GENETIC DISEASES

A First Step Toward Gene Therapy for Hemophilia B?

For the child who has the hereditary bleeding disease hemophilia, treatment with the blood proteins called clotting factors can mean the difference between life and death. But the treatments available today could be considerably better: They do not cure the disease and they have some serious drawbacks. One of the most threatening is that they have the potential to transmit diseases, because most of the clotting factors currently used are prepared from human blood. What's more, they carry a steep price tag—as much as $100,000 per year to treat a patient with severe hemophilia. To circumvent these difficulties, hemophilia researchers would much rather attack the problem at its source, using gene therapy to give patients a good copy of the defective clotting factor gene that causes the bleeding problems of the disease.

Now, a team of researchers from Baylor College of Medicine in Houston and the University of North Carolina (UNC) in Chapel Hill, may have taken a step toward achieving that goal for one form of the disease, hemophilia B. This type accounts for about 15% of the cases and is caused by a defect in the gene for factor IX, one of several proteins needed for normal blood clotting. On page 117, Mark Kay, Savio Woo, and their colleagues report that they have partially corrected the clotting factor deficiency in dogs with hemophilia B. By introducing a copy of the factor IX gene into the animals' livers, the researchers were able to reduce the dogs' clotting times by more than 50%.

Although it offers considerable promise, this method isn't ready for the clinic yet. For one thing, the researchers weren't able to get the dogs' clotting time down to normal because the transferred gene doesn't make enough factor IX. Still, hemophilia and gene therapy experts find the results encouraging. "It's a very important paper," says gene therapy pioneer French Anderson of the University of Southern California. Even though factor IX production is low, the achievement, Anderson says, is "so much better than anything that's been done before. It's the first time there have been significant levels of a clinically important gene product in a large animal for a significant period of time."

Researchers are hopeful that a similar approach might also be used for other hereditary diseases, including hemophilia A, which accounts for the remaining 85% of the cases: All told, about 13,000 people in the United States have hemophilia and might benefit from gene therapy if it becomes a reality.

About a dozen groups are trying to do gene therapy for hemophilia, and most of these, at least in their published work, have taken an indirect route. They put the clotting factor gene into cells in culture, and then transplant those cells into experimental animals, usually mice. But the Baylor group decided to put the factor IX gene directly into the liver, which is the organ that normally makes clotting factors. If this technique works, Woo says, it should be easier to carry out and thus more widely applicable than the indirect procedures, which would require what Kay describes as "incredibly difficult" culture procedures for hard-to-grow human cells.

Kay, Woo, and colleagues started their effort by putting a cloned, functional copy of the canine factor IX gene into a retrovirus. That turned the virus into a biological shuttle for carrying the gene into mammalian cells, where the virus can incorporate itself—along with any genes it carries—into the cellular genome. The virus, which had originally been developed by Dusty Miller of the Fred Hutchinson Cancer Center in Seattle, was modified to prevent it from reproducing and spreading in the body.

WILLIAM BRINKHOUS

Well recovered. This hemophilia B dog, shown with technician Pamela McElveen, had factor IX gene therapy almost 9 months ago.

The next step was to put the retrovirus into the livers of the hemophilia B dogs, beagle hybrids bred by UNC's Kenneth Brinkhous and Marjorie Read that produce no detectable factor IX. But since retroviruses only insert their genetic material into cellular DNA in dividing cells, the Baylor team first had to get the dogs' liver cells to divide. The researchers did this by removing two-thirds of the liver from each of four dogs, because liver cells start dividing when part of the liver is removed in order to regenerate the organ. Then they injected the animals with the factor IX gene-carrying retrovirus, putting it into the hepatic portal vein that leads directly to the liver. One animal died, apparently because of excess liver damage during the surgery. But the remaining three began producing factor IX—although in small quantities. "The level is really quite low, about 0.1% of normal," says Woo. "When I first saw that data, I was not encouraged."

