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INSIGHTS

Death by microglia

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The roles of microglia and ApoE in tauopathies, such as Alzheimer's disease, remain elusive. In this issue, Shi et al. (https://doi.org/10.1084/jem.20190980) demonstrate that microglia-mediated innate immunity collaborates with ApoE to drive neurodegeneration and disease progression in a mouse model of tauopathy.

Changes in microglia, the resident innate immune cells of the brain, have long been associated with tauopathies such as Alzheimer's disease (AD). Microglia are found surrounding amyloid-β (Aβ) plaques and near tau tangles in an activated state that represents an inflammatory response that is distinct from their normal homeostatic condition (Cras et al., 1991; Taipa et al., 2017; Heneka et al., 2015). This activated state is accompanied by changes in the expression of multiple proteins, including apolipoprotein E (ApoE), which is significant because presence of the ε4 allele of the *APOE* gene is the greatest genetic risk factor for late-onset AD (Corder et al., 1993).

In a previous study, Shi et al. (2017) established a link between ApoE and tau-mediated neurodegeneration in the absence of Aβ. They used the P301S tauopathy mouse model, also known as the PS19 line, which overexpresses human tau containing the P301S mutation that has been implicated in frontotemporal dementia (Yoshiyama et al., 2007). The researchers found that P301S tau transgenic mice crossed to homozygous humanized ApoE4 knockin mice had higher tau levels in the brain and exhibited greater neuron loss than the tau transgenic mice carrying either of the other two human ApoE isoforms, E2 or E3. Conversely, complete removal of the ApoE gene was protective against neurodegeneration. In comparing microglial gene expression profiles in the different mice, another key finding emerged—the P301S tau transgenic mice with ApoE4 had the greatest upregulation of genes associated with microglial activation. The conclusions of this study reinforced the importance of taking a closer look at cellular and molecular mechanisms of ApoE action in AD, especially in the context of neuroinflammation. The major question remaining was whether glial activation involving either astrocytes or microglia was in some way involved in ApoE4-driven neurodegeneration related to tauopathy, or whether the observed neuron loss was a direct result of pathological tau protein neurotoxicity.

To address this question, in this issue of *JEM*, Shi et al. decided to take the approach of eliminating microglia completely in the same tauopathy mouse model and assess the effects on neurodegeneration. Microglia have been successfully eliminated from mice in other studies without adverse effects (Spangenberg et al., 2019). In this case, the drug PLX3397 was administered to the P301S tau transgenic mice from 6 to 9.5 months, which represents a critical neurodegenerative period for this mouse model. PLX3397 is a brain-penetrant inhibitor of CSF1R, which is otherwise required for microglial survival. This allows for the use of CSF1R inhibitors to effectively reduce and even deplete microglia almost entirely. To further elucidate the connection between ApoE status and neurodegeneration, the mice were either homozygous for ApoE4 or lacking ApoE altogether.

Remarkably, the tau transgenic-ApoE4 mice depleted of microglia were protected from neurodegeneration and had the same brain volume as non-tau transgenic mice. This is the first reported evidence clearly demonstrating that in a tauopathy model, microglia-mediated innate immunity drives neurodegeneration, rather than direct neurotoxicity from the pathological tau protein itself. It is interesting to note that microglia-depleted tau transgenic mice had similar brain volumes regardless of ApoE status, suggesting that the effect of ApoE on neurodegeneration is mediated through its regulation of microglial function.

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PLX3397 treatment resulted in robust depletion of microglia, although in tau transgenic ApoE4 mice, a small number of Iba1$^+$/CD68$^+$ cells were found in the piriform/entorhinal cortex and hippocampus, suggesting the existence of a few activated microglia based on the presence of these markers. In keeping with the idea that activated microglia fuel neurodegeneration, the presence of CD68$^+$ microglia was associated with higher levels of neurodegeneration as evidenced by reduced piriform/entorhinal cortex and hippocampus volumes. In non–drug-treated mice, there was no difference in the number of Iba1$^+$ cells between tau transgenic mice, regardless of ApoE status, indicating that microglial proliferation alone was not associated with neurodegeneration. However, the tau transgenic ApoE4 mice had more CD68$^+$ microglia, suggesting that while their overall number of microglia remained the same, their microglial activation states were different. Additionally, in non–drug-treated mice, there was a strong negative

