchers' efforts to develop animal models that can mimic the human diseases. In the past, such models offered little more than an exercise in comparative biology. A researcher could see how a biochemical flaw, such as a clotting factor deficiency, affected dogs, say, as opposed to humans. But once the advent of recombinant DNA technology allowed researchers to identify disease genes and attempt to correct the defects they cause by giving patients good gene copies, that all began to change.

Many, although not all, gene therapists are now coming to prize animal models as a way to test the safety and efficacy of their gene transfer protocols before applying them to human patients. "If you have an animal model, you are really remiss if you don't use it before going into patients. You can do in 6 months in mice what takes 6 decades in humans," says one model supporter, molecular biologist Drew Pardoll of Johns Hopkins University School of Medicine. Indeed, the animal work is already proving its mettle by helping researchers win approval for their human trials from the National Institutes of Health Recombinant DNA Advisory Committee (RAC) and the Food and Drug Administration.

What's more, the work is also getting a boost from the genetic engineers themselves. Researchers once had to rely on sharp-eyed veterinarians and animal breeders to spot animals with natural mutations—a hit-or-miss proposition at best. Recently, however, molecular geneticists have devised methods for altering specific genes in animals such as mice, thereby opening the way to creating a wide range of disease models that Mother Nature wasn't thoughtful enough to provide.

Still, most of the dozen or so animal models currently being used in gene therapy experiments were discovered the old-fashioned way, often many decades ago. And while they're not "high-tech" creations of the genetic engineers, they are providing valuable information about what may work—or in some cases not work—when it comes to human gene therapy.

Rabbit "gem." Take, for example, the current animal darling of the gene therapy world, the Watanabe rabbit, which is a model for familial hypercholesterolemia, an inherited disorder that sends blood cholesterol levels skyrocketing and causes the patients to die at an early age—often before their 30th birthdays—from heart attacks. The model owes its existence to Yoshio Watanabe of the University of Kobe in Japan, who in 1973 noticed fat-filled, yellow nodules on the feet of one of the rabbits he was tending. By breeding the animal, Watanabe developed a line of rabbits with extremely high blood cholesterol concentrations.

The Watanabe rabbit first became prominent during the late 1970s and early 1980s in the Nobel Prize–winning work in which Michael Brown and Joseph Goldstein of the University of Texas Southwestern Medical Center in Dallas established the biochemical cause of familial hypercholesterolemia. They showed that both the animals and the human patients have such high blood cholesterol because they have a deficiency of low density lipoprotein (LDL) receptors, which remove cholesterol from the blood for breakdown by the liver. Now the rabbits are making their mark again, helping James Wilson of the University of Michigan in Ann Arbor win RAC approval for a gene therapy trial for familial hypercholesterolemia that is expected to start in May.

In the protocol that the researchers devised for the trial, surgeons will first remove about 15% of each patient's liver. Wilson and his colleagues will grow the liver cells in lab culture and then use a retrovirus that has been crippled so that it can no longer replicate to carry a healthy LDL receptor gene into the cells. The genetically corrected cells will then be injected back into the patients' livers through a major vein. When tested in Watanabe rabbits this protocol produced a substantial reduction in the animals' cholesterol levels—at least 30% for at least 4 months. Indeed, RAC member Scott McIvor of the University of Minnesota hailed that work as a "gem."

Rodent work. Other models currently being used in gene therapy studies also have "gem" potential. Not only do the genetic and biochemical defects closely parallel those of the corresponding human diseases, but like the Watanabe, these models develop symptoms that help researchers assess the effectiveness of their gene therapy protocols. These animals include the Gunn rat, a jaundiced, natural mutant spotted in 1938 and eventually found to be a model for Crigler-Najjar syndrome, a very rare hereditary disease caused by a deficiency of an enzyme called UDP-glucuronyl transferase. As a result of the deficiency, bilirubin, a reddish-yellow pigment, accumulates in nerve cells, causing death in infancy in severely affected individuals.

