Anti-inflammatory clearance of amyloid beta by a chimeric Gas6 fusion protein

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Abstract

Aβ immunotherapy is one of the most promising approach for Alzheimer's disease treatment. Although several Aβ-antibodies can substantially reduce Aβ burden in the brain, their effects on cognitive function remain marginal. Moreover, patients with Aβ-antibody treatment often experience brain edema and microhemorrhage closely associated with immune reactions and inflammation. Here, we develop a chimeric phagocytosis inducer (αAβ-Gas6) for Aβ consisting of a single-chain variable fragment of Aducanumab fused with Gas6, a bridging molecule for clearing dead cells. This αAβ-Gas6 selectively eliminates Aβ plaques mainly through TAM receptor-dependent phagocytosis without NF-kB-mediated inflammatory responses and reactive gliosis. Furthermore, αAβ-Gas6 successfully engaged both microglia and astrocytes to clear Aβ plaques and substantially reduced excessive synapse elimination by microglia in the AD hippocampus to the level of non-AD controls.

Results

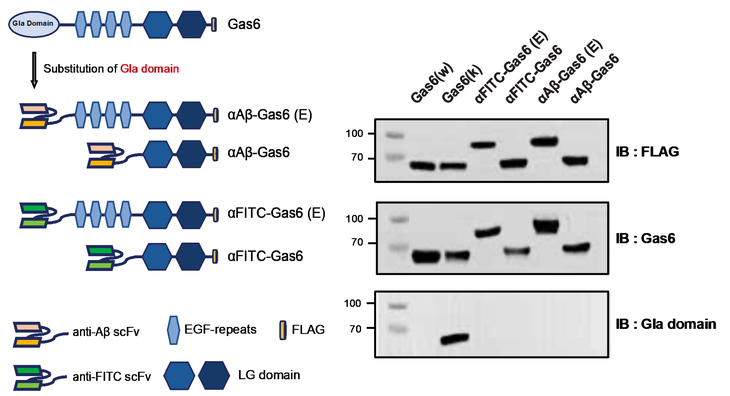

Figure.1 Generation of chimeric Gas6 fusion proteins

Binding of Gas6 to LG1/LG2 domains of TAM receptors induces receptor dimerization and activation, which in turn initiates downstream cascades including phagocytosis and anti-inflammatory responses. To redirect the target-specificity of Gas6 to Aβ, we replaced the Gla domain in the human Gas6 sequence with the single-chain fragment variable (scFv) of Aducanumab.

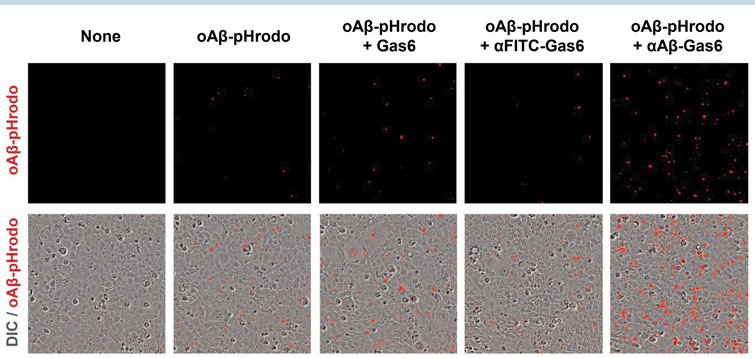

Figure.2 αAβ-Gas6-mediated Aβ uptake by human microglial cells: HMC3

Live-cell imaging analysis showed that only αAβ-Gas6 treated cells have significantly increased red fluorescence compared to Gas6 and αFITC-Gas6 treated cells. This data indicates that unlike native Gas6 which cannot bind to Aβ by itself, αAβ-Gas6 directly induces engulfment and phagocytic degradation of oAβ in the acidic lysosomal compartment.

