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Commentary

β-Amyloid aggregates found in brain plaques are viewed as triggers of cytotoxicity and neuroinflammation in Alzheimer disease (AD). However, the main β-amyloid (Aβ) species and what imbues the aggregates with such toxic potential are still not yet understood. In this issue of the *JCI*, Roy et al. show that Aβ complexed with nucleic acids triggers an antiviral type I interferon response in neuroglia, resulting in complement-mediated synapse elimination in AD models. These findings identify a putative endogenous immune signaling axis that drives neurodegeneration in AD and has strong implications for the development of precise therapeutic strategies.

Intrinsic antiviral immunity drives neurodegeneration in Alzheimer disease

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A role for type I IFNs

The identification of a role for immune cells in the progression of Alzheimer disease (AD) has led to intense focus on the adaptive (1) and innate branches of the immune system (2). Microglia are the main innate immune cells present in the brain and are equipped with the requisite classes of signalling receptors to sense and perpetuate local inflammation (3).

When intracellular innate immune sensors recognize viral or self-derived nucleic acids (NAs), an activated antiviral state produces type I interferon (IFN) and induces interferon-stimulated genes (ISGs). Type I IFN signalling also mediates neuroinflammation in AD, both in animal disease models and human subjects (4), which implies that this signalling pathway activates as an integral response to disease progression.

In the AD brain, the accumulation of aggregated β-amyloid (Aβ) fibrils into AD plaques occurs in concomitance with a chronic neuroinflammatory response (5). This plaque-inflammatory response includes the induction of ISGs, reactive microgliosis, and astrogliosis, as well as increased proinflammatory cytokine production (5). Soluble Aβ oligomers bind with negatively charged factors, such as NAs, thus expediting the formation of insoluble amyloid fibrils (6). Importantly, NA-containing amyloid fibrils potently activate dendritic cells and enable type I IFN production to stimulate systemic autoimmunity in mice (7).

In this issue of the *JCI*, Roy et al. set out to determine if NA-containing (NA^+) amyloid fibrils were able to elicit sufficient production of type I IFN to drive neuroinflammation and neurodegeneration in the AD brain (8).

The authors examined the transcriptional profile of a brain region crucial for human memory and the main target in AD — the hippocampus. They used a series of animal models for AD ($APP^{NL\text{-}G\text{-}F}$, 5XFAD, APP;tTa, and APP-PS1) and showed increased expression of gene markers for microglia (*Aif1*) and astrocytes (*Gfap*). These changes were paralleled by upregulation of genes that were induced by type I IFNs (i.e., *Irf7*, *Cxcl10*, *Oas1*) as well as AD-related proinflammatory factors (i.e., *C3*, *Tnf*, *Il1β*) (8), as shown in previous studies (4).

The in vitro exposure of mixed glia cultures to NA^+ generic amyloid induced a type I ISG signature and led to increased IFN-β secretion in tissue-culture supernatants. This antiviral immune response was markedly reduced when mixed glia cultures were pretreated with a drug that depleted the culture of microglia (liposome-encapsulated clodronate). These results suggest that NA^+ amyloid triggers the activation of type I IFN signaling predominantly in microglia. In vivo, the transcriptional analysis of the hippocampi from wild-type (WT) mice injected with NA^+ generic amyloid revealed an activation profile comparable to that of the experimental AD brains, as above. Indeed, NA^+ generic amyloid triggered antiviral responses onto microglial and astrocytes and in vivo, when injected into the brain parenchyma (8).

Further in vivo validation in the 5XFAD mouse brain (9) revealed that the vast majority of Aβ plaques contained NA inclusions, and that their frequency increased with age. Systematic analysis of these amyloid plaques demonstrated a substantial increase of reactive, phagocytic microglia in close proximity to NA^+ plaques, whereas microglia near to NA^- plaques remained nonphagocytic. NA^+ plaque-associated microglia exclusively expressed the microglial neurodegenerative phenotype (MGnD) marker Clec7a (10), whereas a complete absence of Clec7a expression was observed in microglia associated with NA^- plaques. These data suggest the coexistence of NA^+ plaques and the induction of type I IFN signalling in a subset of (plaque-associated) MGnD that constitute an integral element in the propagation of innate inflammatory responses in the AD brain. While confirming an upregulation of the astroglial marker *Gfap*, this in vivo part of the study

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Conflict of interest: SP is cofounder and chief scientific officer at Cambridge Innovation Technologies Consulting (CITC) Ltd. and iSTEM Therapeutics and cofounder and non–executive director at Asitia Therapeutics.

