

Editorial

Reactive Astrocytes and Alzheimer's Disease

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Ten years ago, scientists at Stanford University challenged the accepted belief that astrocytes respond in the same way to all insults. In a series of elegant studies, glial cell biologist Ben A. Barres and his colleagues showed that genes expressing in astrocytes were different in response to an inflammatory attack than they were on the heels of an ischemic stroke. Since then, the questions have been why and how: Why do astrocytes respond in such different ways, and how are they able to have such different responses? Now, scientists have identified the toxic molecule that gets packed into reactive astrocytes and is responsible for neuronal cell death. It is not a protein, as they suspected, but a long-chain saturated lipid, and it seems to be involved in all neurodegenerative diseases. Blocking the lipid significantly reduces neuronal cell death, and it could be a novel target for treating any neurodegenerative condition.

The publication of this study in *Nature* in October 2021 is bittersweet as Dr. Barres died in 2017 and his laboratory closed down a few years later. But Dr. Barres holds a prominent place on the paper as its senior author and also in the ongoing commitment of his proteges to identify the toxic substance that drives neuronal death in a vulnerable brain. “We did not stop until we solved this puzzle,” said Shane A. Liddelow, PhD, assistant professor in neuroscience & physiol-

ogy and ophthalmology at NYU Langone Medical Center. The first author, Kevin A. Gattenplan, PhD, was a graduate student in the Barres laboratory when this work began. He was unstoppable in unraveling the science that led to the identification of the toxic lipid.

These saturated lipids are not found in the human diet. They are contained in ApoE and ApoJ lipoparticles that mediate the astrocyte-induced toxicity, explained Dr. Liddelow. The story that led to this latest discovery began in the Barres laboratory around 2010. Jennifer Zamanian, PhD, was doing her post-doctoral fellowship and isolated astrocytes in a reporter mouse following two different experimental conditions: One was an acute inflammatory model and the other was an ischemic stroke model. Following each of these two insults she looked at the gene expression profiles of the reactive astrocytes and they were very different. The findings were published in the *Journal of Neuroscience* in 2012. A year earlier, a graduate student in the Barres laboratory, Lynette Foo, was the first author on a paper in *Neuron* where she and her colleagues isolated astrocytes in a dish. This feat had never quite worked because scientists were using serum in their medium, and it changed the properties of astrocytes. She figured out a medium without serum and was able to create non-reactive astrocytes that had the same expression properties as astrocytes *in vivo*.

Shane Liddelow arrived a few years later for his postdoctoral fellowship, and he discovered that three

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specific cytokines, tumor necrosis factor, microglia-derived interleukin-1 alpha, and complement component 1q (C1q), turned a helpful astrocyte into one that was toxic to neurons following systemic inflammation. This served as his recipe to make and study reactive astrocytes in a dish and try to figure out whether they are functionally the same or different in the context of disease. It was clear from this work that once they enter a proinflammatory state they start producing a toxin that kills neurons. The last piece of the puzzle was another complement component, C3. This was upregulated in the toxic astrocytes. First, the proof was in rodent brain cells and then Dr. Liddelow and his colleagues used autopsied brain tissue from people with different neurodegenerative diseases—Alzheimer's, amyotrophic lateral sclerosis, Huntington's—and found that these same reactive astrocytes were responsible for the death of neurons.

But what was the elusive toxin mediating the death of neurons?

The scientists turned to a glaucoma model to find their answer. They realized that if they could stop astrocytes from becoming reactive that could prevent neuronal death. The neurons seem to retain their normal function. Dr. Liddelow left for NYU in 2018 and Kevin Guttenplan was the last graduate student in Ben Barres' laboratory. The funds were running out in the laboratory, and they knew that they did not have much time. Kevin Guttenplan had the conditioned media that contained many compounds and he wanted to fractionate every compound and try to isolate the substance responsible for the reactive astrocytes killing neurons. They all believed they were looking for a protein. But at the end of the long process what he found was an enrichment of lipoparticles such as ApoE and ApoJ. He found that by removing ApoE- and ApoJ-containing lipoparticles, there was a substantial decrease in neuronal toxicity. But if you take lipids from normal non-toxic astrocytes they do not kill neurons. What was it in these lipoparticles in reactive astrocytes that were mediating the death of neurons?

