

Evaluating the therapeutic potential of targeting the CXCR3/CXCL9-11 inflammatory axis in Alzheimer's disease

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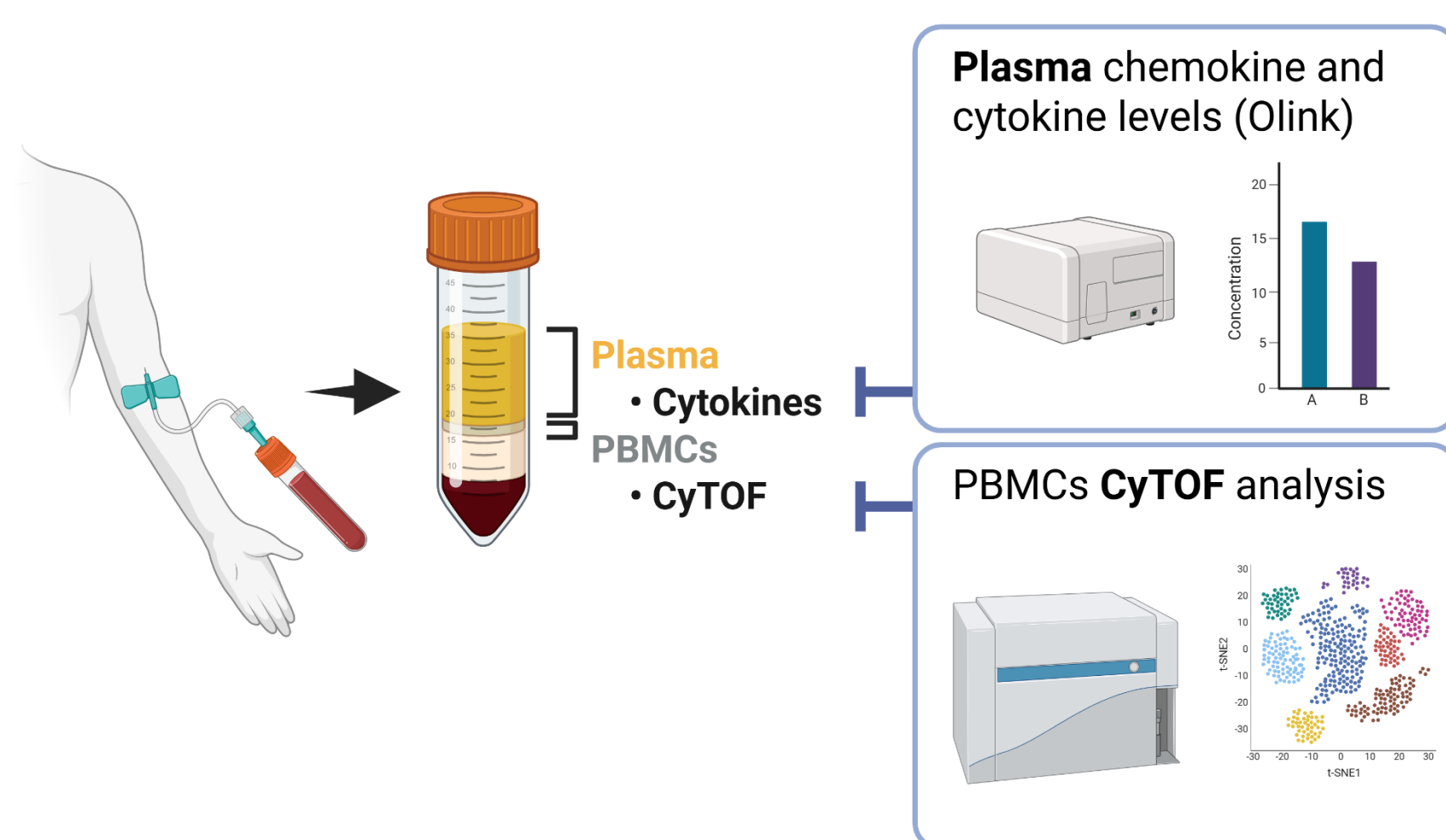
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Background

Peripheral immune dysregulation is increasingly recognized as a modifiable contributor to Alzheimer's disease (AD) and other neurodegenerative disorders, yet the mechanistic pathways linking systemic inflammation to neurodegeneration remain incompletely defined. The pro-inflammatory CXCR3/CXCL9-11 chemokine axis is involved in T cell trafficking to inflamed tissues and represents an inflammatory pathway that can be pharmacologically targeted. Here, we *investigate the systemic activation of the CXCR3/CXCL9-11 axis in AD individuals* and *evaluate the therapeutic potential of CXCR3 blockade in a translational human-mouse framework*.

