

Managing microglia in Alzheimer's

By Lev Osherovich, Senior Writer

Microglia are often found near damaged tissue in Alzheimer's disease patients, but whether the brain's innate immune cells are helpful or harmful in the disease has been an open question. Now, German researchers have evidence that both camps got it right. A pair of studies reveals that microglia play opposing roles in AD pathogenesis: they not only eliminate β -amyloid aggregates via phagocytosis but also kill nearby neurons by causing inflammation and the release of neurotoxic proteases.

Importantly, the reports suggest that the two functions of microglia are controlled by different cell-surface receptors, thus providing a road map for how to clear β -amyloid (A β) plaques without destroying healthy neurons that are in close proximity.

One report makes a case for antagonizing chemokine CX3C motif receptor 1 (CX3CR1), which promotes inflammation and neuron killing by microglia.¹ The other shows that an adrenergic receptor agonist from **Chelsea Therapeutics International Ltd.** and **Dainippon Sumitomo Pharma Co. Ltd.** stimulates a microglial mechanism for clearing A β deposits while suppressing inflammation.²

Together, the studies are "a good demonstration and proof of principle that targeting microglia can be beneficial" if it can be done selectively, said Douglas Feinstein, research professor of anesthesiology at the **University of Illinois at Chicago** and a research career scientist at the **Department of Veterans Affairs**.

Chilling, not killing

The chemokine receptor study, from a team led by Jochen Herms, professor of neurology at **Ludwig Maximilian University of Munich**, connects the dots and orders the events in AD pathology.

The group created an AD mouse model with microglia that lacked *Cx3cr1*, which is known to help microglia migrate to injured neurons. The team monitored the brains of both knockout mice and wild-type controls for 28 days using a recently developed *in vivo* imaging method.³

"We're imaging the fate of individual neurons," Herms told *SciBX*. "This is better than looking at postmortem histology."

Animals with *Cx3cr1* had higher levels of microglial migration around certain neurons than *Cx3cr1* knockout mice. Over time, these neurons died. In contrast, *Cx3cr1* knockouts did not have microglial accumulation or neuron loss.

Blocking microglial recruitment also prevented another key feature of AD pathology: hyperphosphorylation of microtubule-associated

protein- τ (MAPT; tau; FTDP-17). Using postmortem immunohistochemistry, the team found that neurons in *Cx3cr1* knockouts had little detectable hyperphosphorylated tau compared with neurons in wild-type controls.

Together, the findings suggest that recruitment of microglia is necessary for tau hyperphosphorylation and neuron death, and they suggest that CX3CR1 could be a critical target to block this process (see **Figure 1, "New AD targets"**).

"We were really surprised that the *Cx3cr1* knockout rescued neuron loss," said Herms.

Finally, the team found that *Cx3cr1* knockouts had A β levels that were comparable to those in wild-type controls. Results were reported in *Nature Neuroscience*.

Based on those data, Herms believes microglia do not affect the initial formation of A β fragments, which arise from cleavage of amyloid- β precursor protein (APP) near the surface of neurons (see **Box 1, "Stemming the tide of A β "**).

The main effect of disrupting microglial activation is "on neuron loss, not on plaque formation," said Samuel Gandy, professor of neurology and psychiatry at **Mount Sinai School of Medicine**. "This paper shows that turning off a signaling cascade in microglia dampens the production of neurotoxic substances" such as inflammatory cytokines and proteases.

Gandy coauthored a study last year which showed that eliminating the majority of microglia did not prevent A β production,⁴ a finding that is in line with Herms' conclusions.

One outstanding question is what attracts microglia toward A β -producing neurons in AD.

"We can't say yet what activates the microglia, but we know that if the chemokine receptor is missing, then they cannot be activated," said Herms.

One suspect is chemokine CX3C motif ligand 1 (CX3CL1; fractalkine), a neuronal protein that is the ligand for CX3CR1. Herms said that fractalkine "could come from neurons in response to A β accumulation."

He is looking to develop a CX3CR1 antagonist that mimics the effect of deleting the receptor. The chemokine receptor is a G protein-coupled receptor that should be druggable; however, its involvement in lymphocyte migration outside of the brain may complicate the search for a microglial-specific antagonist.

Herms said his team is collaborating with an undisclosed company that has a CX3CR1 blocker in preclinical development. The team plans to treat AD mice with the compound, looking for improvements in neuronal survival and cognition.

Herms did not patent his findings.