He figured out a way to clear all the proteins from the media and collect all of the lipids to isolate the one that was their Trojan horse. They purified the lipids and then added them into wells with neurons and saw that only the lipids from toxic reactive astrocytes could kill neurons. They performed lipidomics, and there it was: A very long-chain fully saturated free fatty acid. If they take these lipids and put them in a dish with neurons, they kill. Other lipids have no such effect.

Digging into the literature they found that there was an enzyme called ELOVL1 that was very important for the formation of these very long chain fully saturated lipids. The scientists in one of these earlier studies worked in the periphery and showed that it was an important defense mechanism in fighting off bacterial infections. It was a lead, and it was not long before Kevin Guttenplan made a knock-out mouse lacking this enzyme and tested it in astrocytes. The astrocytes could still become reactive but were no longer toxic to neurons, due to a significant reduction in these very long chain saturated free fatty acids. It fit together in a dish, said Dr. Liddelow. "But what happens *in vivo*? If there was a way we could stop these very long chain lipids, we could stop or significantly reduce neuronal death."

The scientists had already worked out an *in vivo* mouse model using an acute optic nerve axotomy injury, that is, they crush the optic nerve which in turn activates astrocytes around these neuron cell bodies in the retina. There is a single layer of these retinal ganglion cell neurons, and it happens to be the same layer that also contains astrocytes—by crushing the axons, astrocytes become reactive and secrete toxins onto surrounding neurons. But when the mice no longer produce ELOVL1 they no longer produce the toxic lipids and neurons do not die.

This becomes an intriguing target for neurodegeneration that would not be specific to one disease and could possibly bypass genetic predisposition within a disease. "We now have a full pathway from the initiation of disease to the death of neurons," said Dr. Liddelow. "This target should be applicable to any number of neurodegenerative diseases."

Ben Barres' team has gone on to model the pathway in an acute injury. They were able to stop 80% of the neurons from dying. That it did not confer total protection means that there may well be other cell death signals that they have not found. They are also now looking at neurodegenerative disease genes and risk genes to see whether these mutations change the underlying biology of astrocytes or alter their response to a secondary insult.

Dr. Liddelow said that the findings have created a bit of skepticism in the field. "It almost sounds too good to be true," he added. Still, they share their materials with colleagues in the field and others are replicating their findings.

Dr. Guttenplan completed his graduate degree in neurosciences this year and is now doing a postdoctoral fellowship at Oregon Health Sciences University in Portland. He is working in Marc

Freeman's laboratory. Dr. Freeman is director of the Vollum Institute at OSHU. Dr. Guttenplan continues to study astrocytes and how they influence neuronal pathways, and many in the Barres' laboratory continue to work on this puzzle.

What these scientists learned from their mentor was this: One needs to do everything they can to produce paradigm-shifting work. "This latest finding is a fitting end to Ben's fantastic career," said Dr. Liddelow of their mentor. "He always said that this work was the greatest science in his career, and I am sure he would be so incredibly proud of everything Kevin has discovered in continuing to unravel this complex and important biology."

Francisco J. Quintana, PhD, professor of neurology at the Ann Romney Center for Neurologic

Diseases at Brigham and Women's Hospital and Harvard Medical School, agrees. "Astrocytes have been described to display neurotoxic activity. Shane and Ben had reported a specific subset of astrocytes toxic to neurons, but no one knew what the toxin involved was. It is a beautiful series of studies. Now, the question is whether these astrocytes produce additional toxins, whether this neurotoxic mechanism is used by other astrocyte subsets, and the role of this neurotoxic mechanism in human neurodegenerative diseases."

DISCLOSURE STATEMENT

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