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EDITORIAL HIGHLIGHT

New tool to tackle Alzheimer's disease: amyloid-β protofibril-selective antibody AbSL

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Read the full article *'The conformational epitope for a new Aβ42 protofibril-selective antibody partially overlaps with the peptide N-terminal region'* doi: 10.1111/jnc.14211

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Abbreviations used: AbSL, antibody St. Louis; Aβ, amyloid-β protein; APP, amyloid precursor protein; ELISA, enzyme-linked immunosorbent assay.

Abstract

This Editorial highlights a study by Colvin *et al.* (2017) in the current issue of Journal of Neurochemistry, in which the authors describe the development and characterisation of a new rabbit antibody (termed antibody St. Louis; AbSL) that preferentially recognizes amyloid-β (Aβ) protein 42 (Aβ42) protofibrils over other Aβ species. Two antisera were raised against isolated Aβ42 protofibrils, which had similar immunochemical characteristics. In an indirect enzyme-linked immunosorbent assay (ELISA), the AbSL antibody displayed stronger reactivity with protofibrils than with monomers or fibrils and higher affinity to Aβ42 than to Aβ40. AbSL shoved very low reactivity with amyloid precursor protein (APP) in immunoblots of brain samples. Sandwich and competition ELISAs indicated that the main epitope recognised by the AbSL antibody included the N-terminal region of Aβ42 protofibrils. The new conformation specific antibody to Aβ42 protofibrils have research, diagnostic and potentially therapeutic applications in Alzheimer's disease.

EDITORIAL HIGHLIGHT

[Main text, no separate header]

Slow and initially asymptomatic onset of neurodegenerative disorders, such as Alzheimer's disease, requires clear understanding of the underlying molecular and cellular processes occurring during early stages of the disease. The accumulation and aggregation of Aβ peptides is a key feature of Alzheimer's disease and implicated in neurotoxicity (Ugalde *et al.* 2016). It is now widely recognised that Aβ aggregates are formed through a multistep process, which involves a series of conformational changes before fibrils are produced. A complex and dynamic equilibrium exists between soluble monomers, oligomers and various insoluble aggregates (Fig. 1). Better understanding of this process requires the identification of intermediate states, such as Aβ protofibrils, which are formed during the transition from monomeric proteins to fibrils. Aβ protofibrils are identified as short flexible fibrils (up to 100-200 nm long with a 4-10 nm in diameter; Walsh *et al.* 1999). Previous studies implicated Aβ protofibrils as pathogenic agents (Klyubin *et al.* 2012) and they have been considered as target for immunotherapy in Alzheimer's disease (Lannfelt *et al.* 2014). Therefore, the identification and structural characterisation of Aβ protofibrils is a potentially important step towards understanding the mechanism of neurodegeneration in Alzheimer's disease and other neurological disorders (Abu Hamdeh et al. 2017).

There is a need for better Alzheimer's disease biomarkers that are suitable for the monitoring of disease progression and responses to treatment (Golde 2016). As soluble Aβ oligomers and protofibrils have been implied to be causatives for Alzheimer's disease, they are potentially good biomarker candidates. Due to their selectivity, conformation-specific antibodies are currently the best tools for the detection of particular transient states of Aβ in Alzheimer's disease. These antibodies may also enable the development of highly targeted immunotherapeutic interventions (Westwood and Lawson 2015). Indeed, there are a number of antibody-based immunotherapies targeting Aβ currently in clinical trials (Westwood and Lawson 2015; Cummings *et al.* 2017) and this approach could be improved by immunoreagents that are selective for a particular Aβ conformation.

In the study by Colvin *et al.* (2017), Aβ42 protofibrils were isolated by size exclusion chromatography (Paranjape *et al*. 2013) and used for the immunisation of rabbits. The selectivity of the obtained antisera (AbSL) were characterised using an indirect ELISA and dot blot assay with different forms of Aβ42 and Aβ40, including protofibrils, monomers and fibrils (Fig. 1). Significant selectivity was observed by AbSL antisera for protofibrils compared to monomers and fibrils at lower mass amounts of Aβ42. Using protein samples from C57BL/6 wild-type, amyloid precursor protein (APP) knockout ($APP^{-/-}$; Zheng et al. 1995), and mutant APP/presenilin (APP/PS1; Jankowsky et al. 2004) mice with control anti-APP [22C11 (RRID AB_827115, Millipore), Y188 (RRID AB_2289606, Abcam] and anti-Aβ [4G8 (RRIDAB_662812, Biolegend), 6E10 (RRID AB_1977025, Biolegend)] antibodies, it was established that the AbSL antiserum: (i) does not bind to APP, (ii) it is Aβ conformation selective, (iii)

recognises distinct pathological features in APP/PS1 brain tissue (Colvin *et al.* 2017). Epitope competition between AbSL and other Aβ antibodies (anti-Aβ1-16 monoclonal antibodies Ab9 and Ab5, from Mayo Clinic College of Medicine) revealed that the AbSL conformational epitope and the Ab9/Ab5 linear sequence epitope were distinct, but with some potential overlap in the N-terminal region. Direct and indirect sandwich ELISA using the Ab2.1.3 C-terminal Aβ42-selective antibody (Kukar et al. 2005) confirmed that the protofibril epitope for AbSL does not overlap with the C-terminal end of Aβ42 (Colvin *et al.* 2017).