Microglial fight-or-flight response

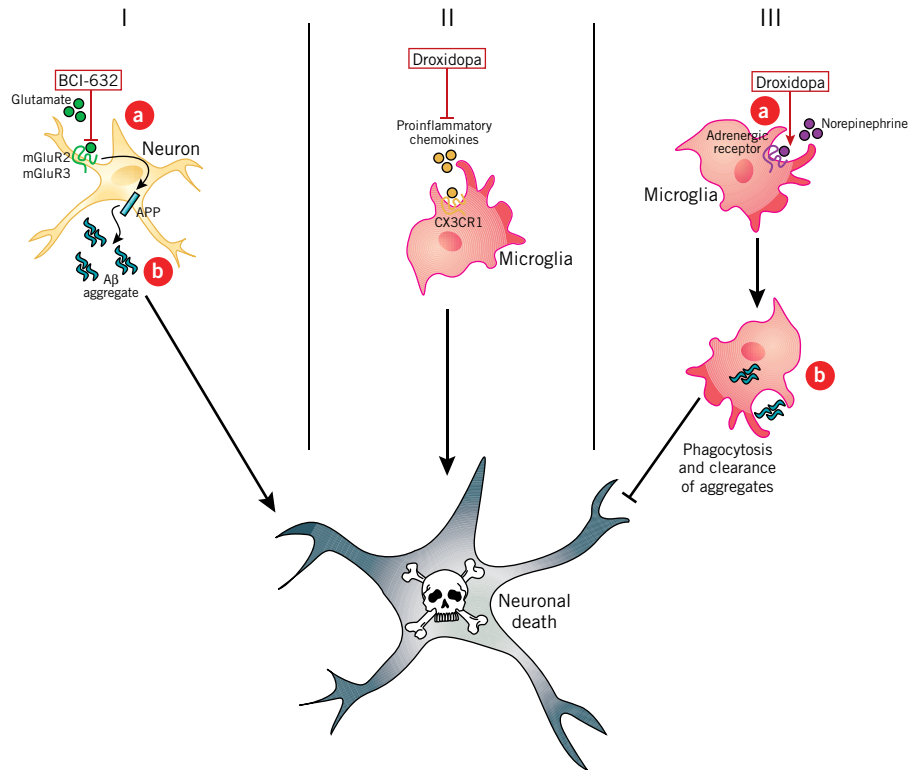
Whereas Herms' and Gandy's studies argue that microglia don't cause the production of A β , other studies have suggested that microglia help to get rid of A β through phagocytosis.⁵ But it has been unclear whether phagocytosis, which is seemingly beneficial, goes hand-in-hand with

Figure 1. New AD targets. Three recent reports suggest that a trio of G protein–coupled receptors could be targeted to treat Alzheimer’s disease.

(I) Kim *et al.* found that activation of metabotropic glutamate receptor subtype 2 (mGluR2; GRM2) and/or mGluR3 (GRM3) by glutamate [a] causes conversion of amyloid- β precursor protein (APP) into β -amyloid (A β) [b]. BCI-632, a group 2 mGluR antagonist now being tested by the researchers in mouse models of AD, is in preclinical development for cognitive disorders and depression by **Taisho Pharmaceutical Co. Ltd.** and **BrainCells Inc.**

(II) Findings by Fuhrmann *et al.* suggest that, in AD mice, activation of microglial chemokine CX3C motif receptor 1 (CX3CR1) by a currently unknown proinflammatory chemokine [a] leads to microglial inflammation and neuron killing by microglia. Heneka *et al.* found that norepinephrine also reduces inflammation, so treatment with droxidopa could protect neurons from microglia-induced death. **Dainippon Sumitomo Pharma Co. Ltd.** and **Chelsea Therapeutics International Ltd.** have droxidopa in Phase III clinical trials to treat neurogenic orthostatic hypotension (NOH) and in Phase II trials to adult attention deficit hyperactivity disorder (ADHD) and fibromyalgia syndrome (FMS). Droxidopa is also marketed in Japan, Korea, China and Taiwan for a variety of cardiovascular and neurological indications.

(III) Findings by Heneka *et al.* suggest that norepinephrine or the norepinephrine precursor droxidopa acts through an adrenergic receptor [a] in nearby microglia to stimulate their migration toward and phagocytic clearance of A β aggregates [b].



the undesirable inflammatory activities of microglia or whether the two functions can be separated.

An answer to this question comes from a study by a **University of Bonn** team led by Michael Heneka, professor of clinical neurosciences. His group has discovered that both microglia-mediated clearance of A β aggregates and inflammatory activation of microglia are controlled by a brain stem structure called the locus ceruleus. They also discovered that the two microglial mechanisms can be uncoupled.

