"Alz 50" Recognizes an Alzheimer's Protein

A research group at the Albert Einstein College of Medicine in New York has characterized a protein that appears to be specific to the brains of Alzheimer's patients. The protein is primarily associated with neurons having neuritic plaques and fibrillary tangles, abnormal structures that are a hallmark of Alzheimer's brain pathology. The protein, which is virtually absent from normal brain, was detected with the use of a monoclonal antibody, "Alz 50."

Peter Davies and his colleagues, Benjamin Wolozin, Alex Pruchnicki, and Dennis Dickson, who presented their work at the recent Society for Neuroscience meeting,* generated the Alz 50 antibody by injecting mice with a crude homogenate from the nucleus basalis, an area of the forebrain that is severely affected by Alzheimer's. They found that Alz 50 preferentially binds to neurons in the hippocampus, temporal cortex, and nucleus basalis, regions that typically show Alzheimer's plaque and tangle formation.

It has been known for some time that a fluorescent marker, thioflavin S, identifies Alzheimer's plaques and tangles but stains nothing in normal brain. Alz 50 antibody recognizes about 95 percent of the plaques and tangles stained by thioflavin S; it also recognizes plaques in the brains of aged Down's patients, whose neuropathology resembles that of Alzheimer's.

About 10 percent of the Alz 50 antibody staining is in cells that are negative for thioflavin S. Wolozin speculates that this staining may be directed toward neurons that will later develop tangles, or it may identify a subset of neurons that do not show tangle formation.

In contrast to the highly insoluble proteins associated with Alzheimer's tangles, the 68 kilodalton Alz 50 protein is very soluble and is easily degraded by trypsin treatment. Wolozin and his colleagues are able to show that Alz 50 also reacts with two normal brain proteins of different molecular weights that exist in only trace amounts. These two proteins do not seem to be present in Alzheimer's brain.

Any relationship these two scarce proteins in normal brain have with the plentiful Alz 50 protein from diseased brain remains to be established, a question that Davies calls "the number one topic of investigation right now." It is possible that the Alz 50 antibody recognizes similar amino acid sequences in the two proteins, or that the normal protein is somehow modified in Alzheimer's disease.

*Annual meeting of the Society for Neuroscience, 20 to 25 October, Dallas, Texas.

Disease Linked to a Faulty Potassium Channel

A research group at the University of California, Irvine, has described what appears to be "the first ion channel abnormality seen in cells of the immune system that is linked to an immune disease," according to George Chandy. Chandy, Thomas DeCoursey, Michael Cahalan, Sudhir Gupta, Norman Talal, and Michael Fischbach have characterized the abnormalities of a type of potassium ion channel in T lymphocytes from mutant mice that spontaneously develop an autoimmune disorder. Furthermore, Chandy says that the work reveals "the first mutation of a single gene locus causing an abnormality of voltage-gated channels in mammalian cells."

Speaking at the neuroscience meeting, Cahalan reported that the abnormal T-lymphocyte potassium channel is the kind regulated by changes in membrane potential. The mutant, or "L channels," require more membrane depolarization to open, stay open longer, allow more potassium ions through, close faster, and have different drug sensitivities than their normal ("N channel") counterparts.

The abnormal T lymphocytes are obtained from mutant mice that develop a disease resembling systemic lupus erythematosus, an autoimmune disorder. Human lupus patients develop antibodies against their own double-stranded DNA, have abnormally high numbers of B lymphocytes, which produce antibodies against some of their own tissues.

There are several mouse models for human lupus, one of which is the MRL strain used by the Irvine group. Chandy confirmed that the immunological properties of T cells from MRL mice having the autoimmune disease are suppressed compared with those of their congenic controls, which differ only in a single gene locus. In diseased mice, a subset of resting T lymphocytes has a unique set of cell-surface markers and, in culture, these cells fail to proliferate with mitogen stimulation.

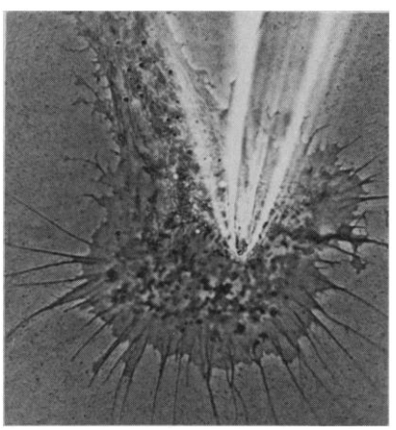
It is this group of T cells that also has abnormal "L"-type potassium channels. In parallel with the development of the disease, quiescent mutant T cells have 20 times more voltage-regulated L channels than resting normal T cells. DeCoursey compared the properties of individual potassium channels in many mutant and normal T cells and found that L channels do not open until the membrane is depolarized to −10 millivolts, in contrast to the −40 millivolts threshold required to activate N channels.

What does all this mean in terms of the mutant mice having an autoimmune disease? The researchers at Irvine have shown that if voltage-dependent potassium channels in normal T cells are blocked, the lymphocytes do not have a normal immune response. Also, if normal T cells are depolarized (by increasing the extracellular concentration of potassium), they are unable to divide. If T cells are to participate in an immune reaction, they must divide, so any defect that blocks their ability to proliferate also inhibits a normal immune response. Additionally, interactions between abnormal T cells that regulate B-cell production of antibodies may result in the production of autoantibodies by B cells which then destroy an organism's own tissues.