In recent experiments, Olivier Danos of the Pasteur Institute in Paris has attempted to correct the Gunn rat's defect by using a retroviral vector to insert the gene for the missing enzyme directly into the animals' livers. The result? Their blood bilirubin levels declined by as much as 60%, Danos says. If further animal tests go well, he hopes to propose a protocol for treating Crigler-Najjar patients by the end of the year.

SOME OF THE ANIMAL MODELS USED IN GENE THERAPY RESEARCH

Animal	Origin	Gene Defect	Human Disease Equivalent
Watanabe rabbit	Spontaneous	LDL receptor	Familial hypercholesterolemia
Gunn rat	Spontaneous	UDP-glucuronyl transferase	Crigler-Najjar syndrome
"Cone-head" mouse	Spontaneous	Beta-glucuronidase	Sly syndrome (a lysosomal storage disease)
mdx mouse	Spontaneous	Dystrophin	Duchenne muscular dystrophy (mouse has no symptoms)
Sparse-fur mouse	Spontaneous	Ornithine transcarbamylase	Urea cycle disorder
Mouse	Knockout	Glucocerebrosidase	Gaucher disease (a lysosomal storage disease)
Mouse	Knockout	HPRT	Lesch-Nyhan syndrome (mouse lacks symptoms)
Golden retriever	Spontaneous	Dystrophin	Duchenne muscular dystrophy
Irish setter	Spontaneous	Factor VIII	Hemophilia A
Beagle hybrid	Spontaneous	Factor IX	Hemophilia B

Another natural mutant, the "cone-head" mouse, is also getting a lot of attention these days. These thick-skulled, short-lived dwarfs have a defect in the gene coding for an enzyme callede beta-glucuronidase. That means the mice are similar to humans with Sly syndrome, one of the "lysosomal storage" diseases in which an enzyme deficiency causes abnormal waste products to build up in the lysosomes—tiny, membrane-enclosed sacs that are supposed to serve as the cell's recycling centers. The result can be serious, even life-threatening, abnormalities in several organ systems. Sly syndrome, for example, is characterized by bone defects and sometimes by mental retardation.

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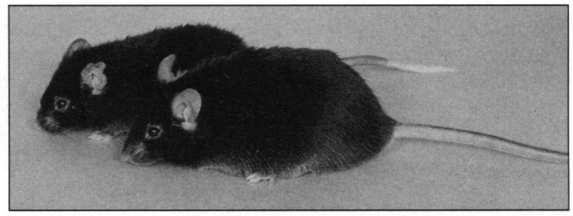

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Disease mimics. Researchers are looking to animals like these hemophilic Irish setters and the "cone-head" mouse *(at right rear)* to perfect their gene therapy protocols.

Edward Birkenmeier of the Jackson Laboratory in Bar Harbor, Maine, the investigator who originally identified the cone-head's defect, is now testing various therapeutic strategies in the animals. Early results suggest the best tactic may be a two-pronged attack in which the animals are given large amounts of replacement enzyme shortly after birth, followed by gene therapy.

And while researchers would prefer learning what gene therapy maneuvers work, the models have also proved useful in steering them away from ineffective approaches. For example, Inder Verma of the Salk Institute in San Diego decided to change his strategy for attempting to correct the defect in hemophilia B, which is caused by a deficiency of clotting factor IX, after a test in hemophilic dogs produced lackluster results.

Verma's original plan was to transfer a good gene for factor IX into dog fibroblasts, a type of cell that forms connective tissue, and then implant the cells in the inner layer of the dogs' skin. Although the liver is the normal site of factor IX formation, Verma reasoned that fibroblasts would do just as well as long as enough of the factor IX they produced got into the dogs' bloodstream to correct their clotting deficiency. Unfortunately, the experiments showed that the fibroblasts didn't make factor IX long enough to do the job. Verma has now decided to try to put the factor IX gene into dog myoblasts, which are immature muscle cells, instead of fibroblasts, in hopes of getting sustained production of the clotting factor.

