suggesting that it is being chopped not by the enzyme that normally cuts the DNA during apoptosis but by some other DNA-degrading enzyme.

MacManus and Buchan argue that the neuron death in stroke is something between apoptosis and necrosis. They see this not as a setback but as an opportunity. "There may be processes in effect here ... that are specific to ischemic neurons," says MacManus. And that, Buchan adds, could "yield us a lot of therapeutic targets, which we badly need."

Others maintain that neurons dying by apoptosis shouldn't be expected to look exactly like other apoptotic cells. Nancy Rothwell of the University of Manchester in the United Kingdom notes that apoptosis was first described in dividing immune cells. "I would be surprised if the criteria established in one type of cell are going to look identical in another," she says, especially in neurons, which do not divide.

The view that apoptosis is the cause of death for some of the neurons killed by stroke got a big boost last year when Michael Moskowitz, Junying Yuan, and their collaborators at Harvard Medical School reported evidence suggesting that caspase activity plays a role in cell death after stroke. The team induced strokes in mice whose caspases had been blocked in either of two ways: by using peptides that inhibit a range of the enzymes, or by a genetic engineering trick that specifically inhibits a particular caspase called caspase-1.

Compared to control animals, caspase inhibition by either method decreased the area of stroke damage by up to 40% or 50%, says Moskowitz. What's more, the neurons were not only saved from dying but seemed to remain in working order; the animals had fewer movement and sensory impairments than controls.

Although many researchers consider caspase activation to be an even more reliable hallmark of apoptosis than the DNA changes measured in the earlier experiments, these results don't settle the issue. Caspase-1, the caspase knocked out in the genetically altered mice and one of those targeted by the peptide inhibitors used, lives a double life. In addition to playing a direct role in apoptosis, it also cleaves and activates interleukin-1β (IL-1β), a signaling molecule of the immune system that triggers inflammation. And inflammation after strokes also causes neuron damage and death.

Rothwell's team at the University of Manchester has shown that IL-1β activity goes up in the brains of rats after stroke and seems to contribute to the neuron damage that ensues. When they block IL-1 action with a protein called IL-1 receptor antagonist (IL-1ra), Rothwell reports, "it reduces ischemic brain damage by greater than 50%." Indeed, she adds, "there is considerable commercial interest" in developing IL-1ra into a possible stroke therapy.

But not all the effects of caspase inhibitors can be explained away by inhibition of inflammation. Moskowitz says his group detected the neuron protection before the time when inflammatory changes typically occur in the brain. What's more, other evidence suggests that caspase-3, which has no direct role in inflammation but is key to apoptosis, also helps kill neurons after ischemia. In May, Moskowitz, Yuan, and their colleagues reported that they had used antibodies that specifically recognize activated caspase-3 to show that caspase-3 activity increases in rodent's brains after stroke. Also in the past few months, Roger Simon and his colleagues at the University of Pittsburgh School of Medicine and a group at Eli Lilly and Co. in Indianapolis reported that caspase-3 gene expression is up as well in rats with global ischemia, the type of brain oxygen starvation that occurs during heart failure. "This puts the apoptosis story on a firmer footing," says Moskowitz.

Researchers will now want to follow up on this work by exploring the safety of known caspase inhibitors for use in stroke therapy, and by searching for even better drugs. And this may not be the only type of ischemia in which they might prove helpful.

Most neuroscientists agree that neurons in the brains of newborn rats deprived of oxygen undergo a death that has all the features of textbook apoptosis, including the intact membranes missing in adult brains. This led neurologist David Holtzman of Washington University to wonder whether caspase inhibitors might prevent cerebral palsy in human babies that have suffered ischemia during difficult births and those born weighing less than 1500 grams, half of whom develop some form of ischemic brain damage due to their immature lungs and brains. If neural apoptosis is to blame, says Holtzman, "one would predict that if you inhibited caspases ... you would protect the brain."

Animal tests of his hunch have looked promising. Holtzman's team reported earlier this year that apoptosis inhibitors given to newborn rats within 3 hours after the researchers had induced brain ischemia protected brain neurons from death. They are now studying rats treated this way to see if the neurons they save go on to function normally. At least one pharmaceutical company is interested in trying this approach to treat infants at risk for cerebral palsy, Holtzman says. So in young as well as old brains, protecting neurons from untimely death may provide a new lease on life.

