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# Microglia are SYK of Aβ and cell debris

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**During neurodegenerative disease, resident CNS macrophages termed "microglia" assume a neuroprotective role and engulf toxic protein aggregates and cell debris. In this issue of *Cell*, two groups independently show how spleen tyrosine kinase (SYK) acts downstream of microglial surface receptors to propagate this neuroprotective program *in vivo*.**

The majority of Alzheimer's disease (AD) risk genes are enriched in microglia, a resident macrophage population of the CNS (Ennerfelt et al., 2022; McQuade and Blurton-Jones, 2019). This genetic link between microglia and AD is coupled with a large breadth of literature showing that microglia surround and engulf cellular debris and protein aggregates, such as amyloid beta (Aβ). Ultimately, these functions play a neuroprotective role in neurodegeneration. Conversely, other populations of microglia assume a more pro-inflammatory role in neurodegeneration and likely propagate neurodegeneration. As a result, microglia have now taken center stage as a key therapeutic target in neurodegenerative diseases. Interestingly, many of the identified AD risk genes enriched in microglia are immune-related surface receptors (*TREM2*, *CD33*, *CD22*, etc.), and genetic perturbation of these receptors modulates neurodegeneration in animal models of disease (Deczkowska et al., 2018; McQuade and Blurton-Jones, 2019). A major question in the field has remained as to what intracellular signaling may be downstream of these receptors in microglia. Previous work has shown in other contexts that spleen tyrosine kinase (SYK) acts downstream of triggering receptor expressed on myeloid cells 2 (TREM2) and C-type lectin domain containing 7A (CLEC7A/Dectin-1) (Mocsai et al., 2010), two surface receptors involved in neurodegeneration. It was also known that SYK regulated microglial responses to toxic protein aggregates (e.g., amyloid beta/Aβ), and pharmacological manipulation of SYK could attenuate neurodegeneration in AD-relevant models. (Combs et al., 1999; Paris et al., 2014; Schweig et al., 2017). In this issue of *Cell*, two independent groups, Ennerfelt et al. (2022) and Wang et al. (2022), build on this existing data to show that SYK is acting downstream of surface receptors TREM2 and CLEC7A in microglia to prevent the spread of neurodegeneration *in vivo*. Genetic ablation of SYK in microglia ($CX3CR1^{CreERT2}/SYK^{fl/fl}$ or $SYK^{i\Delta MG}$) in a mouse model relevant to AD (5xFAD) resulted in heightened Aβ pathology, accumulation of lipid droplets in microglia, enhanced neurodegeneration, and learning and memory impairments. In animal models relevant to multiple sclerosis (MS), Ennerfelt et al. also showed a similar neuroprotective role for SYK in microglia.

It is now well established that when microglia encounter pathological forms of Aβ, as well as other protein aggregates and cellular debris, they adopt a specific disease-associated microglia (DAM or MGnD) transcriptional signature (Keren-Shaul et al., 2017; Krasemann et al., 2017). This DAM signature is necessary for microglia to surround and engulf Aβ plaques, which prevents the spread of Aβ pathology (Krasemann et al., 2017; Wang et al., 2015). Strikingly, both groups identified that SYK-deficient microglia from 5xFAD mice had a diminished DAM transcriptional response, reduced microglial association with plaques, and decreased microglial engulfment of Aβ (Figure 1). In mouse models relevant to MS, Ennerfelt et al. showed a similar failure of SYK-deficient microglia to transition to a DAM phenotype and a failure to clear myelin debris. TREM2 was a clear candidate receptor to work upstream of SYK as it is known to signal through SYK *in vitro* through its co-receptor DAP12. (Mocsai et al., 2010) TREM2 is also a well-established AD risk gene that is critical for microglia to adopt the DAM signature and associate with Aβ plaques (Jay et al., 2015; Krasemann et al., 2017; Wang et al., 2015). By single-cell RNAseq, Wang et al. revealed commonalities—but also some striking differences—when comparing microglia from TREM2-deficient 5xFAD and SYK-deficient 5xFAD mice. Microglia from both genotypes showed an absence of the DAM signature when compared to 5xFAD microglia without perturbation of TREM2 or SYK. However, in contrast to TREM2-deficient 5xFAD microglia, SYK-deficient 5xFAD microglia showed an expansion of proliferating cells and transitioning cells (i.e., prodromal to DAM), which was further supported by bulk RNAseq performed by Ennerfelt et al. Prodromal DAM cells were unique as they expressed some of the core DAM genes (e.g., *APoE*), which are known to be regulated by TREM2, but they lacked the full complement of DAM signature genes. Together, these data demonstrate that, unlike TREM2, SYK is dispensable for microglia to enter a transcriptional program toward a DAM phenotype. Instead, SYK is critical for microglia to transition from a prodromal DAM state to a full stage 2 DAM molecular signature.

