RATIONAL GENERATION OF Aβ OLIGOMER-SPECIFIC ANTIBODIES THROUGH COMPUTATIONAL IDENTIFICATION OF CONFORMATIONAL EPITOPES

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ABSTRACT

Aims

To produce monoclonal antibodies specific for AβO in Alzheimer's disease (AD), by identifying conformation-specific epitopes within amyloid-beta oligomers (AβOs) using steered, computational molecular dynamics simulations considering the biological, structural and thermodynamic properties of the protein.

Method

Proprietary computational algorithms identified 5 distinct AβO-specific epitopes. Peptides containing the epitopes were synthesized, cyclized, conjugated to KLH, and mice were immunized. Using ELISA and Surface Plasmon Resonance Imaging (SPRi) technology, hybridoma supernatants were screened for binding to structured peptides, unstructured peptides, synthetic Aβ monomers and AβOs. Candidate antibodies that were oligomer-selective were purified and validated with immunohistochemical staining of AD brain, as well as SPRi analysis of cerebrospinal fluid and brain extracts from AD patients and age-matched controls.

Results

Immunization of mice with the 5 structured epitopes resulted in 314 hybridoma supernatants. 66 clones were selected for purification based on their ability to recognize the cognate structured peptide and synthetic AβO, with little or no binding to unstructured peptide, KLH-epitope linker peptide, or Aβ monomers. Additional screening identified antibodies that preferentially bound to native soluble AβO in CSF and brain extracts of AD patients compared to controls. Immunohistochemical analysis of AD brain allowed for selection of antibody clones that did not react with plaque.

Conclusion

Immunization with structured peptides synthesized to mimic Aβ oligomer epitopes identified by computational algorithms allowed for the generation of monoclonal antibodies specific for soluble AβO, as demonstrated by selectivity for AβO in human AD tissues, and lack of binding to plaque or monomers.

BACKGROUND

Progress in Alzheimer's disease (AD) immunotherapeutics has been impeded in part by the incomplete neutralization of misfolded Amyloid beta Oligomers (AβO), which are believed to be mediators of the neurotoxic, neuropathologic and behavioral changes characteristic of the disease. Generation of conformationally-selective antibodies that specifically target these toxic and propagative AβOs is paramount to an effective AD immunotherapy that will delay disease onset and progression, and prevent cognitive decline.

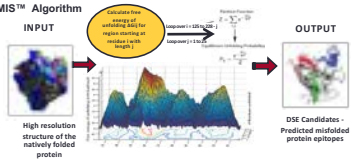
OBJECTIVES

Employing computational molecular dynamics simulations to predict and identify regions of the Aβ sequence that are disrupted or absent in fibrils and monomers but exposed in misfolded toxic AβOs, and to produce conformationally-selective monoclonal antibodies that bind to these regions. To further characterize and develop these mAbs as potential candidates for AD immunotherapeutics and diagnostics.

METHODS

- **Proprietary computational algorithms combining protein biology, structure, and the thermodynamics of conformational change to predict structurally integral misfolding and resulting disease specific epitopes**
  - ProMIS™ discovery process & Collective Coordinates, a complementary methodology
  - Coverage of multiple prion strains

ProMIS™ Algorithm

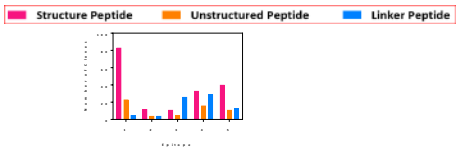


- **Generation and Identification of Lead Monoclonal Antibodies**
  - Epitopes synthesized, structured through cyclization, conjugated to KLH, conjugates validated for sequence and structure, and mice immunized.
  - ELISA and Molecular Affinity Screening System (MASS-1) (Sierra Sensors, Germany), a high throughput surface plasmon resonance imaging (SPRi) analytical biosensor, used to screen hybridoma supernatants for reactivity against structured and unstructured epitopes and synthetic AβOs and monomers.
  - Antibodies preferentially binding structured epitopes and AβOs were expanded and purified.
- **Secondary Screening of Lead Monoclonal Antibodies for Desired Target Profile**
  - SPRi analysis of lead antibodies binding to synthetically-prepared AβOs.
  - SPRi analysis of lead antibodies binding to soluble AβOs in pooled brain extracts and CSF samples from AD patients and healthy controls.
  - Immunohistochemical analysis of lead antibodies for plaque reactivity in well-characterized fresh frozen brain tissue from AD patients. mAb6E10, a pan-Aβ antibody, was included as positive control.
- **Antibody Inhibition of Oligomer Propagation**
  - Monomeric Aβ42 was incubated alone and in the presence of 2 lead monoclonal antibodies and mouse IgG control in a molar ratio of 5:1 (Aβ42 to antibody).
  - Samples were incubated at room temperature and beta-sheet formation monitored hourly for 25 hours with Thioflavin T (ThT) fluorescence.
- **Antibody Inhibition of AβO42-induced Toxicity in vitro**
  - Various molar ratios of AβOs (SynAging SAS, France), lead antibodies, control IgG, vehicle control, AβO alone, antibody alone and a positive control neuroprotective peptide humanin (HNG), were incubated with cultures of mouse embryonic cortical neurons for 24hrs.
  - Cell viability was monitored with the MTT assay.
- **Antibody Inhibition of AβO42-induced Cognitive Deficit in vivo**
  - The efficacy of one lead antibody to prevent the loss of short term memory formation caused by toxic oligomers was investigated in wild-type C57Bl6/J mice.
  - The animals were injected intracerebroventricularly with a mixture of AβOs (SynAging SAS, France), and antibody at day 0.
  - Cognitive performance was monitored using the Novel Object Recognition (NOR) assay at day 8.

