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TAM-ping Down Amyloid in Alzheimer's Disease

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Abstract

The TAM receptor kinases Axl and Mer are critical for microglial recognition and clearance of accumulating amyloid in transgenic models of Alzheimer's disease.

Deciphering how microglia navigate the harried landscape of the Alzheimer's disease (AD)-affected brain to engage amyloid beta (A β) plaques is important for our understanding of early pathology in this disease. These early stages follow a predictable progression, where amyloid precedes tau pathology, and where amyloid accumulation is necessary but not sufficient for full evolution to AD which requires tau spreading in the temporal lobe¹. Thus, targeting early events such as A β plaque deposition in AD development may represent a promising strategy to slow or prevent disease initiation. New data from Huang and colleagues indicate that microglia employ TAM receptor tyrosine kinases, Axl and Mer, to seek out -- and then engulf -- A β plaques². The investigators demonstrate that in preclinical models of AD amyloid deposition, TAM receptors and their ligands form a bridge with phosphatidylserine decorating A β plaques (Figure). Genetic ablation of TAM receptors abolished the ability of microglia to detect, phagocytose and compact A β plaques.

A β peptide -- the main component of A β plaques -- is generated by sequential cleavage of amyloid precursor protein (APP) and is released into the extracellular space where its monomers aggregate in β -sheet conformation to form higher-order assemblies such as oligomers, protofibrils, and fibrils³. Microglia phagocytose A β peptides, and it has recently been suggested that this process may be a necessary step for A β deposition as plaque⁴. This response relies in part on triggering receptor expressed on myeloid cells 2 (TREM2), which enables microglia to surround and compact A β plaques⁵. However, an understanding of the precise mechanisms by which microglia regulate A β plaque deposition had been lacking.

This research group previously demonstrated that the TAM system promoted microglial phagocytosis of apoptotic cells to maintain brain homeostasis⁶. Around the same time, other groups determined that under basal conditions, microglia show high expression Mer with significant increases in Axl expression in inflammatory diseases including AD^{7,8}. Furthermore, cerebrospinal fluid (CSF) analysis showed that soluble concentrations of Axl predicted changes in CSF A β abundance in healthy older adults⁹. Despite these connections, the roles of Mer and Axl in AD had not been studied in great depth. Huang and colleagues therefore hypothesized that the TAM system might play a role in enabling microglial phagocytosis of A β in preclinical models of AD².

To test their hypothesis, the investigators first set out to confirm that brain microglia were equipped with all the components of the TAM system: receptors, ligands, and bridging proteins. By comparing non-plaque-associated microglia (NPAM) to plaque-associated microglia (PAM), they confirmed the latter were Axl^{+} in two separate AD transgenic mouse models. For its part, *Mer* was upregulated in PAM versus NPAM in aged AD model mice. In addition, *Gas6*, the TAM ligand, was enriched specifically around plaques in aged AD mice and in human AD brain with severe amyloid pathology. The phospholipid phosphatidylserine (PtdSer) binds to the amino-terminal Gla domains of *Gas6*¹⁰ and is observed to be externalized on apoptotic cellular debris¹¹. Using a polarity-sensing indicator that fluoresces when bound to PtdSer, the team found that all $A\beta$ plaques were also PtdSer⁺. The investigators then turned to super-resolution microscopy which categorically demonstrated that microglia engage $A\beta$ plaques through a *Gas6*-PtdSer bridge².

Additional insights into the role of TAMs in amyloid pathology were obtained using single-cell RNA sequencing of cells sorted from AD TAM-deficient mice. Microglia were clustered according to their activation state using recently identified microglial activation markers. This analysis revealed the typical heterogeneity of microglia, with the largest cluster representing the homeostatic microglial ground state marked by *Tmem119*, *Cx3cr1*, *P2ry12*, and *Maf*. From this ground state, clusters transitioned to a fully activated cluster marked by *Cst7*, *Lpl*, *Trem2*, and *Csf1*, and resembled disease-associated microglia (DAM) cells previously defined by Keren-Shaul et al.⁷. Accordingly, markers of homeostatic and fully activated clusters resembled NPAM and PAM cells, respectively. *Mertk* was widely expressed across clusters while *Axl* was transcribed only in activated microglia clusters.