But other aspects of the results gave more cause for optimism. For one thing, the factor IX production has persisted for 9 months following gene therapy, indicating that the treatment may produce the long-term effects needed for human use. And even though the animals aren't making very much of the protein, even that small amount has significantly reduced their blood clotting times: from 50 minutes before treatment to about 20 minutes. That doesn't match the normal clotting time for dogs of 6 to 8 minutes, but given the small amount of clotting factor made by the inserted gene, Woo says he was "ecstatic" about the decrease. He estimates that increasing the activity of the transferred gene by 10- to 100-fold ought to be sufficient to provide therapeutic benefit. "If we can approach that, human gene therapy would be thinkable," he remarks.

A 100-fold increase would only bring the clotting factor production up to 10% of normal, but that could be more than enough to help hemophilia patients. Hemophilia specialist Louis Aledort of Mount Sinai Medical Center in New York City notes that patients who make as little as 1.5% of the normal amount of clotting factor do much better than the majority of hemophiliacs, who make less than 1%. In the group making under 1%, bleeding into the joints is a serious problem, despite the availability of clotting factors, since the bleeding quickly leads to a painful and crippling arthritis-like joint degeneration. But those in the less severely affected 1.5% group, Aledort says, "almost never bleed and hardly ever bleed into a joint. Gene therapy will revolutionize the whole field even at 1.5%."

While the current 0.1% level of factor IX production in dogs is a far cry from that magic number, Anderson, for one, is optimistic that researchers will be able to boost the gene activity to clinically useful levels. They

might be able to link the factor IX gene to more powerful regulatory sequences that can push synthesis of the clotting factor more powerfully than the viral sequence used by the Baylor group. Alternatives might be to improve the infection rate of the existing vector, or to find a better vector.

Some other experts in the field think finding a better vector is, in fact, the key. Based on his own group's experiences, molecular biologist Inder Verma of the Salk Institute, who is also trying to do gene therapy for hemophilia B, is skeptical that the retrovirus used by Woo and his colleagues will prove up to the job. Although his approach was different—Verma took the indirect route of putting the factor IX gene into myoblasts and then transplanting the cells into animals—he also used the same virus. But after disappointing results in dogs, Verma says he has switched to a different type of virus, a modified adenovirus that is now under investigation as a gene transfer vehicle in several labs.

Assuming that adequate factor IX production can be achieved, there will still be another major issue to resolve: Is the procedure safe? Aledort points out that the Baylor team's procedure would have to be performed before a child reaches 2 years of age to prevent joint degeneration. And removing part of the liver from a hemophiliac child could be dangerous. In addition, researchers will have to show that whatever viral vector they use will not reacquire the ability to infect other cells or activate oncogenes.

Of course, current clotting factor treatments carry their own risks, so a gene therapy wouldn't have to be perfect to be preferable to the methods now available. Heating clotting factor preparations kills the AIDS and the hepatitis B viruses, which infected many hemophiliacs before manufacturers began using heat treatments. Not all viruses are killed, however, so some risk of contracting a viral disease remains present. Clotting factors made by recombinant DNA technology eliminate this risk, but many patients can't afford to use recombinant materials, which cost about twice as much as the already-expensive products made from human blood. "Any treatment that would get [hemophiliacs] off the need for blood products would be tremendous," says Carol Letendre, associate director of the Division of Blood Diseases and Resources of the National Heart, Lung, and Blood Institute.

Researchers will have a way to go before that's possible, given the modest successes of the gene therapy work so far. But as Brinkhous notes, it reminds him of another modest start: "When the Wright brothers took off at Kitty Hawk, they only went a few hundred feet." So sometimes small beginnings can lead to great things.

–Jean Marx

ASTRONOMY

Are Dark Stars the Silent Majority?

