

TITLE:

RIPK1 promotes inflammation and beta-amyloid accumulation in Alzheimer's disease

[ALTERNATIVE: RIPK1 inhibition attenuates inflammation and beta-amyloid accumulation in Alzheimer's disease model]

David C Rubinsztein^{1,2}

¹Cambridge Institute for Medical Research, Department of Medical Genetics, ²UK Dementia Research Institute, University of Cambridge, Hills Road, Cambridge CB2 0XY, UK.

Email: dcr1000@cam.ac.uk

Alzheimer's disease (AD), the leading cause of dementia, is a major cause of death and a significant economic burden. In 2016, approximately 700,000 Americans aged 65 and over died of AD, and the total health and social care payments for AD in the USA alone exceeded \$230 billion (1). Currently, there are no validated disease-modifying therapies that slow the progression of human AD.

In this issue of PNAS, Junying Yuan's laboratory have provided new insights connecting two key aspects of AD pathogenesis — inflammatory signalling and the consequences of this for deposition of beta-amyloid (4). AD manifests pathologically with extracellular beta-amyloid deposits and intraneuronal tau aggregates. Beta-amyloid is a cleavage product derived from the amyloid precursor protein (APP). Mutations in APP and in processing enzymes that produce beta-amyloid suggest that excessive beta-amyloid is sufficient to cause AD (2). Likewise, tau mutations cause forms of frontotemporal dementia, arguing for a pathogenic role for tau in AD.

At the same time, increases in inflammatory processes are also prominent in AD brains or in response to beta-amyloid and manifest with increased levels of activated microglia (macrophage-like cells in the CNS) and the secretion of chemokines and cytokines (3). Many of the loci implicated in AD risk from genome-wide association studies include genes regulating inflammation (5). However, how systemic immunity, monocytes and brain-resident microglia affect AD pathogenesis has been unclear. Among the cytokines that are elevated in AD, TNF- α has been strongly implicated in inflammation and pathogenesis (8).

One possibility is that microglia phagocytose extracellular beta-amyloid and degrade these toxic molecules in lysosomes. However, beta-amyloid may impair this protective activity (6). Single cell analyses have also revealed a distinct microglial cell type associated with neurodegenerative diseases including AD and a form of motor neuron disease. These disease-associated microglia (DAM) appeared to be concentrated around beta-amyloid plaques and were inferred to protect against neurodegeneration (7).

Yuan and colleagues now provide important links between inflammation, DAM and beta-amyloid metabolism via a kinase called RIPK1 (4). Although RIPK1 is often thought of as a mediator of necroptosis, a form of necrotic cell death, it also promotes inflammation downstream of the TNF α receptor: mice carrying kinase-dead knock-in mutations in RIPK1 are protected against TNF α -induced inflammation (9). In this study, the authors implicate this latter pro-inflammatory role of RIPK1 in AD.

Yuan and colleagues initially observed that the levels of RIPK1 are increased in brains from AD patients and a mouse model of AD (APP/PS1). This was associated with increased RIPK1 autophosphorylation, a marker of its activation (4), in microglia. Interestingly, TNF α levels were also increased in the brains of the AD patients, consistent with previous observations. They then tested if RIPK1 was involved in AD pathogenesis using two complementary strategies: they either treated mice with necrostatin-1 (Nec-1s), a CNS-penetrant RIPK1 inhibitor discovered previously by Yuan's group (10), or crossed the AD mice with a mouse that carries a kinase-dead RIPK1 knock-in mutation. Both strategies decreased the beta-amyloid plaque burden, rescued the hyperactivity of the APP/PS1 mice and improved their spatial working memory in a water T-maze test. Nec-1s did not protect against beta-amyloid mediated cell death in primary cortical neurons, suggesting that it acted independently of necroptosis. However, the numbers of beta-amyloid plaque-associated microglia and the levels of TNF α were reduced in AD mice with incapacitated RIPK1 or in primary mouse neurons exposed to beta-amyloid peptides.

