Extinguishing neuroinflammation

By Lev Osherovich, Senior Writer

U.S. and Australian teams have elucidated the pathway that leads to microglia activation, an early step in inflammatory neurodegenerative disorders. The studies suggest that dampening microglial activity early in disease could spare nearby neurons from destruction by glial cells. The findings could breathe new life into targeting neuroinflammation as a strategy to treat disorders such as Parkinson's disease, a tactic that had fallen out of favor because of a lack of clear targets.

One study describes the anti-inflammatory role of nuclear receptor subfamily 4 group A member 2 (NR4A2; NURR1).[1] The other proposes a mechanism for the proinflammatory activity of purinergic receptor P2X ligand-gated ion channel 7 (P2RX7; P2X7).[2] The challenge now is connecting the dots between these targets and testing whether modulating them can protect against disease progression in animal models.

The nuclear option

Mutations in NURR1 had previously been identified as a cause of a rare hereditary form of PD.[3] Based on that finding, a group at the **University of California, San Diego** (UCSD) sought to examine how NURR1 influenced brain inflammation triggered by bacterial endotoxins like lipopolysaccharide (LPS).

The team suspected that LPS could set off a neuroinflammatory process in glial cells such as microglia and astrocytes that mimicked what occurs in PD. Microglia are non-neuronal cells that protect the CNS from infection and foreign agents; astrocytes play structural roles in the brain.

"How inflammation begins in Parkinson's disease is a wide-open question," said Christopher Glass, professor of cellular and molecular medicine at UCSD. "Inflammatory processes can become self-sustaining. If you look at individuals with years of PD insult, you can see evidence of prolonged inflammation."

In a paper published in *Cell*, Glass and lead author Kaoru Saijo reported that knocking Nurr1 down with small hairpin RNA in mice increased the severity of inflammation caused by LPS compared with that seen in mock-treated controls. Without Nurr1, glial cells exposed to LPS became more active, producing higher levels of inflammatory cytokine–encoding mRNAs and neurotoxic effector proteins such as nitric oxide synthase 2 (inducible) (NOS2; iNOS) than those seen in mock-treated controls.

As a result, LPS-treated mice subjected to glia-specific Nurr1 knockdown rapidly lost dopaminergic neurons, a condition that resembled an accelerated form of PD. Nurr1 knockdown also exacerbated dopaminergic neuron loss in a different mouse model of PD involving mutations in α-synuclein (SNCA), another hereditary PD player.

The team went on to show that in murine cell culture, Nurr1 normally functions to repress the activation of microglia and astrocytes, the abundant glial cells that interact directly with neurons. The

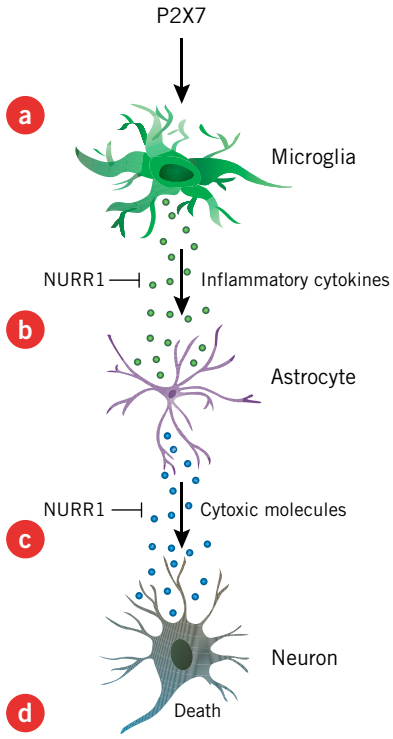

Figure 1. New targets for brain inflammation. Two studies suggest a possible sequence of inflammatory events that contribute to neuroinflammation in Parkinson's disease. Monif *et al.* report that a pore-forming variant of purinergic receptor P2X ligand-gated ion channel 7 (P2RX7; P2X7) promotes inflammation associated with PD through the activation of microglia, non-neuronal cells that protect the CNS from infection and foreign agents. Conversely, Saijo *et al.* found that nuclear receptor subfamily 4 group A member (NR4A2; NURR1) inhibits neurotoxic inflammation in microglia as well as astrocytes, non-neuronal cells that play structural roles in the brain and are also involved in the immune response in the CNS.

Together, the studies suggest that in PD microglia are turned on by P2X7 activity [**a**], leading to the production of proinflammatory cytokines [**b**] that stimulate astrocytes to produce cytotoxic molecules [**c**] that kill dopaminergic neurons [**d**].

P2X7 is being targeted by companies for rheumatoid arthritis (RA). **AstraZeneca plc** has its AZD9056 P2X7 antagonist in Phase II testing, and **Evotec AG** has a P2X7 antagonist in Phase I testing.

researchers worked out how Nurr1 and a complex of other nuclear proteins cooperate to repress the activity of NF-κB, a proinflammatory transcription factor.

The study "reveals a pathway that could be influenced by genetic mutations to make cells more vulnerable to an inflammatory response," said Fred Gage, professor of genetics at **The Salk Institute for Biological Studies** (*see* **Figure 1, "New targets for brain inflammation"**).

Gage, a coauthor of the *Cell* study, added that rather than being the on-off switch for inflammation, NURR1 is more like a fine-tuning knob.

"Just knocking down Nurr1 doesn't have an effect" in the absence of a proinflammatory stimulus like LPS, he noted. "Nurr1 is more of a protective modulator of NF-κB-mediated inflammatory responses."

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>
> ***—Christopher Glass, University of California, San Diego***

The therapeutic implication, said Glass, is that the activity of NURR1 or its transcriptional cofactors could be boosted to tone down the inflammatory component of PD.