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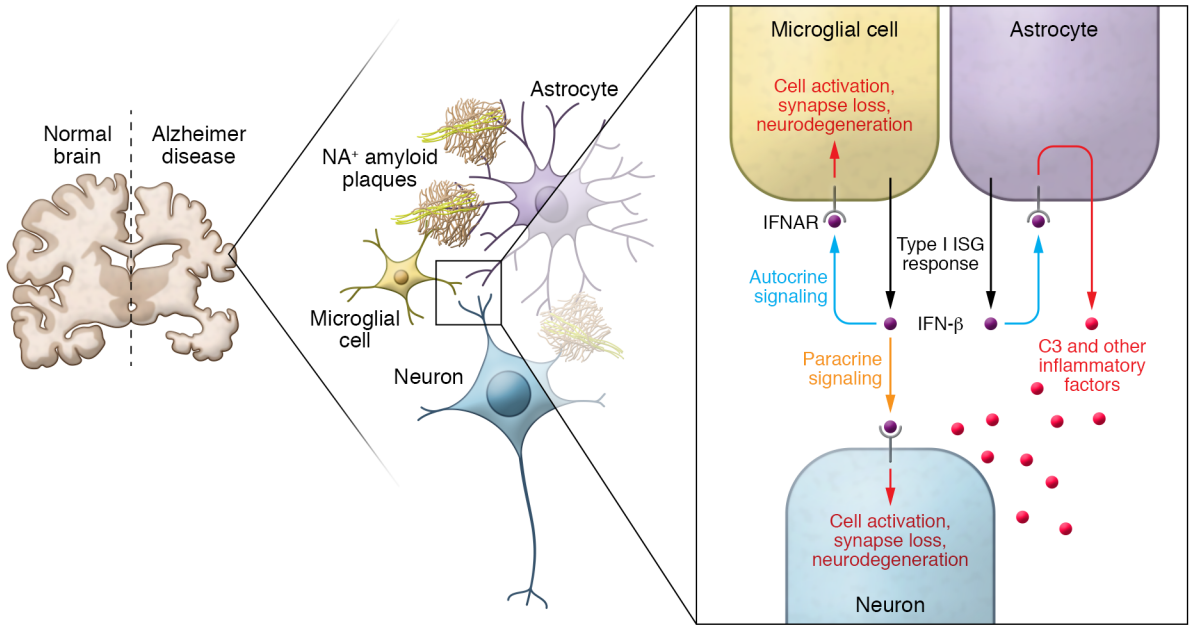

Figure 1. Model of type I IFN–mediated neuroinflammation and synapse loss in AD. NA+ amyloid induces a type I ISG signature leading to increased IFN-β secretion, predominantly in microglia. Type I IFNs act via IFN-α/β receptors (IFNARs) to induce type I IFNs and AD-related proinflammatory factors, such as C3 in astrocytes. Other microglia gene markers are also upregulated. Autocrine and paracrine signaling perpetuates cell activation and drives synapse loss and neurodegeneration.

failed to address the spatial organization of astrocytes in respect to Aβ plaques and NA inclusions (8).

Neuroinflammation and synapse loss

Microglia, which mediate synaptic pruning during brain development (11, 12), are also responsible for synapse loss in neurodegeneration (10). To identify a mechanism linking neuroinflammation with synapse loss in AD, Roy and colleagues injected a potent type I IFN recombinant interferon-β (rIFN-β) into the brains of WT mice and evaluated the cellular and molecular effectors that regulate several innate immune responses (13), including microglia activation (14). Intracerebroventricular (i.c.v.) injection of rIFN-β induced the expression of genes associated with the MGnD phenotype and triggered a cellular response predominantly characterized by increased reactive, phagocytic microglia engulfing synaptic puncta. Conversely, injecting an αIFN-α/β receptor (αIFNAR) blocking antibody into young (3-month-old) 5XFAD mice suppressed type I IFN signalling in reactive microglia, reduced Clec7a expression, and restored synaptic puncta density to control levels. Similar changes in microgliosis were also identified in aged (10- to 12-month old) $APP^{NL\text{-}G\text{-}F}$ mice injected with αIFNAR blocking antibody. Interestingly, αIFNAR blockade failed to alter plaque load in both young 5XFAD and aged $APP^{NL\text{-}G\text{-}F}$ mice (8). Thus, type I IFNs act via IFN-α/β receptors on microglia and signal both autocrine and paracrine pathways to perpetuate cell activation and drive synapse loss and neurodegeneration in the AD brain.