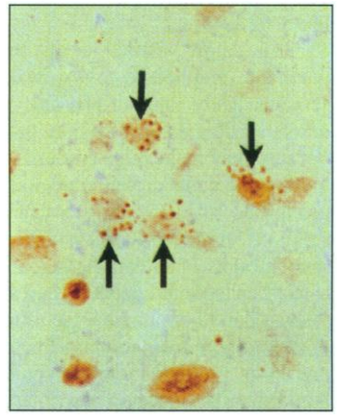
–MARCIA BARINAGA

Is Apoptosis Key in Alzheimer's Disease?

NEWS

Cell suicide may play a role in Alzheimer's disease, although the value of blocking it is not yet known

Researchers studying diseases as devastating as Alzheimer's want to explore every lead that could produce a treatment or cure. So while they have several good potential culprits in the nerve cell death that characterizes the disease—most notably a toxic protein called β amyloid (Aβ)—neuroscientists are also following up on evidence that some of the dying neurons commit a form of suicide called apoptosis. What remains to be seen is whether the cellular equivalent of suicide intervention will help slow the disease's progress.



CARL COTMAN, JOSEPH SU, AND CHRISTIAN PIKE

Telltale dots. Neurons in a brain from an Alzheimer's patient show a dotlike staining pattern characteristic of broken DNA in cells undergoing apoptosis.

Researchers have found that the brains of Alzheimer's patients contain dying neurons that display certain characteristic signs of apoptosis, such as DNA breaks. Even more intriguing, three proteins already linked to Alzheimer's pathology—Aβ itself, along with two others called presenilins—seem to drive cells into apoptosis when conditions are right. Because of the lack of good animal models of the disease, no one has been able to test whether inhibitors of apoptosis can protect against cell death in Alzheimer's, as they can in animal models of stroke (see p. 1302).

But if apoptosis does turn out to play a role in the nerve cell loss, the finding could lead to badly needed new Alzheimer's therapies—a goal that keeps researchers interested despite the uncertainties. "Will [preventing apoptosis] wind up being a good thing?" asks Steven Younkin of the Mayo Clinic in Jacksonville, Florida. "That is an open question ... but I don't think that means you shouldn't pursue [it]."

The notion that apoptosis occurs in Alzheimer's came to light in 1993 with the work of two research teams, Carl Cotman's at the University of California, Irvine, and Gianluigi Forloni's at the Institute of Pharmacological Research in Milan, Italy. The teams showed that Aβ, which builds up in the brains of people with Alzheimer's, caus-

es cultured neurons to die by apoptosis—also known as programmed cell death because it involves the activation of a genetic program for dismantling cells—rather than simply by falling apart in an uncontrolled form of death called necrosis. "This made a prediction that [apoptosis] might be present in human brains" affected by the disease, Cotman says.

Shortly thereafter, his team found signs of the broken-up DNA typical of apoptosis in brains from people who had died from Alzheimer's. But many researchers remain skeptical. "The notion that the neurons that die in Alzheimer's disease undergo apoptosis is a very sound concept that hasn't been totally proven," says Younkin. "There are problems looking in Alzheimer's brains and knowing whether you are looking at true apoptosis or just fragmentation of DNA that resembles apoptosis."

Cotman cites other data, including evidence for the activation of enzymes called caspases, which play a role in apoptosis, that convinces him that programmed death is really occurring in Alzheimer's brains. No one yet knows, however, exactly how Aβ might cause the apoptosis.

But Aβ is only one of the links emerging between Alzheimer's disease and apoptosis. Some researchers studying the presenilins, the protein products of two genes that are mutated in some inherited forms of Alzheimer's, suspect that these proteins might help to regulate apoptosis. Early evidence came from Luciano D'Adamio and his colleagues at the National Institute of Allergy and Infectious Diseases. In 1996, they discovered that cultured neuronlike cells known as PC12 cells that had been engineered to make presenilin 2 are much more sensitive to apoptosis triggers—including Aβ—than normal cells. What's more, PC12 cells engineered to make the mutant form of presenilin 2 that causes Alzheimer's disease were even more likely to die.

