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Trends in Immunology

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Spotlight

APOE4 affects neutrophil–microglia crosstalk in Alzheimer's disease

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Circulating immune cells contribute to the pathogenesis of Alzheimer's disease (AD), but their role is poorly understood. Rosenzweig *et al.* recently identified a subset of interleukin (IL)-17$^+$ neutrophils that inhibit neuroprotective microglia in female APOE4 carriers. Blockade of IL-17 signaling or APOE4 deletion in neutrophils restored microglial responses and reduced murine amyloid pathology.

AD is the most common type of dementia, with an enormous social and economic impact. Classical AD neuropathology is characterized by β-amyloid (Aβ) deposition, tau hyperphosphorylation leading to tangle formation, and the loss of synapses and neurons [1]. However, studies over the past decade show that chronic inflammation mediated by local and peripheral immune cells is also a key feature of AD [2].

Neutrophils are the most abundant leukocytes in human blood, and they rapidly extravasate into inflamed tissues. Under sterile inflammatory conditions, migrating neutrophils release a plethora of potentially dangerous inflammatory mediators (including reactive oxygen species, enzymes, and cytokines) and can deploy toxic structures known as neutrophil extracellular traps (NETs) [3]. Previous studies have shown that neutrophils migrate into the brains of AD patients and transgenic mice with AD-like disease, but can also obstruct blood flow by plugging brain capillaries, thus contributing to disease development through multiple pathological mechanisms [4,5]. Transient neutrophil depletion during early disease in AD animal models reduces neuropathological hallmarks and improves memory, clearly indicating that these cells play a role in the induction of neuroinflammation and cognitive deficits in AD [4,5]. Soluble oligomeric Aβ1–42 peptide triggers rapid integrin-dependent adhesion of human and mouse neutrophils on integrin ligands, inducing an LFA-1 integrin high-affinity state; this is interesting as it suggests that vascular Aβ might favor neutrophil adhesion on brain endothelial cells and enable neutrophil-dependent blood–brain barrier dysfunction [4]. Accordingly, therapeutic blockade of LFA-1 integrin can rescue memory features and reduce the severity of neuropathology in AD mouse models; this suggests that impairing certain neutrophil activities might have therapeutic effects in AD.

Circulating neutrophils have a hyperactivated phenotype in AD patients compared with control subjects [6]. In this context, Rosenzweig *et al.* used single-cell transcriptomics to generate important information about the phenotypic changes of circulating neutrophils in patients with AD or mild cognitive impairment (MCI) [7]. This identified a new blood neutrophil subset associated with cognitive impairment and defined by increased IL-17 and IL-1 co-expression modules specifically present in human female APOE4 carriers (Figure 1). This neutrophil population also upregulated the immunosuppressive cytokines IL-10 and transforming growth factor β (TGFβ), and immune checkpoints such as LAG3 and PD-1, suggesting that these may have an immunomodulatory function in subjects presenting with the APOE4 variant. Flow cytometry supported these data, showing the expansion of IL-17$^+$CD15$^+$CD66b$^+$ neutrophils that are characterized by the expression of immunosuppressive transcriptional factors, immune checkpoint proteins PD-1, LAG3, and TIM-3, anti-apoptotic protein BCL-2, as well as IL-18R1, TGFβ1, interferon γ (IFNγ) and IL-10 cytokines in APOE4 carriers.

Neutrophil–microglia interactions had previously been suggested in two animal models of AD, but the underlying molecular mechanisms were not reported [4]. Now, Rosenzweig and co-workers fill this knowledge gap by demonstrating the increased infiltration of CD66b$^+$ neutrophils co-expressing APOE and interacting with IBA1$^+$ microglia at sites of plaque pathology in AD APOEε3/4 postmortem brains. Moreover, by combining the transcriptomic and bioinformatic analysis of blood neutrophils and microglial cells isolated from subjects with MCI and AD, Rosenzweig *et al.* extrapolated to find that in their protective response to AD pathology in cognitively impaired APOE4 female carriers, IL-17F$^+$ neutrophils suppressed *IL17RA*-expressing neurodegenerative microglia (MGnD). This hypothesis was then elegantly demonstrated in transgenic animal models of amyloid pathology in which APOE4 deletion in neutrophils reduced their suppressive phenotype and IL-17 signaling. Also, APOE4 deletion in neutrophils improved cognition in AD mice and harnessed microglia to limit amyloidosis, suggesting that migrating APOE4 neutrophils suppress the protective MGnD response to neuropathology.