Last week, two groups of astronomers announced the first hints of what may be a silent majority in our galaxy—starlike objects too puny even to shine. These stellar duds, which are thought to form a huge invisible halo around the galaxy, are known by the ironic acronym MACHOs, for Massive Compact Halo Objects. And it took some astronomical moxie to search for them: monitoring the brightness of several million stars night after night for months, waiting for a star to flicker as a MACHO eclipsed it.

That doggedness could have a big payoff. If the two groups—an American-Australian team led by Charles Alcock of the Lawrence Livermore National Laboratory and a French group led by Michel Spiro of the National Center for Scientific Research at Saclay—can find more examples to support their interpretation, they may have done much more than add another exotic creature to the zoo of celestial objects. They may also have accounted for some or all of the invisible "dark matter" that is thought to make up the bulk of our galaxy.

Both groups admit that the small number of examples—three in all—makes them uneasy, but some other researchers are already betting that MACHOs are here to stay. "Any alternative explanation [of these observations] would be even more spectacular," say Princeton University astronomer Bohdan Paczynski, who conceived the search strategy used by both teams. "I think this is definitely the discovery of the year."

Although MACHOs sound exotic, they're actually the least outlandish explanation for a long-standing puzzle. Twenty years ago astronomers noticed that the movements of stars in our galaxy and others could only be explained if there is some 10 times more celestial matter around than can be seen through telescopes of any kind. Since then, researchers have proposed many candidates for this mysterious invisible stuff: rocks, black holes, or mists of elusive particles. MACHOs, stone dead or weakly glowing bodies ranging between the size of Jupiter to about one-third the size of the sun, seemed the most likely candidate, says astronomer Jeremiah Ostriker of Princeton University. Astronomers are already familiar with objects that qualify as MACHOs, he explains—dim stars that only show up because they lie in our immediate vicinity.

Directly detecting these small, dark objects in the far reaches of our galaxy was out of the question, but in 1986 Paczynski proposed an indirect technique: Look at stars outside our galaxy—for example, in the Large Magellanic Cloud, a neighboring galaxy—and watch for signs that they were being eclipsed by MACHOs. You might expect light from the eclipsed star to dim, but Paczynski realized that the star would get brighter because of an effect known as gravitational lensing. Because of its mass, the MACHO would warp the surrounding space into something like a vast magnifying glass, intensifying the background light.

Paczynski's method posed a daunting technical problem, though: Even if our galaxy were swarming with MACHOs, alignments of MACHOs and ordinary stars would be rare in the vast volumes of space. The lensing effect would be so rare, in fact, that astronomers would have to monitor a million stars for months see it happen even once. Says Alcock, "His article attracted almost no attention at the time because it was not technically feasible." But in a couple of years star-monitoring and data-processing technology had advanced enough for both groups to take on the challenge (*Science*, 23 April, p. 492).

The American-Australian team started taking usable data in mid-1992 at the Mount Stromlo and Siding Springs Observatory in Australia, while the French, observing from the European Southern Observatory in Chile, didn't start until early this year. But both groups say they found their first lensing candidates within the last 3 weeks. The Livermore group reported that a computer analysis of their digital CCD images revealed a single star brightening by a factor of 7. The French group, which relied on traditional photographic plates, reported two stars that brightened, one by a factor of 2.5, the other by a factor of 3. The magnitude of the brightening, say the researchers, suggests that the eclipsing objects have between 3% and 30% as much mass as our sun, putting them in the expected size range of MACHOs.

Both groups admit, however, that they may be seeing something else—not gravitational lensing by a massive body but some sort of stellar flares. "We can't exclude that it's some pathological stars," says Spiro, who says he would rather not have announced the results to the public at this stage. The reason for the haste, he says, is that the two groups caught wind of the other's results and agreed that to ensure joint credit for the discovery, they would both announce at the same meeting, in Gran Sasso, Italy, send out press releases at the same time, and publish simultaneous papers (now in press at *Nature*).

In spite of his reservations, Spiro notes that neither his nor Alcock's events fit the profile of any known variable or flaring star. Alcock explains that in his group's event, the