Previous studies had shown that the locus ceruleus undergoes degeneration in AD even though it is far away from the main sites of AD-associated damage in the hippocampus and neocortex.

Heneka’s findings suggest that norepinephrine, a neurotransmitter produced by the locus ceruleus, keeps microglial activity in the hippocampus and neocortex in check. Agonizing norepinephrine signaling could thus be a point of intervention to prevent neurotoxic microglial activity in AD.

“The primary finding is that there’s a small brain area, the locus ceruleus, that has not previously been considered to play a role in AD pathogenesis,” Heneka told *SciBX*. “We find that not having this region can have effects on the neocortex and hippocampus.”

Building on an earlier study that showed norepinephrine reduces A β levels in AD mice,⁶ Heneka’s team tested whether norepinephrine affected the response of microglia to A β .

In cultured microglia, A β induced a range of proinflammatory genes compared with mock treatment. Additionally, treating the microglia with norepinephrine reduced expression of proinflammatory cytokines. Moreover, the microglia given norepinephrine mobilized and devoured A β aggregates by phagocytosis more effectively than the microglia given mock treatment.

These *in vitro* findings suggest that norepinephrine flips the microglial response to A β from an inflammatory mode to a phagocytic mode, which might be better for clearing up extracellular A β aggregates.

In vivo experiments supported the *in vitro* data. Compared with what was seen in mock-treated controls, eliminating norepinephrine by selective destruction of an AD mouse’s locus ceruleus reduced microglial recruitment and A β phagocytosis, leading to increased A β levels.

The defect in A β cleanup was corrected by treating the mice with droxidopa, a norepinephrine precursor in development for cardiovascular and neurological indications by Dainippon Sumitomo and Chelsea.

“We show that in the absence of norepinephrine, microglia are not able to phagocytose A β ,” said Heneka. “This is definitely harmful. If we treat the mice with a norepinephrine precursor, we can reset microglial function and get back phagocytic activity. I believe this could be a therapeutic strategy.”

Box 1. Stemming the tide of A β .

A study led by New York researchers has found that a family of metabotropic glutamate receptors stimulates the release of β -amyloid from synapses in response to neuronal activation, a process that contributes to Alzheimer's disease.⁷ The findings could open up a new indication for BCI-632, a metabotropic glutamate receptor antagonist from **BrainCells Inc.** that is in preclinical development for cognitive dysfunction and depression.

The team, led by Samuel Gandy, professor of neurology and psychiatry at **Mount Sinai School of Medicine**, showed that synaptic preparations secreted β -amyloid (A β) when stimulated with metabotropic glutamate receptor (mGluR) agonists compared with when they were stimulated with a mock control. Other team members were from the **University of Toronto**, the **University of Alberta**, the **University of**

Cambridge and the **James J. Peters VA Medical Center**.

Because there are several types of mGluRs, Gandy's group agonized each of the two major families of mGluRs found in neurons—group 1 and group 2. Agonizing group 1 mGluRs increased production of the relatively benign A β_{40} fragment, whereas agonizing group 2 mGluRs increased levels of the neurotoxic A β_{42} fragment.

Blocking group 2 mGluRs with an experimental antagonist prevented production of A β_{42} compared with that in mock-treated controls. Results were reported in *The Journal of Neuroscience*.

Roberto Malinow, professor of neurosciences at the **University of California, San Diego**, said the new findings are consistent with an emerging model of AD in which A β interferes with synaptic function. Malinow suggested that mGluR activation could set off a cascade that

leads to more A β production and less synaptic transmission.

The key next step, he said, is to test the effect of mGluR antagonists in "a more intact preparation" such as hippocampal slices or live animals.

Gandy is collaborating with BrainCells to test whether the biotech's BCI-632, a group 2 mGluR antagonist, reduces A β . Gandy is repeating the *in vitro* experiments using BCI-632 and hopes to test the compound in mouse models of AD.

BrainCells in-licensed the compound from **Taisho Pharmaceutical Co. Ltd.** in 2007.

Carolee Barlow, CSO of BrainCells, said modulating mGluRs to treat AD "opens up a whole new area" for the company. She noted that the company "would want to see benefit in *in vivo* models of AD" with BCI-632 before entering the AD field. —LZO

Heneka's paper "tells us that norepinephrine downregulates the harmful inflammatory effects but enhances the phagocytic effects" of microglial activation, said Feinstein, who was Heneka's postdoctoral mentor.