As useful as these animal models have been, however, they are not without their problems. "No animal model is a perfect replica of a human. I think we need animal models, but the only way to cure disease in humans is to treat humans," says Steven Rosenberg of the National Cancer Institute, whose own research is aimed at using gene therapy to beef up the tumor-fighting abilities of cancer patients' immune cells.

Rosenberg's remark about no animal model being perfect applies even to the estimable Watanabe rabbit. Since liver surgery is a much different proposition in rabbits than in humans, the Michigan team had to shift to larger animals, baboons with normal LDL receptor activity, to test the surgical and cell cultivation techniques they plan to use in their gene therapy trial.

But some models are much less perfect than others, even though they have mutations that would seem to correspond to those causing human diseases. Take the case of the *mdx* mouse, which lacks dystrophin, the same protein that is missing in people with Duchenne muscular dystrophy. While the human patients suffer progressive muscular weakness that eventually leads to death, the mice do just fine without dystrophin. As a result, says Jon Wolff of the University of Wisconsin in Madison, the animals are virtually worthless for testing treatment efficacy, although they did help him gauge the efficiency of his gene transfer method.

Making models. The greatest limitation of natural animal models, however, is that they simply don't exist for many genetic diseases, including such common ones as cystic fibrosis and sickle cell anemia. That's where efforts to use genetic engineering technology to craft animals that mirror human diseases may help. A few years ago, researchers devised methods for producing mice in which transferred DNA has been inserted in specific genes, thereby disabling—or knocking out—the genes.

So far the results have been mixed. The knockout mutations, just like the naturally occurring *mdx* mutation, don't always cause symptoms. For example, in 1987 two British groups independently created new strains of mice in which the gene for hypoxanthine-guanine phosphoribosyltransferase (HPRT) had been knocked out. Although HPRT is the enzyme defective in Lesch-Nyhan syndrome, a human disease characterized by mental retardation and self-mutilation, the resulting mice did not develop comparable symptoms.

But other researchers have had better luck. Edward Ginns of the National Institute of Mental Health, working with gene transfer pioneer Richard Mulligan of the Massachusetts Institute of Technology, has used knockout technology to create a model for another lysosomal storage disease, namely Gaucher disease, which is caused by a deficiency of the enzyme glucocerebrosidase and is especially common in Jews of Eastern European origin, among whom the incidence is about 1 in every 625 people.

The mice developed symptoms, including lipid accumulation in their tissue macrophages and early death, that parallel those of babies with the most severe type of Gaucher. "A major reason we wanted to develop these mice was to test gene therapy," says Ginns, who notes that it may be possible to correct the defect with bone marrow cells that have been genetically engineered to contain a good copy of the glucocerebrosidase gene.

But even if the gene therapists get all the models they want, they can still encounter logistical problems in breeding and maintaining their animals. Just ask Michael Hayden of the University of British Columbia in Vancouver. The Canadian group wants to use a mutant cat discovered in New Zealand to test gene therapy for lipoprotein lipase deficiency, a disorder that results in sharp increases in blood triglyceride levels. But don't expect the gene therapy studies to get under way any time soon, says Hayden, bemoaning the fact that his female cats each produce only about one live kitten every 2 years.

And cost is also an issue, especially for the larger animals. Kenneth Brinkhous of the University of North Carolina, who's in charge of the colony of hemophilic dogs Verma is using for his gene therapy studies, estimates that the cost is two to three times that of maintaining a normal dog, partly because it's necessary to keep healthy dogs as blood donors for the animals with the clotting disorder.

Nevertheless, the promise of animal models outweighs their limitations for the many gene therapy researchers who have given them a central role in developing their treatment strategies. Says Johns Hopkins' Pardoll: "We are every bit as anxious to have an impact on human disease as clinicians. We feel we will only lose in the end by moving into humans without good animal work."

–Rebecca Kolberg

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