RESULTS

- **ELISA Prescreen of Hybridoma Supernatants**

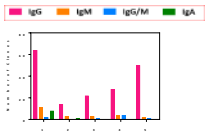
Varied Reactivity to Structured and Unstructured Peptides



- **Immunoglobulin Subclass Distribution of Hybridoma Supernatants**

(Clones specific to structured epitope by ELISA)

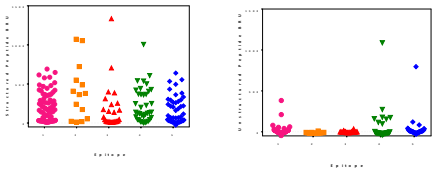
Clones are Predominantly of the IgG Subclass



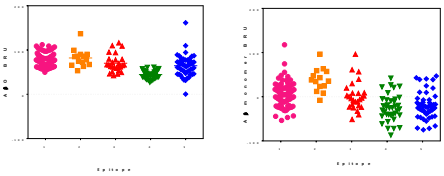
- **Screening of Hybridoma Supernatants by Surface Plasmon Resonance Imaging**

(Clones specific to structured epitope by ELISA)

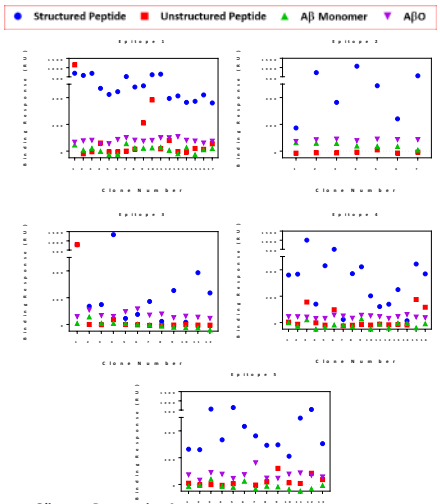
Preferential binding to Structured vs Unstructured peptide



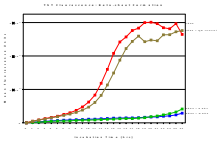
Binding to Aβ1-42 Oligomers. Limited binding to Aβ1-42 Monomers



- **Comparative Binding Profiles for Lead Monoclonal Antibodies**



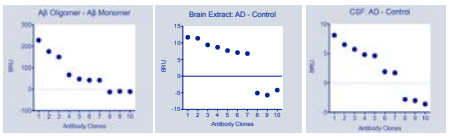
- **Oligomer Propagation Assay**



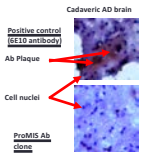
RESULTS

- **Secondary Screening of Lead Clones for Desired Target Profile**

Desired Binding to Soluble Oligomers from AD brain and CSF.
Little or no binding to monomers

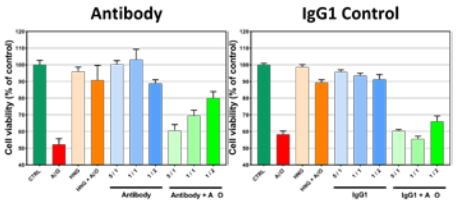


No binding to plaque in AD brain tissue



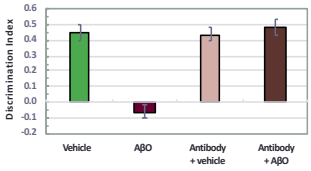
- **In vitro Neurotoxicity Assay**

AβO-selective Lead antibody Protects against AβO-induced Toxicity



- **In vivo Cognition Testing (NOR assay)**

AβO-selective Lead antibody improves Cognitive Function



CONCLUSIONS

- Computational methods using molecular dynamic simulations have predicted 5 conformational epitopes in Aβ Oligomers.
- Conjugates of the structured epitopes are immunogenic.
- Monoclonal antibodies raised against these epitopes can :
  - Bind preferentially to synthetic Aβ Oligomers and negligibly to monomers.
  - Inhibit Aβ oligomer propagation in vitro ;
  - Bind desirably to naturally-occuring soluble Aβ Oligomers in AD brain tissue and CSF.
  - Show no reactivity to plaques in cadaveric AD brain tissue.
  - Protect against Aβ neurotoxicity in vitro.
  - Prevent loss of short term memory formation in a mouse model.