PERSPECTIVES

Structural aspects of Alzheimer’s disease immunotherapy targeted against amyloid-beta peptide

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ABSTRACT
Alzheimer’s disease is the most prominent neurodegenerative disease and has no efficient therapies available so far. Immunotherapy against amyloid-β (Aβ) peptide is one of the currently tested therapeutic approaches. Here we have reviewed the available structural knowledge about antibodies tested as passive anti-Aβ immunotherapy in clinical trials. The therapeutic anti-Aβ antibodies differ in their epitope specificity and in recognition and affinity to different Aβ species present in vivo (Tab. 1, Fig. 1, Ref. 17). Text in PDF www.elis.sk

KEY WORDS: Alzheimer’s disease, amyloid beta, immunotherapy, antibody-amyloid complex.

Introduction

Major histopathological hallmarks of Alzheimer’s disease are senile plaques composed of amyloid-β (Aβ) peptide and neurofibrillary tangles composed of tau protein. The 3D structure of a stable polymorph of amyloid fiber composed from Aβ1-42 peptide, the most toxic and aggregation-prone cleavage product of amyloid precursor protein, was recently determined by solid state NMR and is composed of two molecules per fibril layer that are forming a double-horseshoe-like cross-β-sheet entity (1, 2). However, it seems that the culprits of toxicity are rather the unstable Aβ oligomers that are capable to disrupt cellular membranes by pore formation (3, 4).

One likely mechanism which the passive immunotherapy against Aβ peptide is based on is the peripheral sink phenomenon, where the peripherally administered antibodies bind circulating soluble Aβ species and change the Aβ concentration ratios between CNS and plasma. The gradient in Aβ concentration promotes its export from the brain and dissolution of amyloid plaques (5).

In this review, we summarize anti-Aβ antibodies with a publicly available structure, which have entered the clinical trials (Tab. 1), namely bapineuzumab, gantenerumab, crenezumab, solanezumab and ponezumab. Solanezumab and bapineuzumab did not improve clinical outcomes in patients with mild to moderate Alzheimer’s disease. The publicly available data from Phase II studies for these antibodies indicate that neither of compounds produced a compelling evidence of drug-like behavior that would justify their progression into phase III trials (17). It is considered that it may be an issue of treatment window and therefore the anti Aβ antibodies are further being examined in trials of treatment in at-risk asymptomatic individuals (Dominantly Inherited Alzheimer Network (DIAN) trial, Alzheimer Prevention Initiative (API) trial) (13). Clinical trials of ponezumab were also discontinued in association with Alzheimer’s disease, and the antibody is now in phase II for cerebral amyloid angiopathy (www.AlzForum.org). The reviewed anti-Aβ antibodies, crenezumab and gantenerumab, are in an ongoing phase III of clinical trials.

Antibodies targeting the N-terminus of Aβ peptide

Bapineuzumab is an IgG1 antibody produced by humanization of parent murine antibody 3D6. The Kd of bapineuzumab interaction measured with thermophoresis increases from 89 nM measured with Aβ 1-40, 151 nM with Aβ 1-28, to 4.5 µM with Aβ 1-8 peptide, indicating that a longer peptide sequence is needed for full reactivity (6). Bapineuzumab captures Aβ in a 3α helical conformation stabilized by five intramolecular hydrogen bonds. The N-terminal amine of Aβ is involved in hydrogen bonds with Glu3(α) side-chain carboxyls and Asp1(α) binds to the bottom of bapineuzumab paratope groove, where its side-chain carboxyl makes hydrogen bond with Ser100β(α) and side-chain nitrogen of Trp89(β) . Aβ residues Glu3 and Arg5 form salt bridges with bapineuzumab residues Arg96(β) and Asp27d(α), respectively. The guanidinium group of Arg5(α) side-chain π stacks over the side-chain of Tyr32(β). The hydrophobic side-chain of Phe4(α) is buried and π stacks against the side-chain of Tyr95(β). The structure of Aβ in complex with bapineuzumab is similar to the TFE stabilized solution structures of Aβ determined by NMR. The reactivity of bapineuzumab with plaques suggests the presence of this conformation also in dense Aβ deposits with core cross-β structure (6, 7). Interestingly, in the recent ssNMR structure of Aβ1-42 fiber, Aβ residues 1-15 comprising the epitope of bapineuzumab are not

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tightly bound to the fiber core (2). It has been noted that antibodies against the $\beta_{10}$ helical conformation of $\alpha$-secretase are raised by immunization of mice with short N-terminal $\alpha$-secretase (Aβ1-7 conjugated to KLH in case of 3D6 antibody), whereas immunization with Aβ1-28, Aβ1-42, Aβ fibrils or protofibrils produces antibodies recognizing extended N-terminal $\alpha$-secretase conformation (antibodies 12A11, 12B4, 10D5, PFA1/PFA2, WO) (8, 9). The antibody C706 generated after immunization with Aβ1-5 binds Aβ N-terminus in a somewhat distorted manner, as well as with $\beta_{10}$ helical conformation, and approaches Aβ from a different side (key epitope residues are Arg5A and His6A) (9). It can be concluded that the length of Aβ fragment modulates the preferred conformation of its N-terminus in the conformational ensemble of free peptide.

