Exploiting a window into AD

By Lev Osherovich, Senior Writer

A paper in *Nature* presents the first images of how Alzheimer's disease-associated amyloid plaques initially appear in living brains, and points to microglia, the brain's immune cells, as potential therapeutic targets. The question, however, is how to modulate microglia, as the specific role of these roving immune cells in Alzheimer's disease is still unclear.

Although figuring out how microglia are activated near amyloid will require further experiments, companies with amyloid-targeting AD therapeutics in the clinic could use techniques from the paper to tease out

the specific mechanisms by which their antibodies and vaccines work.

The study by researchers at **Massachusetts General Hospital** and colleagues puts to rest the debate over whether amyloid plaques are a cause or consequence of the activation of microglia, which are drawn to sites of tissue damage in the brain. Using multiphoton microscopy, the researchers repeatedly scanned the brains of living mice engineered to overexpress amyloid precursor protein (APP; also known as presenilin 1) and looked for areas that developed new plaques. The window into the brain showed the initial appearance of β -amyloid plaques, which consist of toxic fragments of APP, in areas without nearby microglia, proving that plaques come first.¹

By focusing on individual newly formed plaques for days at a time, the research team saw the arrival of microglia and the subsequent start of neuronal damage near the plaques. "We've had a chance to see the course of events play out in real time," team leader Bradley Hyman, professor of neurology at Massachusetts General Hospital and **Harvard Medical School**, told *SciBX*.

The paper shows that amyloid plaques grow surprisingly quickly. Hyman's team found that deposits emerge and grow to about 90 μm^2

Figure 1. Key steps in early Alzheimer's disease pathogenesis. Deposits of β -amyloid ($\text{A}\beta$) protein, called plaques, are the primary marker, and likely cause, of Alzheimer's disease.

[a] In AD, fragments of amyloid precursor protein (APP), termed $\text{A}\beta$ peptides, are generated by the sequential action of two enzymes, γ -secretase and β -secretase (BACE). $\text{A}\beta$ peptides accumulate in the extracellular matrix surrounding the affected neurons. Subsequently, soluble $\text{A}\beta$ peptides assemble into β -sheet-rich, highly stable amyloid fibrils.

[b] These bundles of amyloid fibrils then grow into plaques.

[c] The plaques attract migrating immune cells called microglia. Upon arrival, microglia release inflammatory cytokines that attract other immune cells and attempt to destroy the plaques by breaking them with secreted proteases or by swallowing them through endocytosis. Microglia can induce cell death in damaged neurons; researchers are as yet undecided about whether microglial activation antagonizes or exacerbates the consequences of plaque formation.

A report in *Nature*¹ indicates that this sequence of events can happen within days, which is much faster than previously suspected.

A number of companies are pursuing therapeutic approaches that go after targets in these early steps in AD pathogenesis (see **Table 1**, "Targeting β -amyloid").

within 24 hours. It was previously thought that plaques result from the slow accumulation of β -amyloid ($A\beta$) protein over many years.² Hyman's team also saw that fresh plaques induce abnormal curvature in nearby neurites, which connect neurons to one another, indicating that physical damage to neurons begins soon after plaques appear. (See **Figure 1**, “Key steps in early Alzheimer's disease pathogenesis.”)

Microglia: friend or foe?

Another finding of the *Nature* paper is that plaques stop growing at around the time microglia arrive, which is within days of initial plaque formation. This suggests that microglia could restrict the further growth of plaques and thus stall disease progression. However, other studies suggest that activated microglia may not be entirely beneficial.

“Microglia clearly can have toxic effects,” said Li Gan, an assistant professor of neurology at the **University of California, San Francisco's Gladstone Institute of Neurological Disease**. Gan, whose research

focuses on the role of microglia in AD, said the *Nature* paper strongly suggests that plaques stop growing because microglia restrict them, but noted that microglia seem unable to make the plaques shrink.

Indeed, microglial activation may be a double-edged sword. According to Gan, activated microglia secrete proteases and inflammatory cytokines that combat plaques, but they also damage nearby neurons and attract more immune cells, starting a cycle of inflammation that does more harm than good. Gan said that adding microglia to cultured neurons *in vitro* makes $A\beta$ even more toxic.³

Hyman agreed the role of microglia in AD is uncertain. “When microglia are activated, there's a whole repertoire of things they can do. In the early days of recruitment, their role may be critical in setting the course of disease,” he said.