To examine the impact of TAM ablation on microglial processing of amyloid, the investigators turned to two-photon live imaging. By injecting mice with Methoxy-XO4, a brain-penetrant $A\beta$ dye, the team was able to follow the progression of $A\beta$ phagocytosis by the microglial TAM system. As expected, in control AD mice, $A\beta$ plaques were completely enveloped by many tightly bound and morphologically activated microglia. These microglia were classically activated and displayed an amoeboid morphology with larger cell bodies and fewer, shorter processes. In contrast, few TAM-ablated microglia were engulfing $A\beta$ plaques, and plaques were often entirely unattended, with nearby microglia appearing more ramified, suggesting retention of a homeostatic resting state. In fact, TAM-deficient microglia appeared wholly uninterested in $A\beta$ plaques, failing even to orient their processes towards them. Using volumetric reconstructions to quantify internalized $A\beta$, the team found 10-fold lower amounts of engulfed plaque material inside TAM-deficient microglia. They also observed increased cerebral amyloid angiopathy (CAA) and uncleared apoptotic cellular debris surrounding plaques.

One prevailing hypothesis in the field of AD research is that microglia, by virtue of their ability to phagocytose and clear amyloid, should inhibit the growth of $A\beta$ plaques¹². Therefore, given the inability of TAM-deficient mice to phagocytose properly, one might expect a significant rise in the amount of plaque in these mice. However, a detailed work-up revealed that the opposite was the case – TAM-deficient mice had fewer dense-core amyloid plaques. This result was true over the course of plaque development, assessed at multiple time points, and importantly, was not due to differences in expression of *APP*

or concentration of soluble A β peptides. Plaque abundance was similar at early ages, but progressively diverged, with TAM-deficient AD mice showing lower amyloid amounts by 12 months of age. An intriguing question for future study is whether microglial TAMs regulate additional immune processes, distinct from phagocytosis, that could drive additional A β accumulation over time. Together, the data indicate that the TAM system is required for the recognition, response to, and phagocytosis of A β plaque and that TAM-mediated phagocytosis is critical for the formation of dense-core plaques.

Interestingly, TAM ablation phenocopies observations in TREM2 loss-of-function models, notably effects on phagocytosis and packing of A β plaques. While TAM receptors and TREM2 are both highly expressed on plaque-associated microglia, the receptors occupy distinct membrane regions. TAM expression appears to be regulated by TREM2 at the transcriptomic level, as upregulation of *Ax*/mRNA is TREM2-dependent⁷. Future research will hopefully parse out and compare the differences in microglial biology for these two receptors in models of inflammatory neurodegeneration.

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In Alzheimer's disease, plaque-associated microglia (PAM) express high levels of the TAM receptor tyrosine kinases, Axl and Mer. Lemke and colleagues show in preclinical models of AD amyloid deposition that microglia engage A β plaques using the TAM system (inset), whereby microglia TAM receptors are activated by Gas6 in the presence of phosphatidyl serine (PtdSer) bound to the GLA domain. PtdSer⁺ dystrophic membranes are present in fibrillar A β halos surrounding dense core plaques, thus allowing an anchor for TAM-expressing microglia to construct a Gas6-PtdSer bridge in order to detect, phagocytose, and compact A β plaques.

Note to artist on Figure:

We envision a scene within an Alzheimer's brain, that shows angry A β plaques with activated microglia clustered around them (see sketch). These microglia should be classically activated, with shortened arms, enlarged soma, etc. Further away, microglia should have a more ramified shape, with elaborated processes, similar to surveilling microglia. Microglia in close proximity to A β plaques should express Axl and Mer on their surface, while distant microglia should express on Mer, which is constitutively expressed. In some configuration, we would like to include an inset (see PDF attached) to depict the TAM receptor bridge with plaque PtdSer and Gas6.

We want to convey how harried the AD brain environment is – think: No Man's Land, in World War 1. This will hopefully highlight how specialized the TAM system is to be able to function in such a complex environment.