**Gantenerumab** was derived from a synthetic human combinatorial antibody library based on phage display. The epitope mapping using overlapping Aβ decapetides has revealed two discontinuous regions of recognition. The strongest one was the N-terminal decapetide EFRRHDSGYEV9 and the other was a central decapetide VFFAEVDG3N5N. SPR revealed $K_d$ values of 0.6 nM, 1.2 nM, and 17 nM for fibrillar, oligomeric and monomeric Aβ1-40, respectively, showing a preference for high-molecular structures. In structural studies, the gantenerumab Fab fragments were co-crystallized with Aβ 1-11 and Aβ 3-11 peptides. Aβ 1-11 peptide binds in an extended conformation in the groove defined by CDRs H1, H2, H3, and L3. First three aminoacids of Aβ peptide interact with the antibody mainly through their main-chain carbonyl oxygen atoms. The side-chain of Phe4A is deeply buried in a hydrophobic pocket anchoring the Aβ chain. The side-chain of Arg5A is stacked from one side by three antibody tyrosines. The N-terminal part of Aβ peptide is in the proximity of N-acetylglucosamide moiety found at Asn52CDRH2. Ser8A and Gly9A residues point away and do not interact with the antibody. They form a short $\gamma$-turn that is stabilized by a hydrogen bond between the main-chain carbonyl of Asp7A and main-chain nitrogen of Tyr10A. The orientation of Aβ peptide with respect to antibody CDRs is flipped in the gantenerumab complex by 180° when compared to antibodies recognizing the extended N-terminal conformation of Aβ (8, 10).

**Antibodies targeting the mid-region of Aβ**

*Crenezumab* is a humanized monoclonal IgG1 antibody that binds multiple forms of Aβ - monomers, oligomers, fibrils and plaques. The SPR-measured $K_d$ was revealed to have a ten times higher affinity for high-molecular forms, being 0.4–0.6 nM and 3.0–5.0 nM for oligomeric and monomeric Aβ forms (peptide 11–28), respectively (11).

Crenezumab was crystallized as synthetic CreneFab with mutations changing the heavy chain constant domain to IgG1 sequence. The aromatic side-chains of Phe19A and Phe20A are anchored to the bottom of the paratope groove by $\pi$-$\pi$ stacking interactions with Trp96CDRL3 and His34CDRL1. Charged side-chain of Asp23A forms hydrogen bonds with main-chain nitrogen of Gly33CDRH1, whereas Ser52A and Glu22A are engaged in water-mediated hydrogen bonding. Lys16A forms a salt bridge with Asp101CDRH1 and is stacked between Tyr32CDRH1 and Phe27CDRH1. A non-canonical antibody-antigen interaction was observed between His14A side-chain and N-terminal amino group of heavy chain Glu1(11).

**Solanezumab** is a humanized monoclonal IgG1 antibody that recognizes soluble monomeric Aβ with picomolar affinity and does not bind fibrillar Aβ species. The epitope of solanezumab is partially overlapping the epitope of crenezumab. All CDRs are identical in length in solanezumab and crenezumab while L2, L3, and H3 are also identical in composition. Both antibodies therefore show cross reactivity with plasma proteins containing Phe-Phe dipeptide (14). The $K_d$ of solanezumab fully glycosylated at N52CDRH1 was 4 pM, whereas the $K_d$ of a mutated unglycosylated variant drops to 0.8 pM. Nevertheless, these $K_d$ values are not corrected for the avidity of bivalent antibody and therefore they are not directly comparable to other results (12). Solanezumab may bind Aβ monomers with the preference to adopt a helical conformation that was shown by NMR for Aβ in helix promoting agents (13).

While bound to solanezumab, residues 16-18 of Aβ adopt an extended coil conformation and residues following the Phe-Phe dipeptide (14). The $K_d$ of solanezumab fully glycosylated at N52CDRH1 was 4 pM, whereas the $K_d$ of a mutated unglycosylated variant drops to 0.8 pM. Nevertheless, these $K_d$ values are not corrected for the avidity of bivalent antibody and therefore they are not directly comparable to other results (12). Solanezumab may bind Aβ monomers with the preference to adopt a helical conformation that was shown by NMR for Aβ in helix promoting agents (13).