Thus, the fact that plaques surrounded by microglia stop growing may explain why the brains of people who have had AD for 2 years usually show the same plaque size as people with AD for 20 years. On

Table 1. Targeting β -amyloid. A selected list of therapeutics in development for Alzheimer's disease that target early steps in β -amyloid-related pathology.

Therapeutic approach	Company	Product	Description	Status in AD
Anti-inflammatory	Hunter-Fleming Ltd., being acquired by Newron Pharmaceuticals S.p.A. (SWX:NWRN)	HF0220	Oral cytoprotective steroid	Phase II
	TransTech Pharma Inc./Pfizer Inc. (NYSE:PFE)	TTP488 (PF-04494700)	Receptor for advanced glycosylation end products antagonist	Phase IIa
	Reata Pharmaceuticals Inc.	RTA 404	Oral synthetic triterpenoid	Preclinical
β -secretase (BACE) or γ -secretase inhibitors	Myriad Genetics Inc. (NASDAQ:MYGN)	Flurizan ^a	R-enantiomer of flurbiprofen, γ -secretase modulator	Phase III
	Eli Lilly and Co. (NYSE:LLY)	LY450139	γ -secretase inhibitor	Phase II
	Humanetics Corp.	NIC5-15	γ -secretase inhibitor	Phase II
	CoMentis Inc.	CTS-21166	Small-molecule BACE inhibitor	Phase I
	TorreyPines Therapeutics Inc. (NASDAQ:TPTX)	NGX555	γ -secretase modulator	Preclinical
Small molecules preventing β -amyloid misfolding or β -amyloid aggregation	Pipex Pharmaceuticals Inc. (AMEX:PP)	Coprex	Tetrathiomolybdate anticopper agent	Phase II
	Transition Therapeutics Inc. (TSX:TTH; NASDAQ:TTHI)/Elan Corp. plc (NYSE:ELN)	ELND-005 (AZD-103)	Small molecule that disaggregates β -amyloid fibrils	Phase II
mAbs or vaccines targeting β -amyloid	Elan/Wyeth (NYSE:WYE)	Bapineuzumab	Humanized mAb against β -amyloid	Phase III
	Elan/Wyeth	ACC-001	β -amyloid-related immunotherapeutic conjugate	Phase II
	Baxter International Inc. (NYSE:BAX)	Gammagard liquid 10% solution	Immunoglobulin G antibodies plasma-based therapy	Phase II
	Affiris GmbH	Affitope AD01	Vaccine against β -amyloid	Phase I
	Cytos Biotechnology AG (SWX:CYTN)/Novartis AG (NYSE:NVS; SWX:NOVN)	CAD106	Vaccine with a fragment of the β -amyloid protein	Phase I
	MorphoSys AG (FSE:MOR)/Roche (SWX:ROG)	R1450	HuCAL-derived human mAb against β -amyloid	Phase I
	Pfizer	RN 1219	Humanized mAb against β -amyloid	Phase I
	Intellect Neurosciences Inc. (OTCBB:ILNS)	Recall-Vax	Vaccine based on a short fragment of β -amyloid peptide combined with an epitope from tetanus toxin	Preclinical
	Pharmexa A/S (CSE:PHARMX)/H. Lundbeck A/S (CSE:LUN)	PX106	Vaccine targeting β -amyloid-42	Preclinical

^aAlso shows anti-inflammatory properties.

the other hand, Hyman thinks that microglia trying to chew up plaques could free up small A β clusters called oligomers, which are thought to be especially toxic.

Gan suggested that a test for the role of microglia in AD pathogenesis would be to repeat Hyman's experiment in mice with defective microglia. For example, microglia that lack the receptors needed to home in on A β might not be able to restrict the growth of plaques. Alternatively, microglia that cannot secrete proteases or other toxins might limit plaque growth and could cause less inflammation and neuronal damage.

Engineering such mutants, however, is "very difficult technically," said Gan.

Plaque attack

A likely near-term use for Hyman's scanning technique could be to figure out how and where the current crop of AD therapeutics works.