Methods



Plasma and peripheral blood mononuclear cells (PBMCs) were obtained from individuals with AD and age-matched healthy controls (HC) enrolled at the Houston Methodist Nantz National Alzheimer's Center. Circulating cytokines and chemokines levels were quantified using the Olink® Target 48 Cytokine panel. The CXCR3/CXCL9-11 signaling axis was profiled on peripheral immune cell types at single-cell resolution using time-of-flight mass cytometry (CyTOF).



To assess the therapeutic relevance of targeting the CXCR3/CXCL9-11 axis on AD pathology, 5-month-old 5xFAD mice received intraperitoneal injections of the CXCR3 antagonist **AMG487** (5mg/kg) or vehicle as control (placebo) for two months. Post-treatment analyses in 7-month-old 5xFAD mice included immunofluorescence, ELISA, and targeted proteomics (Olink® Target 48 Cytokine panel) to assess the effect of the treatment on neuroinflammation and amyloid pathology.

Result

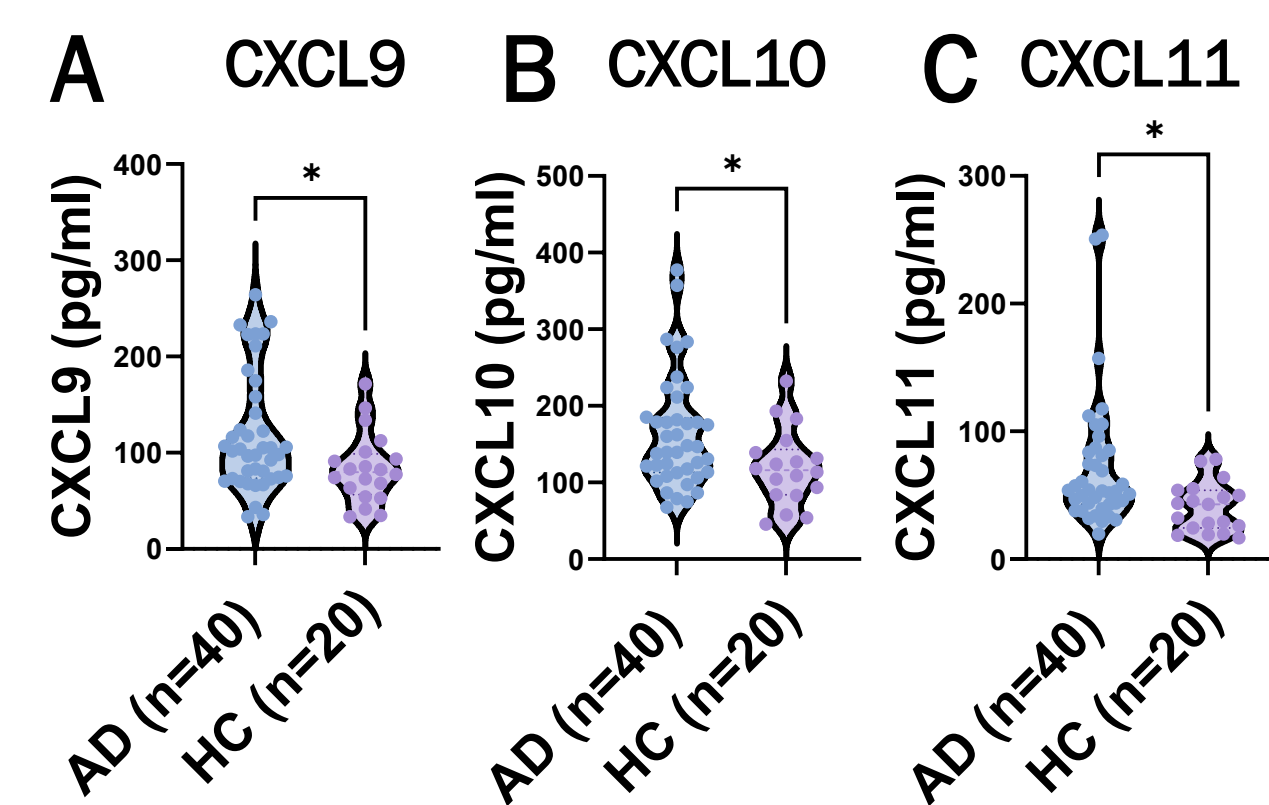


Figure 1. Plasma levels of CXCL9, CXCL10, and CXCL11 are significantly elevated in AD individuals. Concentration of CXCL9 (A), CXCL10 (B), and CXCL11 (C) in AD and HC individuals. Graphs depicting mean $\pm$ SEM; T-student $*p < 0.05$.