The NA+ amyloid–type I IFN–C3 signaling axis

Complement component 3 (C3) is recognized as a central mediator in the pruning of weak synapses tagged with complement proteins by microglial cells expressing complement receptors during development (11, 12). Complement proteins are also substantially upregulated during aging and neurodegeneration — even prior to any evidence of neuronal loss, which anticipates a role for complement-mediated mechanisms in neuronal dysfunction and loss (15). Furthermore, aging promotes sustained expression of type I IFN in the brain, which induces an inflammatory microglia phenotype and leads to increased production of complement factors (16).

What remains to be established is whether signaling through IFN-α/β receptor induces the expression of complement proteins. To this aim, the authors injected stereotaxically rIFN-β into the hippocampi of WT mice and measured C3 at transcript and protein levels. Interestingly, rIFN-β increased C3 expression in astrocytes only. Conversely, the injection of NA+ generic amyloid into the hippocampi of WT mice elicited a substantial induction of the expression of complement genes. The i.c.v. injection of an αIFNAR attenuated the complement response, strongly suggesting that type I IFNs signal through IFN-α/β receptor onto astrocytes for complement activation in response to NA+ amyloid in AD. The authors confirmed these findings in the 5XFAD and $APP^{NL\text{-}G\text{-}G}$ mouse AD models, where blocking αIFNAR with an antibody reduced C3 protein and transcript expression, respectively. While NA+ amyloid as well as the recombinant protein stimulated type I IFN signaling in both in microglia and astrocytes, downstream complement activation occurred only in astrocytes (8). Therefore, the model proposed here suggests that NA+ amyloid is able to induce C3 production in astrocytes but not in microglia (Figure 1).

In experimental demyelination in mice, C3 but not C1q has recently emerged as a major driver of microglia engulfment and

elimination of synapses (17). To address whether C3 was indeed necessary for the type I IFN-triggered synapse elimination in AD, the authors injected cerebral ventricles of *C3*$^{-/-}$ mice with rIFN-β and quantified microglia and synapses. Interestingly, while *C3*$^{-/-}$ microglia exhibited a reactive morphology, *C3*$^{-/-}$ brains were completely protected from postsynaptic puncta loss after rIFN-β compared with controls (8).

Together, these results highlight a critical NA^{+} amyloid–type I IFN complement-mediated signaling axis that triggers and perpetuates neuroglial reactivity and drives synapse loss in AD.

Conclusion

The work by Roy et al. (8) proposes a role for NA^{+} amyloid as a potent inducer of type I IFN signalling and complement-mediated synapse elimination by microglia and astrocytes in AD and potentially other neurodegenerative disorders. At the same time, the study raises a number of questions warranting further investigation.

Further studies are required to establish the specificity and the main source of cell-free NAs (i.e., DNA, RNA), and whether these might represent a potential disease biomarker to be detected and quantified in biological fluids (18). Additional mechanistic work is also indispensable to identify the key components of this antiviral type I IFN–mediated neuroglia reaction, and to address the relative contribution of the autocrine versus paracrine signaling pathways — both mediated by soluble factors and associated with extracellular membrane vesicles (19) — in the perpetuation of neuroinflammation in AD. Nonetheless, it will also be important to determine if this endogenous type I IFN–driven response to NA^{+} amyloid, and its consequences on excessive synapse elimination and neurodegeneration, plays any role in non-AD chronic neurological conditions associated with increased type I IFN serology (20).

In summary, Roy et al. provide compelling evidence that neuroinflammation is a critical feature of AD pathobiology. While demonstrating that NA^{+}-containing amyloid is a potent inducer of type I IFN signalling in neuroglia, this study also addresses the key mechanisms of the induction and propagation of antiviral innate immune responses in the brain, which in turn amplify complement-mediated synapse loss and neurodegeneration (8).

These results not only expand our understanding of the pathology in AD but also identify a putative endogenous immune signaling axis driving neuroinflammation and neurodegeneration in AD, and may have strong implications for the development of precise therapeutic strategies.

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