The whole concept of voltage-regulated ion channels in nonexcitable cells is a relatively new one. As yet, it is not known if the voltage-gated potassium channels in the nerve cells of mutant MRL mice are defective in the same way as their T-lymphocyte channels. According to Chandy, it is also essential to evaluate the T cells from other mouse "lupus models" to see if they also show abnormal channel activity. Whether lupus in some patients can be traced to a single ion channel defect is a more distant objective.

Action Potentials Halt Growth Cone Elongation

What makes growth cones stop growing and "stabilize" so that nerve cells can begin to communicate with each other? One likely mechanism involves electrical activity in the growing neuron. At the neuroscience meeting, Christopher Cohan of the University of Iowa, Iowa City, and Stanley B. Kater of Colorado State University, Ft. Collins, reported that action potentials cause growing growth cones to change their morphology and stop moving. These are events that precede the establishment of functional connections, or synapses, between nerve cells.



Growth cone from a cultured Helisoma *neuron with a patch clamp electrode to record ion channel activity.*

Growth cones are structures that form the tips of elongating neurites, fiber-like extensions produced by differentiating and regenerating nerve cells. Using cultured neurons isolated from the buccal ganglion (that controls feeding) of the snail, *Helisoma*, Cohan and Kater stimulated actively growing cells to fire action potentials.

Within 15 minutes, the cells' growth cones retracted their fine filopodial processes and stopped extending forward. After the electrical stimulation was stopped, the growth cones began growing again.

Cohan and Kater also used delicate patch clamp electrodes to record the activity of single ion channels in the growth cones. They wanted to know if a growing growth cone's ion channels were any different than those in a stabilized growth cone. They discovered that the active ion channels of growing growth cones were "masked" in a silent state, if the structures had stopped growing.

Obviously, electrical activity plays some role in regulating growth cone morphology and elongation. But how action potentials fired in the cell body of a neuron might communicate a change in the activation state of ion channels in a growth cone, is unknown. Kater proposes that electrical events may be transmitted to the growth cones and there activate the masking molecule to regulate growth cone ion channels.

During development, when a growing neuron receives electrical input from another cell, does it stop growing? And why are "active" ion channels conducive to growth cone elongation, but "inactive" channels are not? These, and many other questions, identify directions for future research.

A New in Vitro Model of Seizure Activity

No one knows what causes epilepsy, but some theories suggest that a group of neurons, interconnected to form a "local circuit," begin to fire together in an abnormal fashion. Finding an in vitro model to study the development and spread of "seizure-like discharges" has been the focus of many investigations.

At the neuroscience meeting, Wilkie Wilson and his colleagues, Steven Stasheff and Andrew Bragden, of Duke University Medical Center, in Durham, North Carolina, reported that appropriate electrical stimulation of certain pathways in the hippocampus, under otherwise normal physiological conditions, produces long-lasting electrical discharges similar to those associated with epileptic seizures.

The hippocampus is often studied as a brain area likely to be involved in the generation of seizures. One of the more interesting models of epilepsy is the kindling model first described by Graham Goddard. If repeated trains of electrical stimuli are delivered to the hippocampus of an experimental animal, at a "subconvulsive" level that does not cause seizures immediately, the animal's brain eventually shows abnormal electrical discharges and it develops seizures. The animal is thus "kindled," and the abnormal electrical activity that can be recorded from its brain reflects a permanent change.

Wilson's research group has used brief, high-intensity trains of stimuli to induce epileptic-like discharges in slices of rat hippocampus. (Similar or less intense stimuli would produce kindling in an animal.) They observed electrical phenomena typical of a brain area involved in epileptic activity, including afterdischarges that follow the stimulus trains, spontaneous excitatory bursts, and stimulus-triggered bursting of populations of neurons.

Thus, Wilson's group has taken a phenomenon, seen by other investigators but considered as unrelated to their own experiments, and looked at it closely in a physiological environment. "We're natural," says Wilson, referring to his efforts to keep the experimental environment as similar as possible to an in vivo situation. Unlike the approach used in many other laboratories, Wilson's slices exhibited seizure-like activity in a solution free of drugs and containing normal concentrations of ions.

Wilson notes that "[hippocampal] slice physiology has played a major role in understanding local circuit epilepsy." He believes that the new model, called "STIB" for stimulus train-induced bursting, offers another way to look at the development and expression of seizure-like activity in a local circuit.

Many people who suffer from epilepsy have an initial "focal" seizure, localized to one discrete region of the brain, that communicates its epileptic properties to surrounding healthy tissues, which then "learn" to be epileptogenic. Whether the mechanisms by which such focal seizures become "generalized" seizures are the same as those involved in animal models of kindling is as yet unknown.

An important next step for Wilson's group is to show that the pharmacology of his system is right. Wilson already has some preliminary evidence that indicates his slice cannot be "STIBBED" if certain excitatory amino acid receptors are blocked. He also plans to show whether drugs used to treat epilepsy will reduce or prevent the seizure-like discharges in his model.

DEBORAH M. BARNES