PRIZE ESSAY

FINALIST

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Kevin Guttenplan received his undergraduate degree in Neuroscience and Mathematics from Pomona College. He then went to Stanford University for his PhD, where he worked first in the laboratory of B. Barres and then, after Barres's passing, in the laboratory of A. Gitler and studied the role of astrocytes in disease and injuries of the nervous system. He is now a Helen Hay Whitney postdoctoral fellow in the laboratory of M. Freeman in the Vollum Institute at Oregon Health & Science University, studying the role of astrocytes in neuronal circuits. **www.science.org/doi/10.1126/science.ade2129**

eppendorf & Science PRIZE FOR NEURO BIOLOGY

NEUROSCIENCE

Why do neurons die?

Astrocytes emerge as key mediators of neurodegeneration

By **Kevin Guttenplan**

Why do neurons die in neurodegenerative disease? Despite decades of research, this question remains a fundamental and unsolved mystery of neurobiology. One clue came when 19th-century scientists noticed that astrocytes, cells that normally support the health of neurons, dramatically change morphology after neuronal damage (*1*), a response termed "reactive astrogliosis." Almost 150 years later, researchers found that astrocytes become actively neurotoxic in mouse models of amyotrophic lateral sclerosis (ALS) (*2*, *3*), hinting that reactive astrocytes might play an important role in neurodegeneration.

Despite this evidence, researchers have been historically hampered by a lack of tools to determine how astrocytes become reactive and drive neuronal death (*4*). To unravel the mysterious connection between astrocytes and neurodegeneration, my colleagues and I have pioneered methods to track and prevent astrocyte reactivity and uncovered a mechanism underlying astrocyte-mediated toxicity.

To better understand what reactive astrocytes do, we first sought to determine what factors make nonreactive astrocytes become reactive. We characterized the gene expression profile of reactive astrocytes in vivo and screened for molecules that could replicate this reactivity signature (*5*, *6*). We found that the cytokines interleukin-1α (IL-1α), tumor necrosis factor–α (TNFα), and complement component 1q (C1q) together are able to instruct astrocytes to become reactive. To test this model in vivo, we generated a triple-knockout mouse lacking IL-1α, TNFα, and C1q and found that the reactive-astrocyte response after neuronal injury was eliminated. Importantly, this molecular cascade is highly conserved in humans: We discovered a marker of this type of reactive astrocyte in our mouse models and observed that the same marker was substantially up-regulated in brain tissue from patients with Alzheimer's disease, Parkinson's disease, ALS, and multiple sclerosis (*5*, *7*). Through these experiments, we revealed a molecular mechanism that drives reactive astrogliosis in diverse human diseases and established genetic tools to investigate the influence of astrocytes on disease pathogenesis.

Next, we asked what role reactive astrocytes play in disease and injury. We started with a simple mouse model of axonal injury. Retinal ganglion cells (RGCs) are neurons that project from the eye into the brain, and acutely injuring RGC axons leads to the death of these neurons. Surprisingly, our triple-knockout mice that lack the reactive astrocyte response were completely resistant to this injury, and none of their RGCs died after axon damage (*8*). This demonstrated that factors that drive reactive astrocyte formation also drive neurodegeneration after acute injury and that loss of these factors prevents neuronal death.

We then explored a mouse model of glaucoma in which elevated intraocular pressure leads to progressive RGC death. Just as in acute injury, inactivating the factors that induce reactive astrocytes prevented the death of neurons in this chronic injury model (*8*). Further, morphological and electrophysiological analysis of surviving neurons revealed that they retained electrical responses to light stimulation and dendritic targeting in the retina. Together, these results raise hope that damaged neurons could be reincorporated into their endogenous neural circuits and, in the case of glaucoma, restore vision (*8*).

Finally, we tested the role of astrocytes in a mouse model of ALS. By inhibiting reactive astrocytes using our triple-knockout strategy in the *SOD1*^{G93A} mouse model of ALS, we slowed the progression of motor impairment and extended the overall life span of the mice by more than 50% (*9*). This result represents one of the longest preservations of life span ever reported in *SOD1*^{G93A} mice. Combined with our data in mouse models of acute and chronic injury, this protection highlights astrocytes as drivers of neurodegeneration and could propel therapeutic strategies that target reactive astrogliosis.

Once we knew that astrocytes were involved in the brain's response to acute injury and neurodegeneration, we wondered what mechanism they used to drive neuronal death. Our first clue was that media conditioned by reactive astrocytes, but not by nonreactive astrocytes, was actively toxic to neurons (*2*, *3*, *5*). This effect indicated

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that reactive astrocytes were secreting something that could kill neurons. To identify this factor, we biochemically fractionated reactive astrocyte–conditioned media and identified candidate toxic proteins by mass spectrometry (*10*). Unexpectedly, all toxic fractions contained apolipoprotein E (ApoE) and apolipoprotein J (ApoJ). ApoE and ApoJ normally shuttle lipids between cells in structures called lipoparticles, and mutations in these genes are known to increase the risk of developing Alzheimer's disease. Notably, using antibodies to deplete ApoE- and ApoJ-containing lipoparticles from reactive-astrocyte media blocked toxicity. Although ApoE- and ApoJ-containing lipoparticles appeared to be required for astrocytes to kill neurons, additional experiments suggested that the lipoproteins themselves were not toxic. Instead, our data suggested that the toxic factor was a lipid carried within these lipoparticles.

What, then, is the lipid responsible for astrocyte toxicity? We performed mass spectrometry to compare lipids secreted by reactive versus control astrocytes and found an increase in abundance of long-chain saturated free fatty acids and phosphatidylcholines. These lipids are normally present at very low levels in the cell and can induce a form of cell death known as lipoapoptosis. To our surprise, we found that these lipids are largely responsible for astrocyte toxicity—we could kill neurons in vitro with long-chain saturated lipids and could prevent astrocyte toxicity by eliminating mediators of lipoapoptosis. We then inactivated an enzyme required for the synthesis of toxic long-chain lipids specifically in astrocytes in mice and showed that it reduced the death of RGCs after axon injury, establishing the same mechanism of lipid-mediated neuronal death in vivo.

Through this work, we developed tools to track and inhibit reactive astrogliosis, a common feature of neurodegeneration. We used these tools to reveal that reactive astrocytes drive neuronal death and disease progression in mouse models of acute injury and neurodegenerative disease. Finally, we discovered a long-sought-after and unexpected mechanism—the secretion of a lipid rather than a protein—by which astrocytes can kill neurons. These findings highlight that reactive astrocytes and the secretion of toxic lipids are powerful mediators of neurodegeneration in vitro and in vivo and are promising targets for future therapies. ■

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10.1126/science.ade2129

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Science, 378 (6619), • DOI: 10.1126/science.ade2129