> Apoptosis in Alzheimer's disease "is a very sound concept that hasn't been totally proven."
>
> —Steven Younkin

That report verified what other researchers had also observed when they engineered cells to make presenilins, says Alzheimer's researcher Rudy Tanzi of Harvard Medical School in Boston. He and others speculated, however, that the engineered cells might be dying because they make huge amounts of presenilin, which could be overloading the cells' protein transport pathways. Dora Kovacs in Tanzi's group has ruled out that explanation, at least for the mutant presenilins. She recently found that cultured neurons making just normal amounts of mutant presenilin 1 look healthy but are still more easily pushed into apoptosis by various forms of stress.

Just how the mutant presenilins might increase neuronal susceptibility to apoptosis remains unclear. One possibility is that normal presenilin protein actually helps keep the brakes on apoptosis, and that the mutations somehow interfere with that function. Results reported last month by Jean-Pierre Roperch, Adam Telerman of the Fondation Jean Dausset-CEPH in Paris, and their colleagues support that picture. They found that two apoptosis-promoting proteins, p53 and p21, both turn off presenilin 1 production, and that turning off presenilin 1 by other methods also favors apoptosis. "If a cell wants to undergo apoptosis, it has to turn presenilin 1 down," says Tanzi. "That suggests presenilin 1 is normally antiapoptotic."

That's also suggested by an observation reported last year, first by Tanzi's group and then by D'Adamio's group and a team led by Helmut Jacobsen at Hoffmann-La Roche in Basel, Switzerland. These researchers found that in cultured neurons undergoing apoptosis, presenilins are cut by the caspases, protein-cleaving enzymes that are activated as part of the death process. One interpretation is that "not only does the cell turn off expression of new presenilin 1," says Tanzi, referring to the French team's results, "but it takes existing presenilin 1 and renders it inoperable by cutting it with caspase." If the presenilins are antiapoptotic, then cells engineered to make the normal proteins presumably are dying not by a specific activity of the presenilin but by the protein glut that some researchers suspect.

The caspase studies also suggest why mutant forms of presenilin might be less protective than the normal proteins: Researchers have observed that they are clipped more readily than their normal counterparts. Taken together, says Tanzi, the results suggest that "if you don't allow the presenilin to be clipped by the caspase, you could attenuate the amount of cell death." Such a strategy might lead to a new therapeutic approach, designed to block presenilin cutting. An approach like that might protect against both familial and the much more common nonhereditary Alzheimer's, if caspase cleavage of normal presenilin plays a role in that form of the disease.

But that is a big "if," and indeed the scenarios about possible presenilin function are very speculative and have yet to be tested. The majority of Alzheimer's researchers think that if apoptosis is involved in Alzheimer's, it is induced by Aβ, and the effect of the mutant presenilins is simply to raise Aβ levels. Several Alzheimer's labs have shown that the mutant presenilins cause neurons to make more Aβ, particularly the most harmful form, which contains 42 amino acids. "That would suggest that mutations in presenilins cause Alzheimer's by generating more Aβ 42," says Alzheimer's researcher Ben Wolozin. "Occam's razor says you don't need to invoke other things to explain it. Why make it more complicated?"

In the face of these differing views and unproven hypotheses, more work is clearly needed to resolve how—and even whether—apoptosis figures in the development of Alzheimer's disease. But even if apoptosis is established as contributing to Alzheimer's pathology, many neuroscientists doubt that blocking it will slow or halt the progress of the disease. "I think [apoptosis] is very far down the cascade of events driven by these pathogenic [Aβ] molecules," says Alzheimer's researcher Sam Sisodia of the University of Chicago.

Sisodia and others believe that by the time apoptosis occurs, Aβ has already damaged the neurons so severely that they are not salvageable and indeed that blocking apoptosis, a very neat way of eliminating damaged cells, could make things worse, leading to messy necrotic death, which triggers harmful inflammation.

The lack of good animal models for Alzheimer's makes this a difficult controversy to resolve. But given the possibility of a good therapeutic payoff, Alzheimer's researchers will undoubtedly continue to try.

–MARCIA BARINAGA

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