Single-cell proteomic analysis in human PBMCs identified CD14⁺ monocytes as the primary peripheral source of CXCL9, CXCL10, and CXCL11, while CXCR3 expression was enriched on CD4 and CD8 T cells

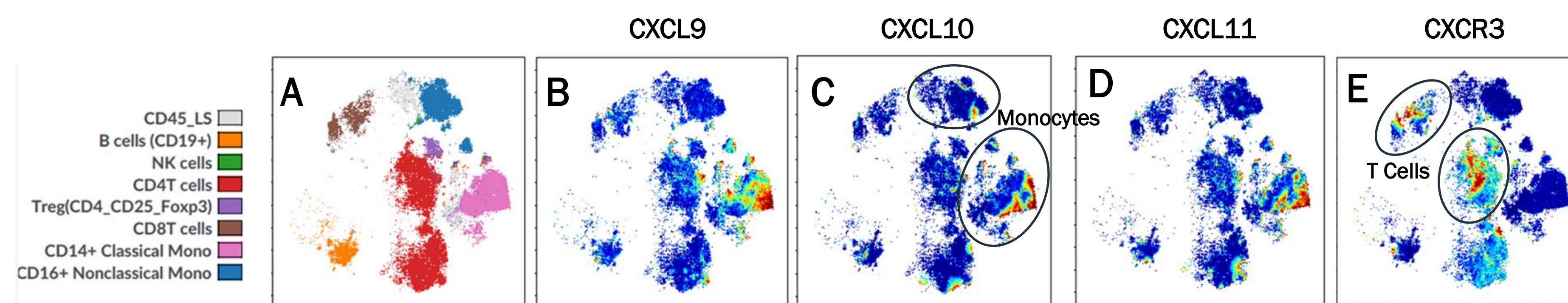


Figure 2. CXCL9-11/CXCR3 protein expression in human peripheral immune cells by CyTOF analysis. A) tSNE graph depicting immune cell clusters. B-E) tSNE graphs illustrating that CD14⁺ and CD16⁺ monocytes highly express the chemokines CXCL9, CXCL10, and CXCL11 (pink and dark blue clusters in A), whereas the receptor, CXCR3, is mainly expressed in CD4 and CD8 T cells (red and brown clusters in A).

Pharmacological blockade of CXCR3 in a mouse model of AD significantly decreased amyloid pathology, attenuated neuroinflammation, and limited the infiltration of T cells to the brain