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correlation between CD68$^+$ area and piriform/entorhinal cortex and hippocampus volumes, suggesting that ApoE status impacts microglial activation, which ultimately mediates neurodegeneration. Interestingly, the absence of ApoE caused increased Iba1$^+$/CD68$^-$ (homeostatic microglia) cell survival in PLX3397-treated mice, suggesting that ApoE may be involved in CSF1R signaling and related critical downstream functions in microglia.

Another significant finding from this study is that tau pathology is linked to microglia-mediated innate immunity. ApoE status was previously shown to impact tau levels and result in distinct patterns of ptau spreading in the brain. In the current study, removing microglia prevented this ApoE-dependent effect, suggesting that in addition to neurodegeneration, tau pathogenesis and disease progression are also exacerbated by microglial activation (Shi et al., 2019).

The study by Shi et al. (2019) establishes the role of microglia-mediated innate immunity as a link between ApoE status, the greatest genetic risk factor for AD, tau, one of the major pathophysiological hallmarks of AD, and neurodegeneration, thereby strengthening the case for further examination of nonneuronal cell types to elucidate mechanisms of neurodegenerative disease. This represents a paradigm shift in how we view the mechanism of neurodegeneration and incontrovertibly demonstrates, perhaps for the first time, the non-cell autonomy of the toxicity of tauopathy. The publication of this work comes at a time when the intersection of the fields of innate immunity and neurodegenerative disease is gaining attention for its potential to identify novel therapeutic targets and advance our understanding of mechanisms of disease. While much of the research focus has been on neurons, it has become apparent that other cell types play critical roles in disease pathogenesis.

Microglia, in particular, have presented a unique challenge in our ability to understand neurodegenerative disease states. As the resident innate immune cells of the central nervous system, they are required for normal functioning of the brain and respond accordingly to disease and injury. On the one hand, in the case of AD, microglia have been implicated in clearance of both Aβ and tau from the brain (Lee and Landreth, 2010). On the other hand, sustained microglial activation has been shown to result in a chronic pro-inflammatory state that is detrimental to neuronal survival and even causative in the spread of Aβ and tau (Venegas et al., 2017; Asai et al., 2015). A number of alleles that are risk factors for AD are upregulated in activated microglia, including *APOE4* and *TREM2*, and have been implicated in inflammation in tau models (Keren-Shaul et al., 2017). A recent publication using a drug similar to PLX3397 to deplete microglia in an amyloid mouse model reported that Aβ plaques did not develop in the absence of microglia, but whether this is ultimately beneficial or harmful to disease pathogenesis remains unclear (Spangenberg et al., 2019). Some studies have pointed to the beneficial role of microglia in compacting Aβ plaques and protecting neurites against damage (Condello et al., 2015; Wang et al., 2016; Yuan et al., 2016). From the research that has emerged, it is becoming clear that microglia take on multiple roles in neurodegenerative disease states and that whether these function to prevent or exacerbate disease progression is largely dependent on the stage of disease.

Extensive tauopathy in AD is associated more with later stages of disease pathophysiology, and as such, the work by Shi et al. (2019) is instrumental in shedding light on our understanding of immune function in advanced-disease brains. This study opens the door to further understanding of this important mechanism, and eventually to novel therapeutic approaches that specifically target microglia-mediated innate immunity and the influence of ApoE in tauopathy-related neurodegeneration, an area of great relevance for AD. The importance of this becomes apparent when we consider that most patient diagnoses of neurodegenerative disease occur long after the initial stages of disease, at a point when tau pathology is already prevalent in the brain and significant neuronal loss has already occurred. Therapeutic targets for later stages of disease that could slow down or halt further progression are urgently required in order to improve the lives of patients and caregivers. The findings from this study emphasize the importance of further exploration of therapeutic strategies targeting microglia-mediated innate immunity, which may be effective at preventing the progression of neurodegeneration and tauopathy in AD and other dementias.

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