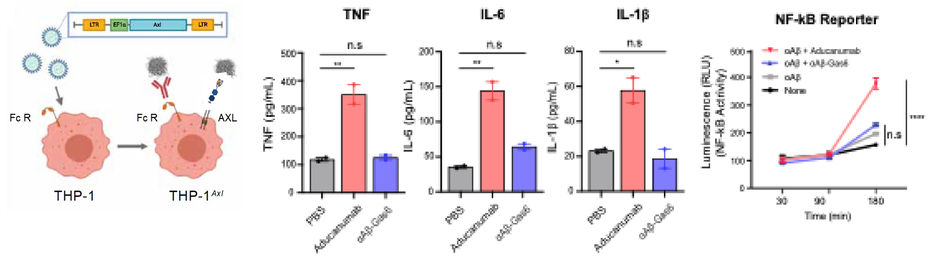

Figure.3 Anti-inflammatory effects of αAβ-Gas6: THP-1^{Axl}

Next, to directly compare the effects of αAβ-Gas6 with Aducanumab, we generated THP-1^{Axl} cells that express both AXL and Fc-gamma receptors. Importantly, only Aducanumab treated THP-1^{Axl} cells secreted significant amounts of pro-inflammatory cytokines, such as TNF, IL-6, and IL-1β. Consistent with this data, Aducanumab, but not αAβ-Gas6, showed strong NF-kB reporter expression in THP-1^{Axl}.

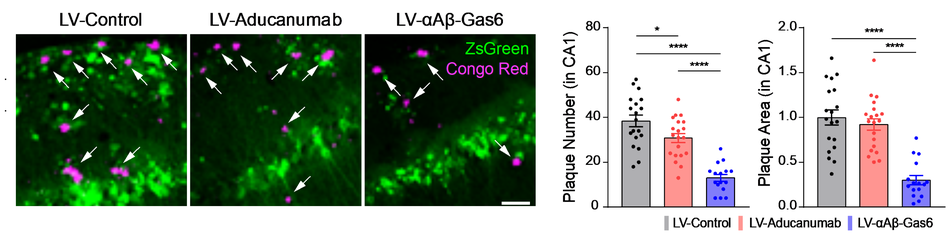

Figure.5 In vivo analysis of αAβ-Gas6 using 5XFAD: Gene Therapy

We examined whether and to what extent microglia and astrocytes were involved in plaque clearance. We bilaterally injected LV-Aducanumab and LV-αAβ-Gas6 into the hippocampal CA1 regions of 8 month-old 5xFAD mice. When we examined LV-injected 5xFAD mouse brains 4 weeks after injection, we found that different LV-injected groups had a similar number of ZsGreen-expressing cells in the CA1. Importantly, when we analysed Aβ plaques in the CA1 by Congo Red staining, we found that both the number and total area of Aβ plaques were significantly decreased in the 5xFAD CA1 injected with LV-αAβ-Gas6.

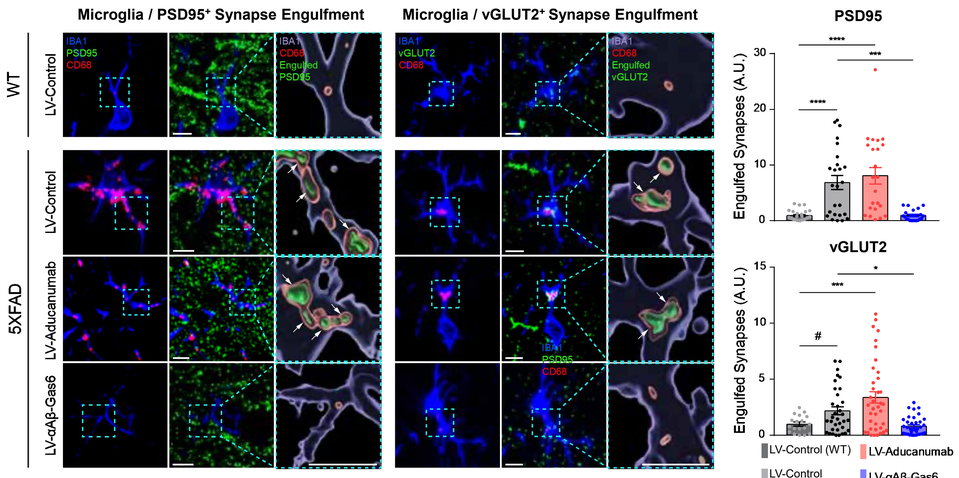

Figure.6 αAβ-Gas6 prevents excessive synapse elimination in 5XFAD AD mice

As reactive microglia are highly associated with excessive synapse elimination, we investigated the extent of NPAM (Non plaque-associated microglia)-mediated synapse elimination in LV-injected WT and 5xFAD brains. Strikingly, we found that LV-αAβ-Gas6 injection significantly reduced excessive synapse elimination by microglia to levels comparable to the non-AD WT brain group.

