Anti-N-Terminal Aβ Mab 3A1 Preferentially Recognizes Aβ Aggregates and Does Not Cross-React with APP

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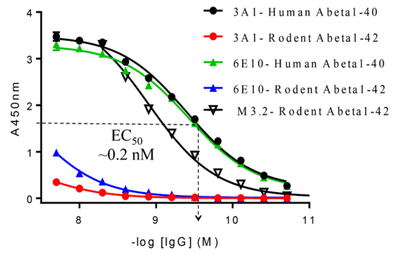
Introduction

Aβ's N-terminus is highly immunogenic and antibodies that target this region are standard reagents for Alzheimer's disease (AD) research, and have been utilized as investigational AD therapeutics. A murine anti-N-terminal Aβ monoclonal antibody (mAb), 3A1, was generated against dityrosine cross-linked Aβ1-40 protein species (CAPS), and its epitope mapped to the peptide's first 15 amino acids. 3A1 has demonstrated activity *in vivo* by decreasing plaque burden and increasing the levels of plasma Aβ in an APPswe/PS1ΔE9 transgenic mouse model of AD [Frost *et al.* (2015) *Neurobiol Aging.* 36(12): 3187]. To better understand the mAb's specificity for Aβ we established *in vitro* the antibody's ability to recognize Aβ1-40 conformers (monomers, dimers, protofibrils), APP, and rodent Aβ.

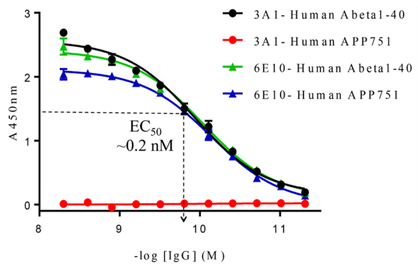
Methods

3A1's avidity for Aβ conformers, APP and rodent Aβ were determined using ELISA, and Western blot assays. Anti-N-terminal Aβ mAb, 6E10 [Kim *et al.* (1988) *Neurosci. Res. Comm.* 7:113], was used as a positive control. Aβ conformers were generated as previously described [Welzel *et al.* (2012) *PloS One* 7(11):e50317].

MAb 3A1 Recognized Human Aβ and Did Not Bind to Rodent Aβ or to Human APP



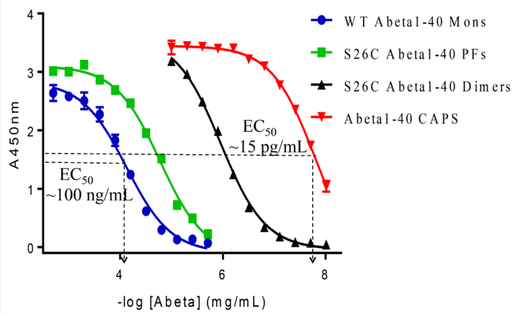
Both anti-N-terminal Aβ mAbs, 3A1 and 6E10, bound to plate-immobilized human Aβ1-40 with EC50 of ~0.2 nM. The mAbs had low to no binding to rodent Aβ.



Mab 3A1 did not bind to plate-immobilized human recombinant APP751 protein. Mab 6E10 bound similarly to Aβ1-40 and APP751 with EC_{50}'s of ~0.2 nM.

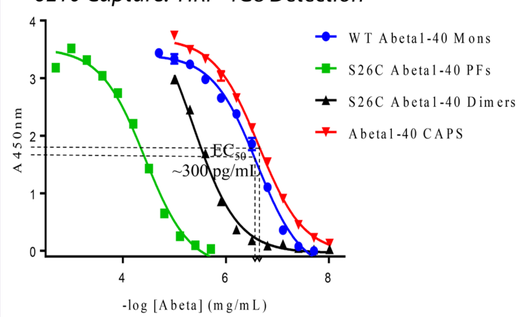
MAb 3A1 Preferentially Captured Aggregated Aβ

