BIOMEDICAL RESEARCH

Umbilical Cords: Turning Garbage Into Clinical Gold

Ordinarily, one of the least significant products of a birth is the umbilical cord: The cord is usually just discarded with other detritus. But recent research is changing all that. What was a discard has become valuable—indeed priceless to many children with leukemia, and perhaps in the future to children with AIDS and autoimmune diseases, such as diabetes and rheumatoid arthritis.

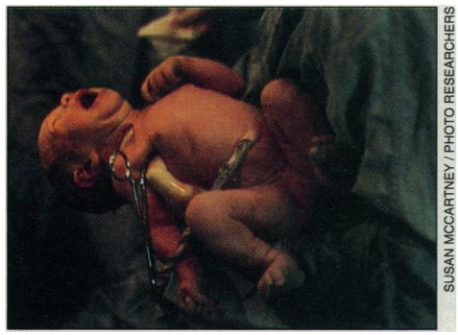
What makes umbilical cords so special? The answer is that they contain many stem cells bearing the CD34 cell marker, which normally reside in bone marrow. These cells are the factory of the blood system—continually regenerating themselves and differentiating into all other types of blood cells. With their myriad potential, stem cells are the key to bone marrow transplants. The advantages of using stem cells from cord blood rather than from bone marrow for such transplants are becoming increasingly clear.

Among those advantages: If the umbilical cord blood is stored (it can be stored indefinitely), it can be transplanted back into its owner, removing the need to find a matched donor. But cord blood could also be donated to an unrelated recipient, because it has reduced immunoreactivity and so lessens the risk of rejection by the recipient's immune system or of a devastating side effect known as graft-versus-host disease (GVHD). With such benefits, cord blood transplantation is rapidly catching on: Some 75 cord blood transplants have been performed worldwide, and cord blood banks are springing up at medical centers across Europe and the United States. Later this month Tufts University medical school is holding a major meeting on cord blood transplantation.

And the success of the transplants has sparked another idea: If cord blood stem cells can reconstitute bone marrow, why not use them for gene therapy? By adding a gene to the stem cell before transplantation, it should theoretically be possible to express the gene in every blood cell. "I think the application of cord blood to the clinic is going to be much larger for gene therapy than for strict transplants or malignant disease. We are talking about hundreds of thousands of patients a year," says hematologist David Harris of the University of Arizona, Tucson.

Others are not quite as gung-ho. John Barrett, head of the bone marrow transplant unit at the National Heart, Lung, and Blood Institute (NHLBI) in Bethesda, Maryland, concedes that cord blood therapy is "a good idea," but adds that "I'm a bit skeptical. I'm waiting to be convinced there will be less GVHD."

The reason cord blood contains more CD34 cells than adult blood is that a fetus generates large quantities of CD34s in its liver and bone marrow during development. Cell biologist Hal Broxmeyer of Indiana University in Indianapolis says these stem cells seem more active than normal: "Cord blood seems to have a greater proliferative capacity than the same cells you find in bone marrow." And that capacity for rapid increase is what gives cord blood an advantage over bone marrow. Says hematologist David Linch of University College London: "Bone marrow is rather a waste. You could throw most of it away, as all you are really after is the primitive stem cells that have both the self-renewal capacity and the capacity to repopulate and produce mature blood cells."



SUSAN MCCARTNEY / PHOTO RESEARCHERS

Don't toss it! Umbilical cords are finding new uses in bone marrow transplants.

The qualities of cord blood as a transplant agent were first put to the test in 1988 when Eliane Gluckman and her team from Hôpital St. Louis in Paris successfully used it to treat a child with a potentially fatal genetic disorder called Fanconi's anemia, which affects red blood cells. The team took whole cord blood from the child's unaffected sibling and transfused it into the patient. When the power of the technique was realized, researchers began developing the techniques needed to obtain and freeze umbilical cord blood.

In 1992, Harris set up one of the first cord blood banks at the University of Arizona, using his son Alexandre's umbilical cord as the first deposit. Since then his bank has ballooned; it is currently receiving five to 10 samples of cord blood a day. "We did our first cord blood transplant at the end of 1993, and in the middle of 1994 we opened our cord blood bank to the public," says Harris. People can make voluntary donations of their children's cord blood, or can pay to have blood stored for their later use, at a cost of $100 per year. Several other centers in countries such as Israel, France, and the United Kingdom now have cord blood banks or are in the process of setting them up with funding from charitable foundations.