If not SYK, what is downstream of TREM2 to initiate the DAM transcriptional program in microglia? To begin to address this question, Wang et al. provided evidence in cultured macrophages that SYK is activated via TREM2 and its co-receptor DAP12. However, TREM2 signaling

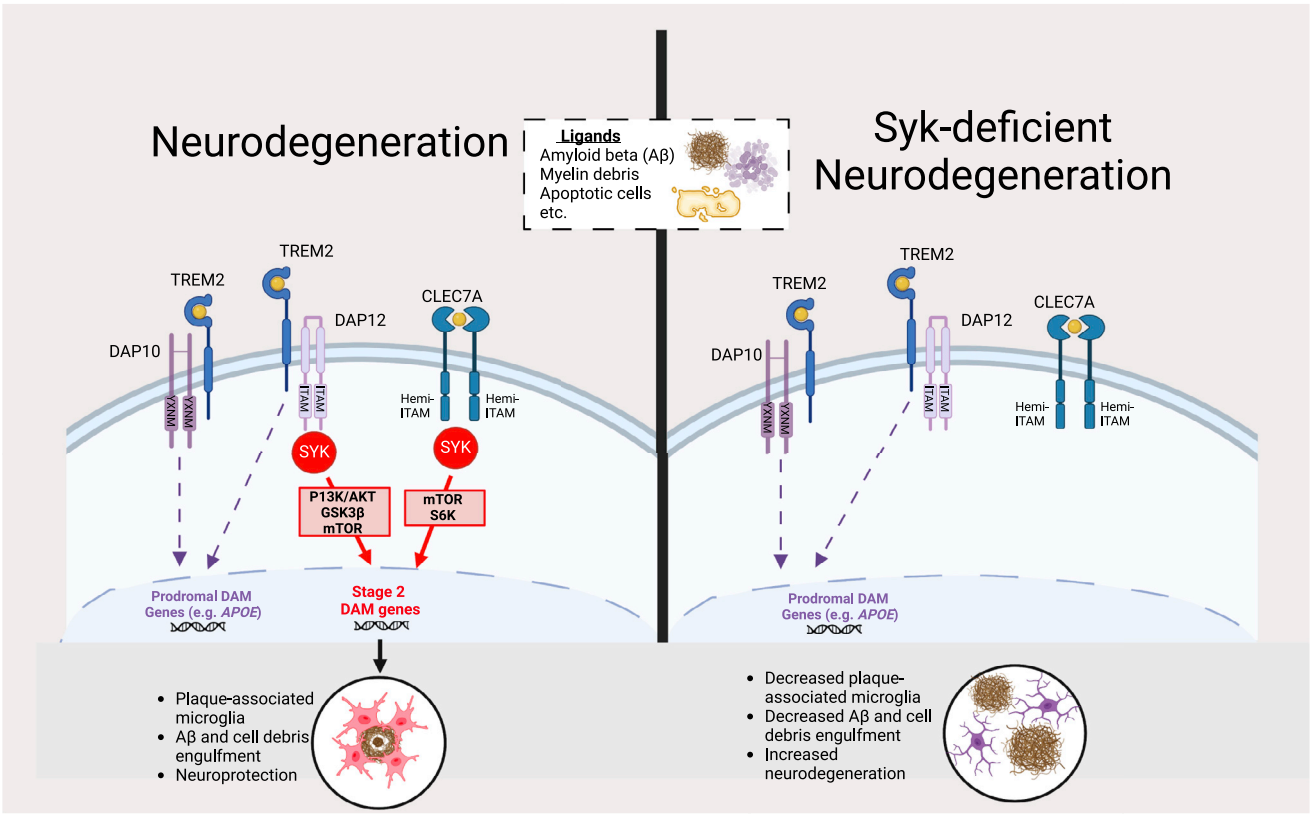


**Figure 1. SYK acts downstream of TREM2 and CLEC7A in microglia to regulate the DAM transcriptional program**

In mouse models of neurodegeneration and neuroinflammation (e.g., 5xFAD, experimental autoimmune encephalomyelitis, etc.), microglia assume a triggering receptor expressed on myeloid cells 2 (TREM2)-dependent stage 2 disease-associated microglia (DAM) signature. This signature is required for microglia to engulf cellular debris and surround and engulf Aβ plaques, which are neuroprotective. Both groups showed *in vivo* that spleen tyrosine kinase (SYK) is required for microglia to adopt the full stage 2 DAM molecular signature downstream of TREM2 and CLEC7A activation. SYK-deficient microglia (right) still entered a prodromal/transitioning DAM transcriptional program and expressed *APoE* but failed to transition to the stage 2 DAM signature. This ultimately impaired the ability of SYK-deficient microglia to surround and engulf toxic Aβ aggregates in the 5xFAD mouse model and engulf myelin debris in mouse models of demyelinating disease. Additionally, SYK-independent TREM2 signaling is necessary to initiate the entire DAM program in microglia. This SYK-independent transcriptional program could be through TREM2/DAP10 or a parallel pathway via TREM2/DAP12 (dotted purple arrows).

through its other co-receptor DAP10 regulated macrophage proliferation and Aβ phagocytosis independent of SYK. It remains to be determined if similar SYK-independent TREM2/DAP10 signaling occurs in microglia *in vivo* during neurodegeneration and, if so, if TREM2/DAP10 is critical to initiate the SYK-independent initiation of the DAM molecular program in microglia.

In addition to TREM2, both groups converged on CLEC7A as another upstream receptor of SYK in microglia to regulate the DAM phenotype. CLEC7A is a core DAM signature gene in microglia (Keren-Shaul et al., 2017; Krasemann et al., 2017) known to activate SYK in other contexts (Mocsai et al., 2010). Ennerfelt et al. showed that injection of a CLEC7A ligand into the hippocampus of 5xFAD mice decreased Aβ load, which was not apparent in SYK-deficient 5xFAD hippocampi. Similarly, Wang et al. used a monoclonal antibody against CLEC7A (mAb2A11) to crosslink the protein and induce activation of SYK. Upon administration of the CLEC7A antibody to 5xFAD mice harboring a mutation in TREM2 that enhances Aβ load (*TREM2*$^{R47H}$-*5xFAD* mice), Aβ phagocytosis by microglia increased, and filamentous Aβ plaques decreased. Thus, enhancing CLEC7A signaling through SYK in microglia was sufficient to protect the spread of Aβ pathology and is a viable therapeutic strategy to block the spread of neurodegeneration. It will be important to understand further whether CLEC7A and TREM2 are acting in parallel pathways or if there is crosstalk via SYK. In addition, identifying other receptor signaling is upstream of SYK in microglia will be key.

To complete the signaling cascade, both groups also looked at signaling downstream of SYK in microglia in response to Aβ. They provide evidence for decreased phosphorylation of AKT/PI3K and GSK3β in SYK-deficient 5xFAD microglia. Ennerfelt et al. further showed that GSK3β phosphorylation was required for phagocytosis of Aβ. Wang et al. provided additional *in vitro* evidence for mTOR-mediated metabolic dysfunction and increased autophagy downstream of decreased AKT phosphorylation in SYK-deficient microglia.

In summary, two independent groups identify SYK as an intracellular signaling molecule critical for microglia to fully transition into a neuroprotective stage 2 DAM

phenotype during neurodegeneration (Figure 1). As many of the identified AD risk genes (*TREM2, CD33, CD22,* etc.) and DAM signature molecules contain motifs that bind and activate SYK, this places SYK at the center of microglia-mediated inflammation and neurodegeneration across multiple diseases. Thus, targeting SYK for therapeutic intervention may have broad implications. Consistent with this idea, Ennerfelt et al. show that SYK deficiency in microglia prevented the DAM phenotype in AD-relevant mouse models as well as mouse models of demyelinating disease relevant to multiple sclerosis. Further emphasizing the importance of SYK, the *Drosophila* homologue performs a similar function in glial cells to regulate cell debris clearance (Ziegenfuss et al., 2008). Thus, SYK appears to be an evolutionarily conserved mechanism necessary for clearance of cellular debris and toxic protein aggregates. Collectively, these results open several exciting new areas of investigation and will serve as the basis of novel therapeutics for years to come. Better understanding the full repertoire of receptors that are upstream of SYK to regulate microglia and identifying the SYK-independent signaling downstream of TREM2 to regulate the initiation of the DAM program will be critical going forward.

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## DECLARATION OF INTERESTS

Dr. Dorothy Schafer is a consultant for Neurocrine Biosciences.

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