3A1 Capture: HRP-4G8 Detection



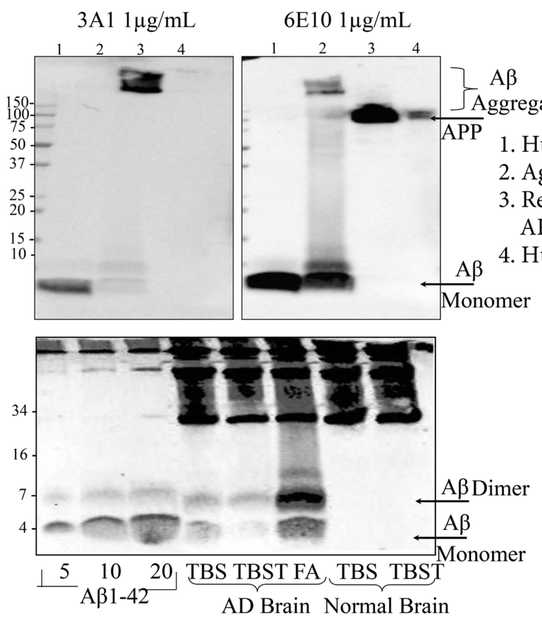
MAb 3A1 preferentially recognized Aβ aggregates compared with the monomeric peptide, with ~700-fold stronger binding to dityrosine cross-linked Aβ1-40

6E10 Capture: HRP-4G8 Detection



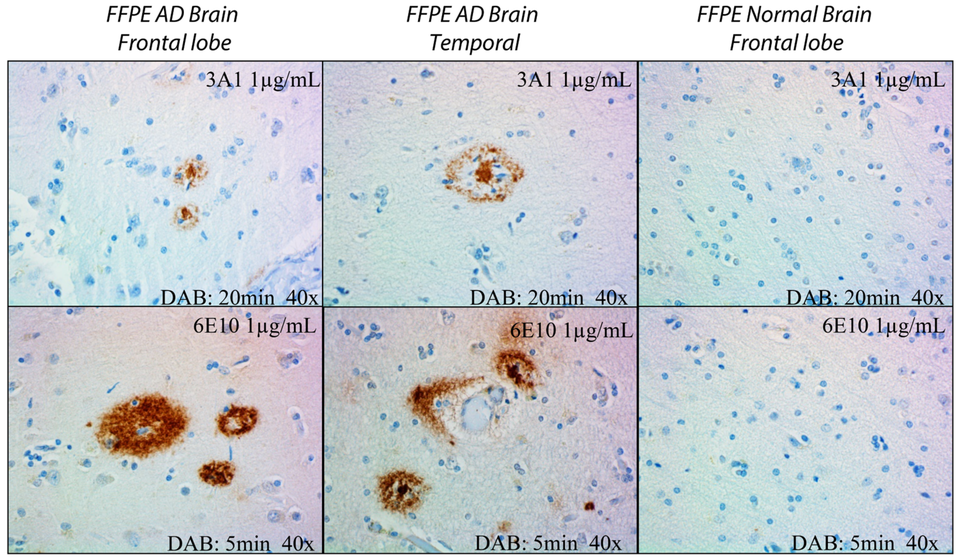
MAb 6E10 similarly bound to Aβ monomers and CAPS, but bound significantly weaker to S26C Aβ dimers and proto-fibrils (PFs).

MAb 3A1 Preferentially Detected Aggregated Aβ and Did Not Recognize APP



Upper blots confirmed mAb 3A1's preference for Aβ aggregates. The lower blot confirmed that 3A1 IP'd Aβ from AD brain extracts, but did not capture the peptide in normal brain extracts. WB was performed using anti-Aβ (21F12/2G3).

3A1 Stained Aβ Plaques in AD Brain Tissues



IHC was performed using 88% formic acid antigen retrieval, and by incubating the primary antibodies overnight at 4°C.

Summary & Conclusions

MAb 3A1 is a novel anti-N-terminal Aβ antibody that specifically detected human Aβ conformers in ELISA, IP/WB, and IHC applications.

The antibody demonstrated up to a ~700-fold preference for aggregated compared with monomeric Aβ in Capture/Sandwich ELISA. In contrast, another anti-N-terminal Aβ antibody, 6E10, did not preferably capture Aβ aggregates.

3A1's utility as an antibody research tool for AD was further demonstrated by its ability to avidly bind to human Aβ conformers without appreciably cross-reacting with human APP or rodent Aβ.

Acknowledgement

We would like to thank the University of Tennessee Graduate School of Medicine for their contributions to this poster.