In addition to having a higher efficiency than bone marrow, cord blood is less likely to trigger rejection and GVHD, which can result when transplanted marrow comes from a donor who is slightly mismatched immunologically. The reason is that cord blood has a muted immune system and "naive" T cells, which are not yet educated to attack specific antigens. "We don't know why the immune reactivity is decreased," says Gluckman. "It could be that the naive T cells are more tolerant, or there may be a suppressive effect by the maternal cells still floating in the cord blood."

Whatever the reason, hematologist John Wagner, who helped set up the cord blood bank at the University of Minnesota, sees this reduced immunologic antagonism to the host as a major selling point for cord blood transplants. He thinks it will obviate the need for the painstaking immunologic matching required for bone marrow transplants—which can take 6 months if no relatives are available as donors. To put that belief to the test, he is going to extend the bank to cover transplants other than between siblings. "This summer we are launching an unrelated cord blood bank where we expect to collect 10,000 cord bloods for unrelated transplants," he says. Arnon Nagler, head of the Jerusalem cord blood bank at Hadassah University, agrees that there could be huge benefits. "With cord blood you can take it and freeze it and so have the product available within 2 weeks or a month—this is especially useful for patients with acute lymphocytic leukemia or aplastic anemia who can't wait," he says.

For all its success so far, cord blood transplantation has yet to be demonstrated in adults, because there may not be enough blood in an umbilical cord. According to hematologist Jill Hows of Southmead Hospital in Bristol, U.K., who hopes to open Britain's first cord blood bank, "you can collect ... blood equivalent to 15×10^8 nucleated cells [from a cord]—a tenth of that needed for an average adult patient. The stem cells will have to be 10 times as good if you are to reconstitute an adult." Hows, among others, is working to optimize cell collection from cord blood samples and develop ways to expand the CD34 population.

But if you can completely regenerate bone marrow with cord blood, why not correct genetic defects or introduce whole new properties to the bone marrow? Donald Kohn of the Children's Hospital of the University of Southern California in Los Angeles made the first attempt at gene therapy

with cord blood in the spring of 1993 and announced his results at a gene therapy meeting in Steamboat Springs last month. Kohn's aim was to introduce a missing gene into three children suffering from adenosine deaminase deficiency, or ADA, a potentially fatal defect that cripples the immune system. His team separated the CD34 cells from each child's cord blood just after birth and then used a retrovirus as a vector to carry the adenosine deaminase gene into the CD34 cells. Kohn reinfused the cells back into the children when they were 3 days old.

The children have been followed for 23 months, and so far the experiment has been partially successful: The gene is expressed in about 1% of the bone marrow population, and messenger RNA for the enzyme is being produced—a sign that the gene is being expressed and that the enzyme is being made in the cells. "We probably only got the gene into 1% of the stem cells we treated, and they were probably diluted another 100 times by the rest of the bone marrow. Our main concern is to optimize the number of cells with the gene," Kohn says. Researchers in Amsterdam, Netherlands, carried out a similar procedure on children with ADA last year and are awaiting the results.

Many other therapies are in the pipeline, although large obstacles remain before some of the most exciting can be put into practice. Chris Walsh of NHLBI wants to treat Fanconi's anemia and thalassemia, but he needs to figure out, he says, how to turn on the relevant gene only in the right blood cells—for example to get red blood cells to express hemoglobin in anemia sufferers. "You'd like to know the regulatory mechanisms for these genes so they are turned on in the appropriate lineages," he says.

Perhaps the most ambitious proposed therapy comes from Anthony Ho and Flossie Wong-Staal of the University of California, San Diego, who would like to use Kohn's technique to treat children with AIDS. Stem cells are not infected by HIV, so the researchers' idea is to extract CD34 cells from the cord blood of babies born to HIV-positive mothers and insert into those cells an anti-HIV gene—for a ribozyme that can cleave HIV RNA. Expression of this gene would inhibit the reproduction of HIV in the child's cells. If the child eventually became HIV-positive, the HIV-resistant stem cells could be transfused back into the child, where they should produce HIV-resistant T cells and macrophages. The team hopes to start a clinical trial early next year.

