Scientists Coax Nerve Fibers to Regrow After Spinal Cord Injury

In tests on rats, researchers at Johns Hopkins and the University of Michigan have developed a treatment that helps spinal cord nerves regrow after injury. The findings were published in the July 18 issue of the Proceedings of the National Academy of Sciences.

The researchers treated experimental nerve injuries in rats with the enzyme sialidase. Four weeks later, more than twice as many nerves in the spinal cords of sialidase-treated rats grew new nerve fibers compared to nerves in untreated rats. The experimental injury in rats mimicked an injury in humans that may occur during childbirth or in motorcycle accidents when an arm is pulled violently away from the body. This injury causes nerves to be yanked out of the spinal cord. Without these nerves, the arm loses feeling and muscle tone. Without muscle tone, the body cannot support the weight of the arm, and many health problems can develop.

While surgeons can sometimes reattach the yanked nerves to the spinal cord, this treatment is not as effective as physicians or patients would like. This is, in part, because nerves in the brain and spinal cord, unlike those in the rest of the body, fail to grow new nerve fibers.

"Molecules in the environment of the injured spinal cord are specifically instructing the nerve end not to regrow," says the study's director, Ronald Schnaar, Ph.D., professor of pharmacology and neuroscience in the Institute of Basic Biomedical Sciences at Hopkins.

"The brain and spinal cord are extremely crowded with nerves and nerve fibers, which may be why we have developed careful controls that tell cells to stop making new connections. The crowded central nervous system has ways to say 'OK, we're done' to keep nerves from sprouting willy-nilly and making inappropriate connections. But in gaining the ability to crowd nerves close together, we have given up flexibility - the ability to heal after injury."

Axon regeneration inhibitors, or ARIs, are molecules in the spinal cord that stop nerve fibers from growing. "Treatments that eliminate ARIs might allow the nerve ends to regain their natural regenerative abilities, as they do in the periphery, and improve recovery," says Schnaar.

The researchers surgically severed nerves that normally extend from the spinal cord to the shoulder of anesthetized rats. They then transplanted a nerve from the hind leg of the same animal into the spinal cord to reconnect the injured nerve ends. To coax the injured nerve ends to grow fibers and connect to the transplanted nerve, they used an implanted pump to bathe the area with one of three different enzymes known to destroy ARIs. Four weeks after transplantation and enzyme treatment, the researchers injected dyes into the nerves to see whether, and how many, nerve fibers grew from the injured cells of the

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Key Process in Cell Death Occurs As Single, Quick Event

The release of mitochondrial intermembrane space proteins into the cytosol is a key event that occurs during apoptosis. Using *in situ* fluorescent labeling, scientists at St. Jude Children’s Research Hospital have now demonstrated that this phenomenon occurs as a single, quick event, rather than as a step-by-step process.

Results of the study indicate the formation of pores in the mitochondrial membranes is a rapid process that allows a nearly simultaneous, rather than sequential, release of many apoptosis proteins, according to Douglas Green, chair of the St. Jude Department of Immunology. Green is senior author of a report on this work that appears in the August 1 issue of *Proceedings of the National Academy of Sciences*.

The process of pore formation, or mitochondrial outer membrane permeabilization (MOMP), allows mitochondrial intermembrane space proteins to escape and orchestrate the cell’s destruction. MOMP is controlled by a family of proteins called Bcl-2; some of these support apoptosis and others interrupt the process. The pro- and anti-apoptotic Bcl-2 proteins cooperate to weigh and balance cell signals that promote survival or death. During apoptosis, these proteins are either already on the mitochondrial membranes or migrate to the membranes, where they trigger MOMP.

Using *in situ* fluorescent labeling of proteins tagged with a short tetracysteine-containing sequence, the researchers were able to follow the release of the apoptotic proteins Smac, Omi, adenylate kinase-2, cytochrome c, and apoptosis-inducing factor (AIF) during apoptosis.

The team found that, after cells were treated with a chemical that triggers apoptosis, it took 3 to 10 minutes for cytochrome c, Smac, Omi and adenylate kinase-2 to escape together immediately following MOMP. However, AIF escaped from the mitochondrial membrane much more slowly and incompletely, starting with the release of cytochrome c but continuing during the next few hours. The researchers concluded that, while AIF is known to regulate other cellular processes, the protein itself is not involved in triggering apoptosis.

“The slow, continuous release of apoptosis-inducing factor (AIF) suggests that the pore formed during MOMP remains open for many hours,” Green said. “Our finding of nearly simultaneous, rather than sequential, release of the mitochondrial membrane proteins helps to explain the timing of the movement of these apoptosis proteins following MOMP. The findings also suggest that release of these proteins is not controlled by multiple levels of regulators, but rather occurs as a single event.”

The study also highlights the importance of the Bcl-2 family in regulating the formation of pores in the mitochondrial membrane and emphasizes how critical the formation of these pores is to the regulation of apoptosis, Green said.

ASBMB member Douglas R. Green holds the Peter C. Doherty Endowed Chair of Immunology at St. Jude Children’s Research Hospital in Memphis, Tennessee. He received both his B.S. and Ph.D. in biology from Yale University in 1977 and 1981 respectively. He was tenured at the University of Alberta in the Department of Immunology from 1989 to 1991, then served there as Adjunct Professor until 1993. From 1990 to 2005 Green served as member and head of the Division of Cellular Immunology at the La Jolla Institute for Allergy and Immunology. He currently worked as Adjunct Professor in the Department of Biology at the University of California, San Diego, from 1994 to 2005.

Green is a prominent scientist in the field of apoptosis and is well known for his research on how a breakdown in this process can trigger cancer in lymphocytes and other types of cells. In 2002 he received the MERIT Award from the National Institute of General Medical Sciences. Green holds several patents and is a member of the American Association of Immunologists and the American Association for Cancer Research.