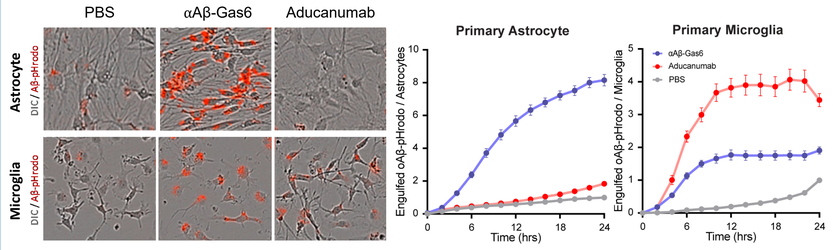

Figure.4 Increased astrocyte-mediated Aβ uptake by αAβ-Gas6

We found that both αAβ-Gas6 and Aducanumab significantly induced phagocytosis of oAβ-pHrodo in primary microglia, albeit with lower levels with αAβ-Gas6, most likely due to different expression levels of TAM versus Fc-gamma receptors in cultured microglia. Importantly, in sharp contrast to microglia, only αAβ-Gas6, but not Aducanumab, was able to significantly increase astrocyte-mediated oAβ phagocytosis.

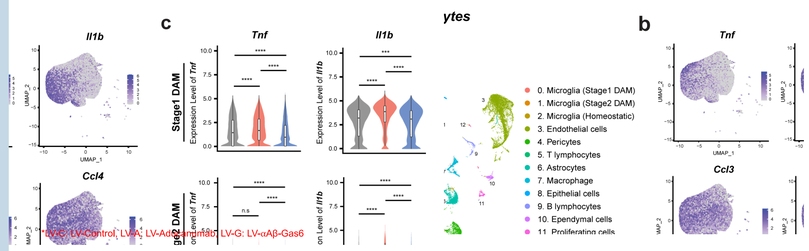

Figure.7 *In vivo* scRNA-seq reveals anti-inflammatory phagocytic effects of LV-αAβ-Gas6

Tnf and *Il1b* were significantly upregulated in DAM cluster of the LV-Aducanumab group, but were suppressed in the LV-αAβ-Gas6 group to levels even lower than the LV-control injected group. Concomitant with reduced expression of pro-inflammatory genes, microglia from LV-αAβ-Gas6 injected group upregulated different gene sets related to interferon-alpha responses and MHC class II related genes (*Cd74, H2-Aa, H2-D1, H2-k1, H2-Eb1,* etc.). Furthermore, interestingly, some of DAM markers (*Spp1, Itgax, Lgals1*) were also distinctly upregulated in the LV-αAβ-Gas6 injected group.

Similar to microglia, we found that astrocytes from the LV-Aducanumab injected group significantly upregulated several reactive astrocyte genes (*Gfap*, *C4b*, and *Cd14*) as well as proinflammatory chemokines and cytokines compared to the LV-αAβ-Gas6 injected group.

Conclusion

- We developed a novel chimeric fusion protein, αAβ-Gas6, by engineering Gas6 protein, a bridging molecule for the clearance of dead cells via TAM (Tyro3, Axl, and MerTK) receptors.
- αAβ-Gas6 selectively eliminates Aβ plaques mainly through TAM receptor-dependent phagocytosis without inducing NF-kB-mediated inflammatory responses.
- αAβ-Gas6 can induce synergistic clearance of Aβ by activating both microglial and astrocytic phagocytosis.
- αAβ-Gas6 prevents excessive synapse elimination.
- Our results suggest that αAβ-Gas6 can be a novel immunotherapeutic agent for AD beyond conventional antibody therapy.

Acknowledgements

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