Both Feinstein and Heneka are testing whether droxidopa reduces overall A β levels and the behavioral defects of AD mice. Feinstein hopes to start an investigator-sponsored Phase I trial of droxidopa to treat AD this year.

Chelsea CMO William Schwieterman noted that "there has been some previous speculation that norepinephrine deficiencies exist in AD," adding that the Heneka paper shores up the evidence that modulating norepinephrine signaling could be therapeutic.

In 2006, Chelsea acquired rights to develop droxidopa outside of Asia from Dainippon Sumitomo, which markets the generic drug in Japan, Korea, Taiwan and China to treat hypotension. Chelsea is testing droxidopa in Phase III trials to treat neurogenic orthostatic hypotension (NOH) under the trade name Northera and in Phase II trials for adult attention deficit hyperactivity disorder (ADHD) and fibromyalgia syndrome (FMS).

Schwieterman said droxidopa has good CNS safety and availability and can be given orally. He said Chelsea is focused on the NOH program, but he noted that Heneka's findings mean that "in the future, we would consider it" as an AD candidate.

Gandy cautions, however, that norepinephrine's many roles in the nervous system could mean that a norepinephrine mimic like droxidopa could elicit side effects. Elsewhere in the brain, norepinephrine and the related neurotransmitter epinephrine influence heart rate, energy utilization and alertness as part of the fight-or-flight response to stress.

"Norepinephrine has a psychoactive effect, increasing anxiety and leading to behavioral concerns," said Feinstein. "It affects sleep, short-term memory, circadian cycles and attention."

An alternative strategy would be to selectively target norepinephrine receptors on microglia, but the precise identity of these receptors remains unknown.

"From *in vitro* studies, we know that the effects of norepinephrine are mediated by β_2 adrenergic receptors, but there could also be β_3 adrenergic receptors" involved in norepinephrine's effect on microglia, said Feinstein.

Heneka plans to pursue mechanistic studies of how the locus ceruleus controls microglial activity. He has not patented his discoveries. Feinstein has filed patents on the use of droxidopa to treat AD.

Combination approach

Together, the two new studies paint a picture of microglia as a double-edged sword, cleaning up A β deposits and killing nearby neurons. Although both functions are likely to be necessary in a healthy brain, the studies argue that an imbalance in these activities can exacerbate neurodegeneration in AD.

Therefore, rather than blocking microglia outright, the best route may be to fine-tune microglial activity with a combination of CX3CR1 antagonists and norepinephrine agonists.

Heneka thinks microglial modulation could be a good adjunct to therapies aimed at reducing A β levels, such as A β -directed mAbs and vaccines.

He noted that postmortem analysis of brains from patients in **Elan Corp. plc**'s failed Phase III trial of the A β vaccine AN-1792 showed high levels of active microglia despite a reduction in A β plaques. This suggests that overactive microglia were still killing neurons even when A β levels fell.

Herms also noted that modulating microglial activity could slow down neurodegeneration even in the presence of A β .

Microglia's normal function is to eliminate injured neurons, but "in AD there is too much destruction," said Herms. "Slowing the killing of neurons that aren't completely damaged may be beneficial."

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Contact: Jochen Herms, Ludwig Maximilian University of Munich, Munich, Germany
e-mail: Jochen.Herms@med.uni-muenchen.de
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Contact: Michael T. Heneka, University of Bonn, Bonn, Germany
e-mail: michael.heneka@ukb.uni-bonn.de
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Contact: Samuel Gandy, Mount Sinai School of Medicine, New York, N.Y.
e-mail: samuel.gandy@mssm.edu

COMPANIES AND INSTITUTIONS MENTIONED

BrainCells Inc., San Diego, Calif.
Chelsea Therapeutics International Ltd. (NASDAQ:CHTP), Charlotte, N.C.
Dainippon Sumitomo Pharma Co. Ltd. (Tokyo:4506; Osaka:4506), Osaka, Japan
Department of Veterans Affairs, Washington, D.C.
Elan Corp. plc (NYSE:ELN), Dublin, Ireland
James J. Peters VA Medical Center, Bronx, N.Y.
Ludwig Maximilian University of Munich, Munich, Germany
Mount Sinai School of Medicine, New York, N.Y.
Taisho Pharmaceutical Co. Ltd., Tokyo, Japan
University of Alberta, Edmonton, Alberta, Canada
University of Bonn, Bonn, Germany
University of California, San Diego, La Jolla, Calif.
University of Cambridge, Cambridge, U.K.
University of Illinois at Chicago, Chicago, Ill.
University of Toronto, Toronto, Ontario, Canada