Glass and Gage also noted that NURR1 and related proteins may play wider roles in inflammation beyond PD. Previously, Gage's lab reported that the absence of NURR1 made embryonic stem cells susceptible to toxicity caused by mutations in superoxide dismutase 1 (SOD1), an enzyme linked to amyotrophic lateral sclerosis (ALS).[4]

"There's evidence that NURR1 relatives influence inflammation in the cardiovascular system and can antagonize atherosclerosis," said Glass. "We're speculating that it could be involved in a variety of inflammatory diseases. This pathway seems to be broadly utilized."

The problem, he said, is that agonizing NURR1 could be more complicated than targeting most nuclear receptors because it has no known ligands and appears to be constitutively active. Thus, any agonist would have to increase the protein's activity beyond an already high level.

As a result, Gage suggested that some of the biochemical cofactors that work together with NURR1 could be better targets. These include lysine (K)-specific demethylase 1 (KDM1; LSD1), v-rel reticuloendotheliosis viral oncogene homolog A (RELA; p65) and REST corepressor 1 (RCOR1; COREST).

Glass and Saijo, a research scientist at UCSD, have not filed for patents covering their findings.

Pore it over

Meanwhile, a team at **The University of Melbourne** reported in the *Journal of Neuroscience* how P2X7 helps activate microglia. "We think we've found the receptor at the initial step of the whole pathway" described in the *Cell* paper, said David Williams, professor of physiology at the university and corresponding author on the *Journal of Neuroscience* paper.

According to Williams, P2X7 on the surface of microglial cells was thought to influence the innate immune cells' activation and inflammation, but how it did so was unclear because the protein has two distinct biochemical activities.

"This is an unusual purinergic receptor that has two modes—it mainly conducts calcium and sodium ions in response to ATP, but upon prolonged activation it switches into a pore for larger molecules," said Williams.

In a mutagenic screen for P2X7 variants, Williams' team identified a mutation that blocked the protein's ability to form a pore without affecting the ATP-gated ion channel activity. This allowed the group to determine which conformation of the protein—the pore or the channel—was most important for microglial activation.

The important conformation, the team discovered, was the pore. Cultured murine microglia expressing the pore-deficient mutant P2X7 had lower levels of proliferation and activity in response to LPS stimulation than controls expressing wild-type P2X7.

"The proliferative and proinflammatory effects of microglia go hand-in-hand," said Williams.

The team then showed that microglial activation by the pore-specific form of P2X7 required ATP binding and could be rendered inactive by a non-hydrolyzable analog of ATP. This provides some hope that the receptor could be targeted to prevent microglial activation.

Altogether, the findings suggest that excessive ATP levels force P2X7 to become a pore, leading to microglial activation. It is still unknown, however, what molecules enter or leave microglia through this pore as part of the activation process.

Mastura Monif, a graduate student at the university and the study's lead author, said the results highlight the role of extracellular ATP as an exacerbating factor in PD.

"Dying neurons can release high concentrations of ATP, which would stimulate the P2X7 receptor," said Monif. "Although their original intention is to protect nearby neurons, activated microglia set up a cascade that leads to degeneration of other dopaminergic neurons."

Williams' team is now testing whether adding microglia with wild-type or mutant P2X7 alters the course of inflammation and neurodegeneration in brain slices.

Williams and Monif also did not patent their discoveries.

Target practice

The two studies make a case for targeting microglial activation to treat PD, an area not occupied by many companies, perhaps because the specific molecular players in the process have been unknown until now.

Gage told *SciBX* that his team's earlier discoveries about NURR1 had been licensed by cardiovascular company X-ceptor Therapeutics Inc., which was acquired by **Exelixis Inc.** in 2004. Exelixis is no longer pursuing the target.

Håkan Eriksson, principal scientist in the Department of Molecular Pharmacology at **AstraZeneca plc**, told *SciBX* that the company previously tried to target NURR1 in PD. The project was canceled, he said, "because of strategic circumstances that made us leave Parkinson's disease."

Going forward, Eriksson thinks the *Cell* paper will rekindle interest in NURR1 agonists.

"With these data linking NURR1 to an anti-inflammatory pathway, I'm very confident that there will be a lot of effort to understand

this protein in wider fields, including chronic neurodegenerative conditions," said Eriksson. NURR1 has previously been "discussed in peripheral inflammation, but in the neuronal context this is a step forward," he added.

Eriksson believes that the UCSD researchers must show that NURR1 can be agonized to ameliorate pathology in animal models of PD. "There was definitely an indication that in this disease context, Nurr1 was not maximally activated and there was room for more activation," he said.

Gage said his team is already screening for anti-inflammatory molecules that influence the activity of the NURR1 pathway. Meanwhile, Glass is testing whether NURR1 activity in the periphery could be a predictive biomarker for greater PD risk.

At the suspected front end of the pathway, P2X7 is already being targeted with anti-inflammatory compounds, albeit in the periphery. AstraZeneca's AZD9056, a P2X7 antagonist, is in Phase II testing to treat rheumatoid arthritis (RA). **Evotec AG** has an unnamed P2X7 antagonist in Phase I for RA and inflammatory bowel disease (IBD).

Eriksson said that if AstraZeneca's compound can cross the blood brain barrier, it could be worthwhile to test it as a PD candidate.

"I don't know at this stage how such a program would be prioritized, but AstraZeneca should consider this very carefully," he said.

Eriksson noted that it remains to be seen whether P2X7 works in the same pathway as NURR1 or in a parallel pathway. The answer, he said, could come from a more detailed genetic characterization of the mutations and knockdowns used in the two studies.

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COMPANIES AND INSTITUTIONS MENTIONED

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