"A lot more work can now begin to examine changes in amyloid deposition, microglial recruitment and neurite curvature" in response to therapeutics, said John Lin, senior director of neuroscience at **Pfizer Inc.**'s Rinat research unit. "This paper is absolutely great."

In addition to therapeutics aimed at preventing the production or aggregation of A β , there are at least nine different vaccines and anti-A β mAbs in clinical or preclinical development (see **Table 1**, "Targeting β -amyloid"). Vaccines work by triggering the production of host antibodies against fragments of A β protein, whereas mAbs are engineered to bypass the host immune system and directly bind A β .

In mouse models of AD, both types of immunotherapeutics delay the appearance of amyloid plaques, and in certain cases can cause pre-existing plaques to shrink.⁴ However, academic and industry researchers are still debating exactly how vaccine-elicited antibodies and mAb immunotherapeutics prevent or break up plaques, according to Hyman and Gan.

One view is that antibodies somehow evade the blood-brain barrier, bind to plaques and recruit immune cells such as microglia. According to Gan, microglia obtained from A β -vaccinated mice are full of A β fragments, suggesting that microglia can swallow plaques that have been flagged by the antibodies the mouse produces in response to the vaccine. However, direct evidence that microglia are required for AD vaccines to work has not yet surfaced.

"It's clear that microglia are a key mediator of plaque clearance," said Markus Mandler, head of the neurodegeneration department at **Affiris GmbH**. "It would be very interesting to see what active immunization does to the plaques" using Hyman's technique. Affiris' Affitope AD01 vaccine against A β is in Phase I testing to treat AD.

Another view is that antibodies trap soluble or oligomeric A β that leaks out of the brain before forming plaques, possibly dumping the neutralized protein somewhere in the body.

Lin believes Pfizer's anti-A β mAb, RN 1219, falls into the latter camp. The antibody is "immunologically inert, i.e., devoid of ability to activate microglia," he said, and thus is likely to function by trapping A β that leaves the brain. RN 1219 is in Phase I testing.

Hyman's technique could resolve the debate between the two camps. Therapeutics that bind up soluble A β should block or delay the initial formation of plaques, whereas antibodies that modulate the brain's immune response to plaques could change the speed or extent of microglial

recruitment after plaques have already formed.

Erroneous inflammation

Whether microglia play a role in antibody-based AD therapeutics, researchers agree that the *Nature* paper makes the immune cells attractive drug targets, although the specific approach is up for debate.

"Ideally, we would want to enhance the ability of microglia to metabolize misfolded protein aggregates but diminish the pathways leading to secretion of toxic enzymes," said Gan.

According to Pfizer's Lin, the key is to figure out how to modulate immune activation without eliciting overt inflammation of the CNS. That side effect caused **Elan Corp. plc** and partner **Wyeth** to halt a Phase IIa trial of their AN-1792 vaccine against A β in 2002.^{5,6}

One tactic to control inflammation involves nonsteroidal anti-inflammatory drugs (NSAIDs), which could limit the damage caused by excessive microglial activation. However, NSAIDs have a mixed record as AD therapeutics. Epidemiological studies suggest that over-the-counter NSAIDs might ward off clinical AD,⁷ but prospective studies of several such drugs (**Merck & Co.**'s Vioxx rofecoxib, as well as naproxen and diclofenac) did not show a clear benefit.⁸

Moreover, the targets and mechanisms of NSAIDs are unclear, at least in the case of AD.⁹ Part of the problem, said Chris Wigley, VP of research at **Reata Pharmaceuticals Inc.**, is that the products of cyclooxygenase (COX) enzymes can either promote or antagonize inflammation, depending on the context.

Wigley expects that Reata's RTA 404 works by tipping the balance toward anti-inflammatory molecules. RTA 404 is a triterpenoid that mimics the anti-inflammatory cyclopentone prostaglandins, which are synthesized as a result of COX activity. The compound is in preclinical development.

"The clearly demonstrated relationship between plaque formation and microglial activation and recruitment [shown by Hyman's paper] emphasizes the importance of investigating molecules such as RTA 404 that suppress inflammation," said Wigley.

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COMPANIES AND RESEARCH INSTITUTIONS MENTIONED

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