While the epitope recognised by AbSL has not been identified precisely, it is clear that this antibody preferentially interacts with Aβ42 protofibrils (Colvin *et al.* 2017). Therefore, the new antibody, in combination with other previously developed immunoreagents to other forms of Aβ (Westwood and Lawson 2015), will be useful for detecting Aβ42 protofibril formation in Alzheimer's disease as well as distinguishing between different forms of Aβ42. This study supports the notion that there are significant structural differences between Aβ protofibrils and other Aβ oligomers which can be detected with conformational epitope-specific antibodies. A systematic identification of amino acids that form the conformational epitope of AbSL in combination with the recently established fibril structure of Aβ1-42 (Gremer *et al.* 2017) would provide further information about Aβ protofibril structures. This information would be beneficial for the development of new diagnostics and therapeutics for Alzheimer's disease.

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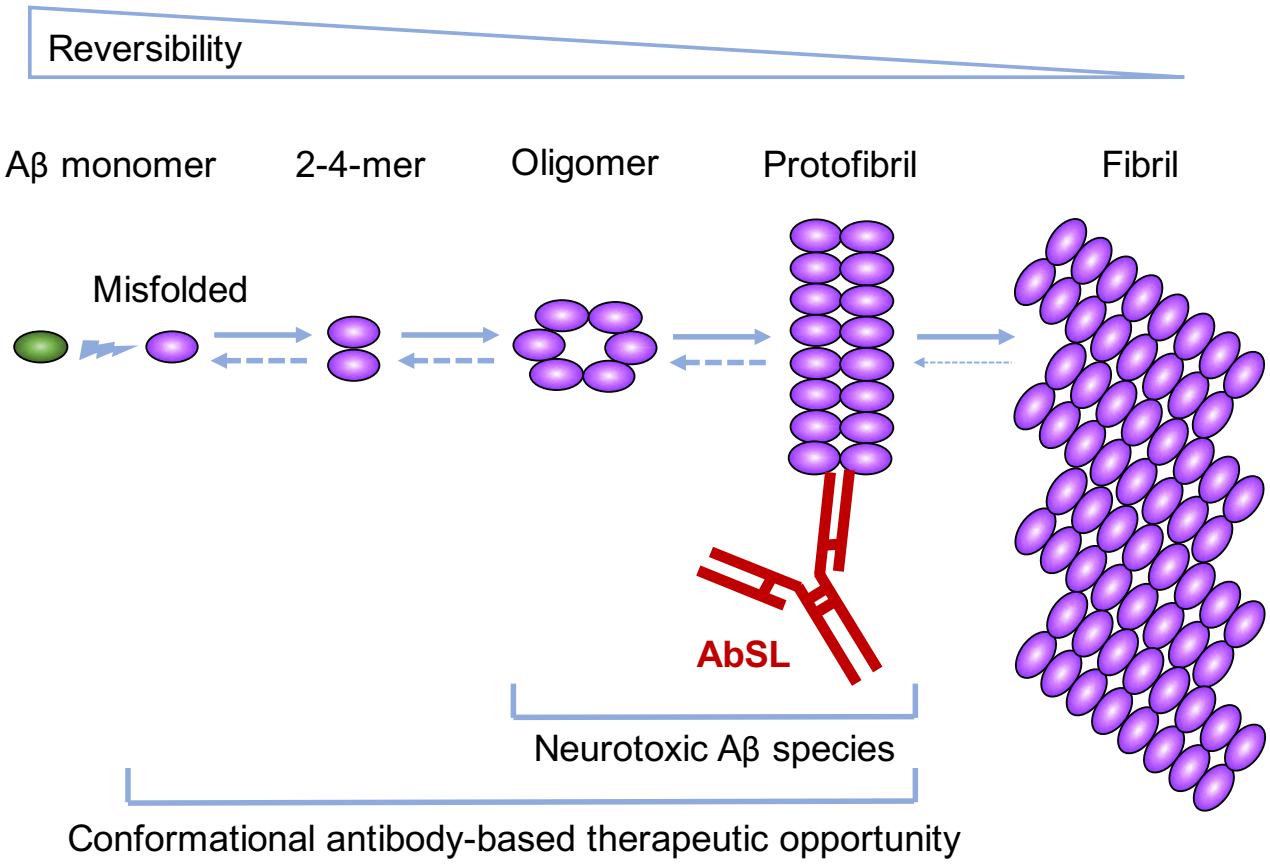
Figure legend

Fig. 1. **The AbSL antibody preferentially interacts with Aβ42 protofibrils** (Colvin *et al.* 2017). Schematic representation illustrates the Aβ aggregation process initiated by misfolded, monomeric proteins, which undergo several conformational transitions and aggregation before they reach mature fibril states (Westwood and Lawson 2015).

Fig. 1.


Reversibility
Aβ monomer
2-4-mer
Oligomer
Protofibril
Fibril
Misfolded
AbSL
Neurotoxic Aβ species
Conformational antibody-based therapeutic opportunity