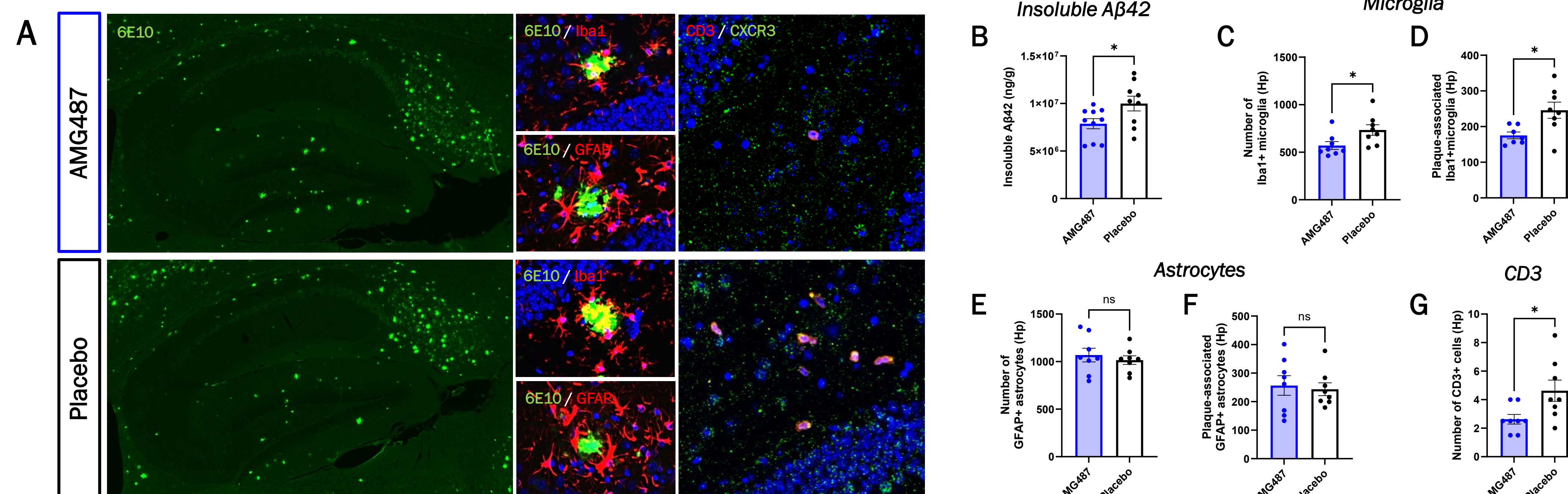


Figure 3. AMG487 treatment in 5XFAD mice significantly reduced amyloid pathology, microglia response and the number of T cells in the brain. A) Representative images of 6E10 (total A β), Iba1 (microglia), GFAP (reactive astrocytes), and CD3 in the hippocampus. B) Brain insoluble levels of A β 42 (measured by ELISA) were significantly decreased in AMG487 treated mice compared to placebo group. C, D) Quantification of numbers of total and plaque-associated Iba1 microglia in the hippocampus. E, F) No significant changes in the number of total or plaque-associated GFAP astrocytes were observed among the groups. G) Quantification of numbers of CD3 T cells in the hippocampus. Graphs depicting mean $\pm$ SEM; T-student $*p < 0.05$; ns= non-significant.

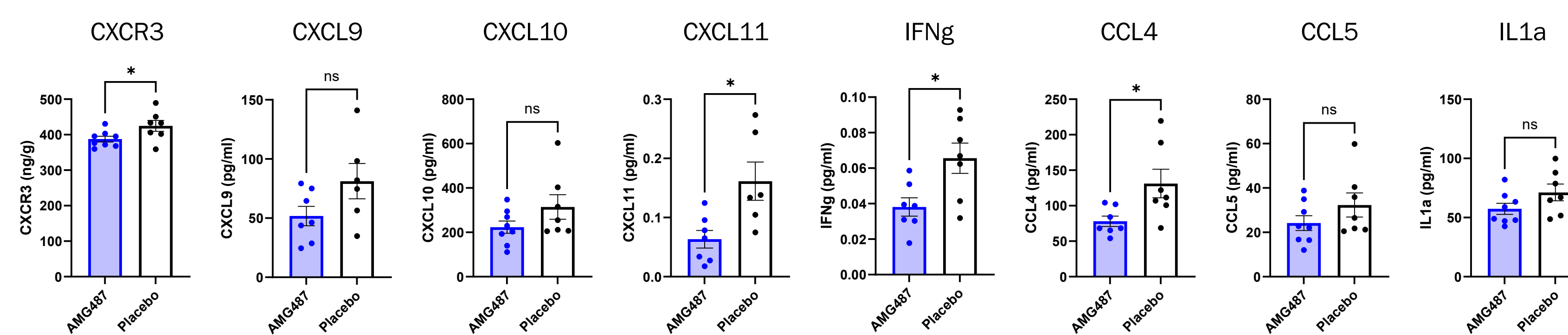


Figure 4. Brain levels of inflammatory chemokines and cytokines decreased following blockade of CXCR3 with AMG487 in 5XFAD mice. Quantification of CXCR3, CXCL9-11, IFN γ , CCL4, CCL5 and IL1a in brain homogenates of AMG487-treated and placebo 5XFAD mice. Graphs depicting mean $\pm$ SEM; T-student $*p < 0.05$.

Conclusions

- Our findings identify systemic activation of the CXCL9-11/CXCR3 axis as a mechanistic link between peripheral inflammation and neurodegeneration in AD
- The pharmacological inhibition of CXCR3 using AMG487 in a mouse model of AD (5xFAD) mitigates T cell brain infiltration, neuroinflammation, and insoluble A β 42 levels, supporting CXCR3 as a promising, druggable target for translational immunomodulatory strategies in AD

Acknowledgments

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