Despite the obstacles, the components of cord blood look set for some close scientific scrutiny. Indeed, it's already true that doctors should think twice before discarding them.

–Clare Thompson

Clare Thompson is a medical writer in London, U.K.

CHEMISTRY

New Compounds Make Light of Pests

Low-flying helicopters, on a regular basis, spew dense clouds of a necessary evil across the California countryside. The clouds contain the insecticide Malathion, necessary because it kills Mediterranean fruit flies (Medflies) that threaten billions of dollars of California crops, but evil in the eyes of many Californians, who fear that Malathion—a nerve toxin—poses a threat to human beings as well as flies. Although toxicologists believe the health threat from the typical exposure to the insecticide is minimal, growing public outrage over Malathion's use has sent state officials scrambling for alternatives.

At last month's American Chemical Society meeting in Anaheim, California, researchers from the U.S. Department of Agriculture (USDA) reported that they may have found one. Field tests of two compounds that become toxic in the digestive tract of insects—but not mammals—showed they are highly effective against the Medfly and its equally destructive cousin, the Mexfly, or Mexican fruit fly.

The compounds, the chemical dyes phloxine B and uranine, have been used for decades to color drugs and cosmetics; they have already been deemed safe by the U.S. Food and Drug Administration. "We're excited about the potential of using these dyes as alternatives to Malathion," says Ted Batkin, a physicist who heads California's Citrus Research Board.

The dyes' selectivity stems from the fact that they turn toxic only after exposure to light. "Insects like fruit flies have translucent guts" that are easily penetrated by sunlight, says James Heitz, a biochemist at Mississippi State University. As a result, when flies eat a protein-based bait laced with the dyes, the compounds are activated in the flies' digestive systems. But in humans, other mammals, fish, or birds, the dyes pass through the digestive system without seeing the light of day.

The dye molecules' facility at absorbing

D. BURROWS / GAMMA LIAISON

Powerful protest. Controversy over the insecticide Malathion has spurred development of a light-sensitive replacement.

energy from photons starts a deadly chain of reactions in the insect, Heitz says. That excess energy gets nabbed by nearby energy-hungry oxygen atoms. The energy excites and destabilizes one electron orbiting the oxygen, transforming the atom into a form known as "singlet oxygen" that more easily binds to surrounding molecules. In insect tissues, singlet oxygen typically binds with lipids in cell membranes and amino acids in proteins, changing their structure and therefore interrupting their functions. This process, once started, kills cells and eventually the organism itself. (In cosmetics or on leaf surfaces this chain of events is broken, Heitz thinks, because reactive oxygen doesn't have a chance to penetrate cells before it reacts with other airborne compounds.)

Although these compounds don't kill Medflies as quickly as Malathion does, in lab tests the dyes—which seem to work best together—have proven quite lethal. At the Anaheim meeting, two groups of researchers from the USDA reported that the dyes appeared to be very effective in small field trials as well. One group, led by Bob Mangan and Daniel Moreno at the USDA's Agricultural Research Service in Weslaco, Texas, hung bait consisting of plant proteins laced with the dyes from grapefruit trees in a large cage containing 2000 Mexican fruit flies. Nearly 90% of the flies were killed within 4 days.

In the first uncaged field trial, Nicanor Liquido, a research entomologist with the USDA's Tropical Fruit and Vegetable Research Laboratory in Hilo, Hawaii, reported that on a 10-acre plot of coffee bushes sprayed with a mixture containing the dyes, the fruit fly population dropped 50% compared with an unsprayed plot. The population of flies in the sprayed plot would have dropped even further, notes Grant McQuate, a technician on the project, but the plot was surrounded by 1000 acres of untreated plantation, allowing flies from untreated areas to migrate into the test site.

Despite these promising results, the dyes are not ready to replace Malathion, according to David Bergsten, a toxicologist at the USDA's Animal and Plant Health Inspection Service in Riverdale, Maryland. Researchers need to show that the dyes don't break down into compounds that can harm crops. If they can, sunlight may soon not only help fields grow but keep them fly-free as well.

–Robert F. Service