PERSPECTIVES

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

Cehlar O1, Skrabana R1, Revajova V2, Novak M1

Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia.
ondrej.cehlar@savba.sk

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 \( K_d \) of bapineuzumab interaction measured with thermophoresis increases from 89 nM measured with Aβ1-40, 151 nM with Aβ1-28, to 4.5 μM with Aβ1-8 peptide, indicating that a longer peptide sequence is needed for full reactivity (6). Bapineuzumab captures Aβ in a 3_2 helical conformation stabilized by five intramolecular hydrogen bonds. The N-terminal amine of Aβ is involved in hydrogen bonds with Glu3^Nε side-chain carboxyls and Asp1^Nε binds to the bottom of bapineuzumab paratope groove, where its side-chain carboxyl makes hydrogen bond with Ser100\(^6\)C^O\(\beta\) and side-chain nitrogen of Trp89\(^6\)C^O\(\beta\). Aβ residues Glu3 and Arg5 form salt bridges with bapineuzumab residues Arg96\(^6\)C^O\(\beta\) and Asp27\(^6\)C^O\(\beta\), respectively. The guanidinium group of Arg5^Nε side-chain \( \pi \) stacks over the side-chain of Tyr32\(^6\)C^O\(\beta\). The hydrophobic side-chain of Phe4^Nε is buried and \( \pi \) stacks against the side-chain of Tyr9\(^5\)C^O\(\beta\). 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...
tightlly bound to the fiber core (2). It has been noted that antibodies against the 3_{10} helical conformation of Aβ peptide are raised by immunization of mice with short N-terminal Aβ peptide (Aβ1-7 conjugated to KLH in case of 3D6 antibody), whereas immunization with Aβ1-28, Aβ1-38, Aβ fibrils or protofibrils produces antibodies recognizing extended N-terminal Aβ conformation (antibodies 12A11, 12B4, 10D5, PFA1/PFA2, W0) (8, 9). The antibody C706 generated after immunization with Aβ1-5 binds Aβ N-terminus in a somewhat distorted manner, as well as with 3_{10} helical conformation, and approaches Aβ from a different side (key epitope residues are Arg5^{40} and His6^{40}) (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 EFHGSYVEV^{12} and the other was a central decapetide VFFAEVGSN^{17}. 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 Phe4^{40} is deeply buried in a hydrophobic pocket anchoring the Aβ chain. The side-chain of Arg5^{40} is stacked from one side by three antibody tyrosines. The N-terminal part of Aβ peptide is in the proximity of N-acetylglucosamidase moiety found at Asn52^{CDRH2} Ser8^{40} and Gly9^{40} residues point away and do not interact with the antibody. They form a short γ-turn that is stabilized by a hydrogen bond between the main-chain carbonyl of Asp7^{40} and main-chain nitrogen of Tyr1^{10}. 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 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).

Crenezumab was crystallized as synthetic CreneFab with mutations changing the heavy chain constant domain to IgG1 sequence. The aromatic side-chains of Phe19^{40} and Phe20^{40} are anchored to the bottom of the paratope groove by π-π stacking interactions with Trp^{96}_{CDRH1} and His^{34}_{CDRL1}. Charged side-chain of Asp^{23}_{CDRH1} forms hydrogen bonds with main-chain nitrogen of Gly^{33}_{CDRH1}, whereas Ser^{52}_{CDRH1}Oγ and Glu^{22}_{40} are engaged in water-mediated hydrogen bonding. Lys^{16}_{40} forms a salt bridge with Asp^{101}_{CDRH1} and is stacked between Tyr^{32}_{CDRH1} and Phe^{27}_{CDRH1}. A non-canonical antibody-antigen interaction was observed between His^{14}_{40} sidechain and N-terminal amino group of heavy chain Glu1^{11}.

Solanezumab is a humanized monoclonal IgG1 antibody that recognizes solubel 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 N5^{2}_{CDRH1} 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^{19} and Phe^{20} dipeptide, Ala^{21} Aβ and Ser^{26} Aβ, adopt a helical conformation (11). In contrast, crenezumab CDRH1, in position 33, occupies glycine, which is not able to make a side-chain hydrogen bond. Aβ conformation recognized by crenezumab is therefore more open and relaxed in comparison with solanezumab, which can account for different reactivity of antibodies to fibrillar forms of Aβ.

The largest Aβ side-chain difference between crenezumab and solanezumab-bound Aβ conformation is found for the side-chain of Glu^{22}_{40}. In complex with crenezumab, Glu^{22}_{40} plunges into a volume surrounded by CDRs H1, H2, L3 and a water cluster. In contrast, this region is blocked in solanezumab by side-chains
of Ser33$^{\text{CDRH1}}$ and Gln50$^{\text{CDRH2}}$, and Glu22$^{\beta}$ does not interact with solanezumab. However, the first putative helix stabilizing the hydrogen bond (between Phe20$^{\alpha}$ backbone carbonyl and Asp23$^{\alpha}$ 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
crenezumab (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 crenezumab 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-β31 and Tyr96C-β31. The N-terminal part of Aβ peptide (residues 30-Ala-Ile-Ile32) is stabilized by the constant domain of second Fab molecule present in asymmetric unit and probably does not correspond to the situation in solution. The SPR-measured Kd of ponezumab 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 crenezumab 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 vasogenic 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.

References


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