In complex with solanezumab, Asp23A forms two hydrogen bonds with Ser33CDRH1 and Glu22A are engaged in water-mediated hydrogen bonding. Lys16A forms a salt bridge with Asp101CDRH1 and is stacked between Tyr32CDRH1 and Phe27CDRH1. A non-canonical antibody-antigen interaction was observed between His14A side-chain and N-terminal amino group of heavy chain Glu1(11).

**Bapineuzumab** is a humanized monoclonal IgG4 antibody that binds multiple forms of Aβ - monomers, oligomers, fibrils and plaques. The SPR-measured $K_d$ was revealed to have a ten times higher affinity for high-molecular forms, being 0.4–0.6 nM and 3.0–5.0 nM for oligomeric and monomeric Aβ forms (peptide 11–28), respectively (11).
of Ser$^{33}_{\text{CDR1}}$ and Gln$^{50}_{\text{CDR2}}$, and Glu$^{22}_{\beta}$ does not interact with solanezumab. However, the first putative helix stabilizing the hydrogen bond (between Phe$^{20}_{\beta}$ backbone carbonyl and Asp$^{23}_{\beta}$ backbone nitrogen) is present in both structures and is even shorter in the crenezumab complex structure.

Recently, Zhao et al. have investigated the different specificity of solanezumab and crenezumab by using computational methods, namely homology modeling, molecular docking and molecular dynamics simulations (14). They have simulated an interaction of solanezumab, CreneFab (used for crystallization) and

**Fig. 1. Structures of anti $\alpha\beta$ antibodies.** $\alpha\beta$ peptide is shown as sticks with carbon atoms colored magenta while the first amino acid of $\alpha\beta$ peptide observable in the complex is marked. Antibody paratopes are shown as a white transparent surface and sticks with carbon atoms colored green. N-acetylglucosamine (NAG) present in gantenerumab is shown in sticks with green carbon atoms. Other atoms, namely oxygen, nitrogen and sulphur are colored red, blue, and yellow, respectively. Bottom right part of the figure shows the amino acid sequence of $\alpha\beta$1-42 peptide.
cerepezumab (homology modeled) with Aβ 12-28 monomer, and
docked models of Aβ 11-42 oligomer (5-mer) and fibril (16-mer) 
derived from ssNMR structure of Aβ. Their results have shown 
that cerepezumab recognizes N-terminally shifted hydrophilic 
and cationic epitope around residues 13-16 on different oligomeric 
Aβ forms, which was not observed for solanezumab. They also 
pointed out the influence of Fab constant domain on Aβ binding 
through entropy redistribution.

**Antibody targeting the free C-terminus of Aβ1-40**

Ponezumab is a humanized monoclonal antibody that binds to 
C-terminus of Aβ1-40. Aβ40 residues 30 to 40 visible in the 
complex structure form of four β turns (31-34, 33-36, 35-38, 
and 36-39). The C-terminal Val40Aβ with its charged carboxy- 
terminus interacts with ponezumab most extensively, forms nine 
interactions, and buries 35 % of the total binding interface. The 
C-terminal carboxyl interacts with ponezumab residues Arg50C-
DR32 and Tyr96CDRH1. The N-terminal part of Aβ peptide (residues 
30Ala-Ile-Ile32) is stabilized by the constant domain of second Fab 
molecule present in asymmetric unit and probably does not cor-
respond to the situation in solution. The SPR-measured Kₐ of 
ponsezumab binding to immobilized Aβ 17-40 peptide was 0.3 nM 
and it binds neither to Aβ 17-42 peptide nor to Aβ 17-40 peptide 
with amidated C-terminus (15).

**Conclusions and outlook**

The knowledge of detailed atomic resolution and complex 
structures of therapeutic antibodies with their targets is indispensable 
for correct elucidation of the therapeutic mode of action. The 
example of cerepezumab and solanezumab complex structures 
shows how slight structural changes are manifested in different 
binding properties and selectivity. The structures of all mentioned 
antibody-Aβ complexes are shown on Figure 1.

In phase III of clinical trials there is also a fully human IgG1 antibody named aducanumab. It is derived from healthy aged 
donors and its structure is not available. The antibody may bind 
with conformational epitope on Aβ. However, 41 % of patients 
receiving the highest tested dose of 10 mgkg⁻¹ of aducanumab have developed ARIA-E (amyloid-related imaging abnormalities such as 
asosgenic edema) abnormalities early in the course of treatment (16). A dose lowered due to the side effects may be too low 
to exhibit beneficial clinical outcomes in passive immunotherapy 
targeting Aβ peptide.

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