The authors showed that immunosuppressive neutrophils were recruited into the brain by IL-17F; this was demonstrated by experiments in which IL-17F was directly injected into the brain hemisphere of Ragγc [*Rag2*$^{-/-}$*γc*$^{-/-}$; B, T, and natural killer (NK) cell-deficient] female mice. Supporting these data, flow cytometry showed reduced neutrophil infiltration in the brain and choroid plexus – but not the meninges – of 5×FAD: APOE4 AD mice following IL-17F blockade. Interestingly, a similar mechanism was previously reported in response to *Trypanosoma cruzi* infection in mice during which IL-17RA

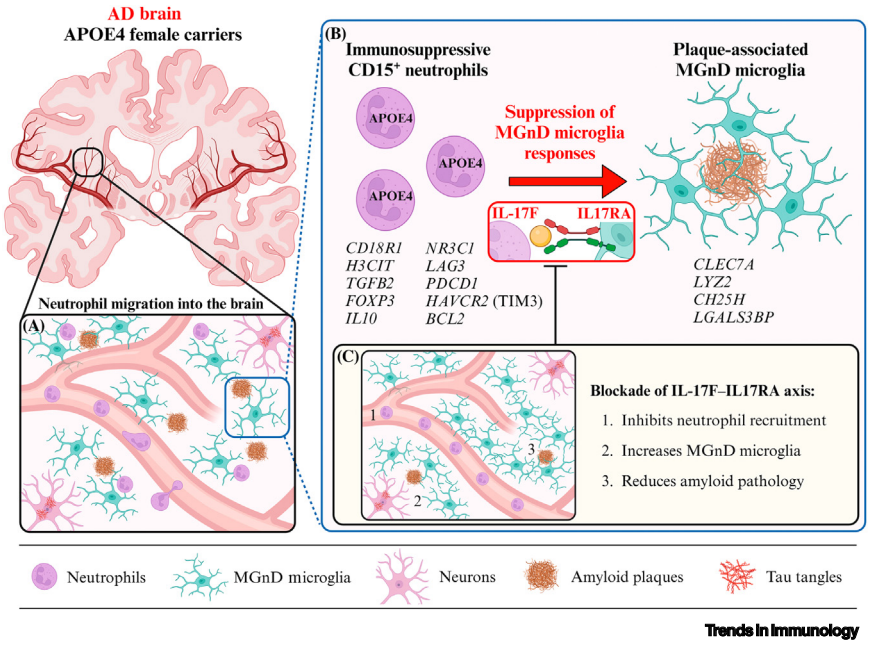

Figure 1. Role of the interleukin (IL)-17F–IL-17RA axis in the interplay between neutrophils and microglia in female APOE4 carriers with Alzheimer's disease (AD). (A) Circulating neutrophils migrate into the brain of APOE4 female carriers with AD characterized by the presence of extracellular amyloid plaques and intraneuronal tau tangles. (B) Inside the brain parenchyma, immunosuppressive CD15$^+$ neutrophils release IL-17F, which binds to IL-17RA expressed on plaque-associated neurodegenerative microglia (MGnD), suppressing MGnD responses to AD pathology. (C) A blockade of the IL-17F–IL17RA axis in mice with amyloidosis inhibits neutrophil recruitment, increases the abundance of MGnD in the brain, and reduces amyloid pathology [7]. Figure created with BioRender.

and IL-17F directly mediated the recruitment of IL-10$^+$INFγ$^+$ immunosuppressive neutrophils to the site of infection [8]. IL-17 triggered NETosis and, accordingly, Rosenzweig *et al.* showed increased myeloperoxidase (MPO) and citrullinated histone 3 (CitH3) expression in CD15$^+$ neutrophils isolated from the blood of APOE ε3/4 female carriers with MCI [7,9]. Furthermore, neutrophils co-expressing CitH3 and IL-17 were detected near amyloid plaques in the brains of APOE ε3/4 female carriers, supporting a role for these cells in AD. Notably, these data are in line with earlier results showing in human and mouse AD models increases in the accumulation of intravascular and intraparenchymal neutrophils, as well as the presence of NETs and IL-17$^+$ neutrophils in the AD brain cortex and hippocampus [4]. Current data therefore suggest that IL-17 and NET release by brain-infiltrating neutrophils may damage the blood–brain barrier and cause neuronal injury during AD [4,7].

The single-cell RNA sequencing analysis of the human dataset by Rosenzweig and colleagues showed a significant correlation between MGnD clusters and tau pathology, and between neutrophil degranulation signaling and tauopathy in microglia from APOE ε3/4 female carriers. However, only animal models of amyloidosis (APP/PS1 and 5×FAD) were used in this study, and the effects of IL-17F blockade on tau pathology were not evaluated. Previous studies demonstrated that neutrophil depletion in 3×Tg-AD mice, which develop both tau and amyloid pathology, significantly reduced amyloid load and tau hyperphosphorylation [4]. Moreover, the APOE4 variant has been shown to exacerbate tau pathology in a mouse model (TE4), in which upregulation of *Clec7a* (a signature of MGnD) was also observed in microglial cells [10]. These findings suggest that the APOE4 variant, together with brain-invading neutrophils, helps to activate microglia during tauopathy, but further studies are needed to better understand the link between APOE4, tau pathology, and neutrophil-dependent damage in AD.

In conclusion, Oleg Butovsky's research team demonstrate a key role for the IL-17F–IL-17RA axis in mediating neutrophil–microglia interactions in female APOE4 carriers with cognitive impairment and in mice with amyloidosis. Presumably, targeting IL-17F might benefit female APOE4 carriers (who respond poorly to current anti-Aβ therapies). Nevertheless, future studies are needed to explain in more detail the sexual differences between AD patients and to better define clinical therapeutic strategies in AD.

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Declaration of interests

The authors declare no conflicts of interest.

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