Consistent with these *in vivo* studies, beta-amyloid activated RIPK1 *in vitro*. Their data also suggested that RIPK1 inhibition may also enhance microglial beta-amyloid degradation, which could account for lower beta-amyloid levels in the treated mice. They identified an intriguing link between these phenomena by showing that the mRNA expression of the *Cst7* gene encoding cystatin F, which is expressed in microglia, was upregulated in response to RIPK1 activation, and could be attenuated in the AD mouse microglia by RIPK1 inhibition. This is exciting as cystatins are endogenous lysosomal cathepsin inhibitors and upregulation of this enzyme inhibitor would be predicted to impair lysosome function, as the authors observed when they overexpressed cystatin F in cell lines (4).

These data suggest that the induction of cystatin F levels in disease-associated microglia via RIPK1 activation impairs microglial removal of beta-amyloid via phagocytic-lysosomal pathways. It is likely that this is one of many pathological processes induced by elevated RIPK1 activity. But it will be very difficult to show that it is a predominant pathway, short of repeating all the experiments on a cystatin F-null background. It is interesting that deletion of another cystatin, cystatin B, ameliorated autophagic-lysosomal pathology, reduced beta-amyloid levels in an AD mouse model and improved learning and memory (11); this supports the current model and the importance of cystatin levels in disease. Indeed, cystatin F levels appear to increase in other mouse models of neurodegenerative diseases, including motor neuron disease caused by mutated SOD1 and prion disease (4, 7, 12), as well as in DAMs in human AD.

A major model of AD pathogenesis, the so-called amyloid hypothesis, posits that beta-amyloid accumulation is a primary driver of pathology and that tau aggregation and/or spreading may follow this (2). While AD mutations in this pathway in rare AD cases supports the model, the failure of beta-amyloid lowering strategies in patients has undermined it (although other reasons may account for these failures) (2). The pathogenic model proposed in the current paper introduces potentially important new steps into the amyloid hypothesis. This shows that beta-amyloid accumulation in mice is sufficient to activate RIPK in DAMs. This, in turn, causes cystatin F upregulation, lysosomal impairment and impaired phagocytic clearance of extracellular beta-amyloid

by microglia. This would accelerate beta-amyloid deposition through a positive feedback loop operating between neurons and microglia. However, in addition to these effects, activated RIPK1 results in excessive release of cytokines like $\text{TNF}\alpha$ and IL6, which will likely have additional deleterious consequences beyond the clearance of beta-amyloid. Indeed, the authors identified genes besides that encoding cystatin F whose expression are modified in a RIPK1-dependent fashion in their AD model (4), such as CH25h (cholesterol 25-hydroxylase). Furthermore, the impaired lysosomal activity triggered by RIPK activation in the microglia will affect degradation through multiple pathways, including autophagy and endocytosis. This would create an additional positive feedback loop in microglia themselves, as the authors show that lysosomal inhibition causes RIPK1 activation. Thus, the amyloid cascade is unlikely to be a simple linear series of events.

It would be interesting to consider whether the mechanisms reported by Yuan and colleagues also impact tau biology in AD. Tau is thought to spread from neuron to neuron in a prion-like manner and microglia may also phagocytose tau and enhance tau spreading (13). Interestingly, tau appears to be an important mediator of microglial activation, altered expression of inflammatory genes and inflammation-induced behavioural abnormalities (14). Thus, it is likely that RIPK1 inhibition may be effective in models of both tau and beta-amyloid pathology.

This new study suggests that RIPK1 is an important mediator of neuroinflammation in response to beta-amyloid. The resulting microglial activation is deleterious because it promotes the release of cytokines, some which will further enhance RIPK1 activation. In addition, RIPK1 activation inhibits lysosomal activity by upregulating cystatin F expression. This blunts the normally protective phagocytic activity of microglia towards beta amyloid. These results are exciting as they identify a key link in potential positive feedback loops that may accelerate pathology in AD. Moreover, this link is druggable as several RIPK1 inhibitors have been identified. Thus, in addition to providing important insights into AD biology, this study may have revealed a valuable therapeutic target for AD that tackles both inflammation and beta-amyloid.

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Figure legend:

Schematic diagram illustrating possible feedback loops whereby extracellular beta-amyloid stimulates RIPK1 activation in microglia, leading to increased expression of cystatin F, lysosomal impairment and reduced beta-amyloid clearance, hence feeding the cycle. Additional deleterious consequences of some of the events are illustrated. Nec-1s and other RIPK1 inhibitors can break the cycle.

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