Assessing Aβ clearance aided by mass spectrometry

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Introduction

Amyloid beta (Aβ) derive from processing of amyloid precursor protein, with amino acids (aa) conventionally numbered from aa 1 to 42. Amyloidogenic properties are particularly linked to the longer Aβ 1-42 form, but neurotoxic and inflammatory properties may be retained even in fragments restricted to the 25-35 mid-domain aa (Meda et al, 1996). Catabolism in brain innate immune cells; microglia and peripheral blood (PB); monocytes, contribute to Aβ clearance (Simard et al, 2006). but assays measuring catabolic activity is lacking.

Earlier studies suggest that mid-domain fragments may be relatively retained during intracellular catabolism, and that surrounding cleavage (peptide bonds 20-23 and 33-34) may be particularly relevant for intracellular clearance (Rogeberg et al, 2014).

Goal

Our ultimate objective is to produce a Aβ catabolism assay. Here we explore sensitivity and specificity newly developed antibodies targeting the aa 34-ending Aβ (Aβ x-34) peptides. In an accompanying abstract we present results from a Simoa (Quanterix, MA, USA) immunoassay technique showing levels of target peptides in PB monocytes (CTAD poster Wettergreen et al, 2019).

Methods

Standard immunoassay techniques and immunoprecipitation combined with reversed phase nano liquid chromatography mass spectrometry (IP nLCMS, shown in Figure 1) was performed to screen for endogenous Aβ catabolism peptides and to select the best-performing antibodies. The antibodies tested were proprietary sheep monoclonal antibodies (mAbs) and recombinant human antibodies (HuCal) from Biorad (USA). The antibodies shown here are the best performing pair; mAb 2A9 and HuCal H3, which were used as capture and detection antibody, respectively, in the immunoassay format experiment and both were linked to beads in the IP nLCMS studies (see Figure 1). Either peptide standard solutions or an aliquoted CSF pool served as samples for these investigations.

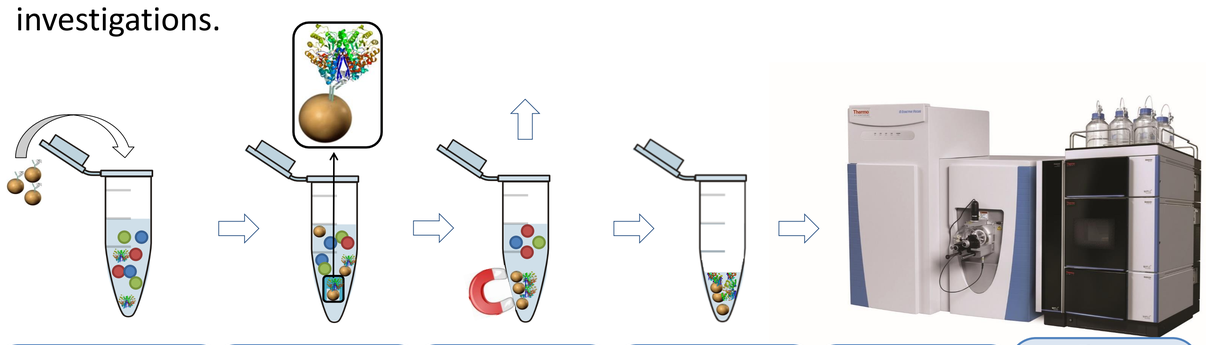

Figure 1. The IP LCMS workflow. The biological samples are cleaned up and enriched by use of antibodies linked to magnetic beads. Subsequently, nLCMS analyses are performed and a database containing the entire human proteome is searched by use of MaxQuant software. The outcome is determination of all the human derived peptides in each sample.

Results

Figure 2 shows the immunoassay response for simple solutions containing a range of Aβ peptide variants, where only Aβ x-34 peptides gave responses exceeding noise. Additionally, a preference towards shorter mid-domain peptides is shown for the anti-complex immuno-sandwich (Aβ 20-34 > Aβ 1-34 and Aβ -2-34).

Figure 3 shows that IP with H3 in CSF only detects one Aβ peptide (condition 1), while CSF preincubation with 2A9 prior to IP with H3 leads to detection of 35 different Aβ peptides (condition 2). Thus, Aβ x-34 captured with 2A9 is needed for H3 affinity. Further on, we see that 2a9 functions better as a capture antibody than H3, leading to detection of more CSF Aβ peptides (condition 2 versus 3, numbers in blue frames in Figure 3). The nLCMS signal intensities (reflecting amount of peptides) are only about 5 to 30 % for IP with H3 with 2A9 in CSF, compared to IP with 2A9 in CSF (condition 2 versus 3; data not shown). Similar percentages were found for Aβ x-34 peptides (data not shown).

Despite the weaker capture properties of H3 compared to 2A9; the number of Aβ x-34 peptides were about 60 % higher for IP with H3 with 2A9 in CSF, compared to IP with 2A9 in CSF (condition 2 versus 3, numbers in red frames). This implies a preference for Aβ x-34 peptide variants.

IP nLCMS showed that non-Aβ peptides comprised less than 6 % of total identified peptides, with the nLCMS signal making up less than 0.4% of total signal (data not shown here).

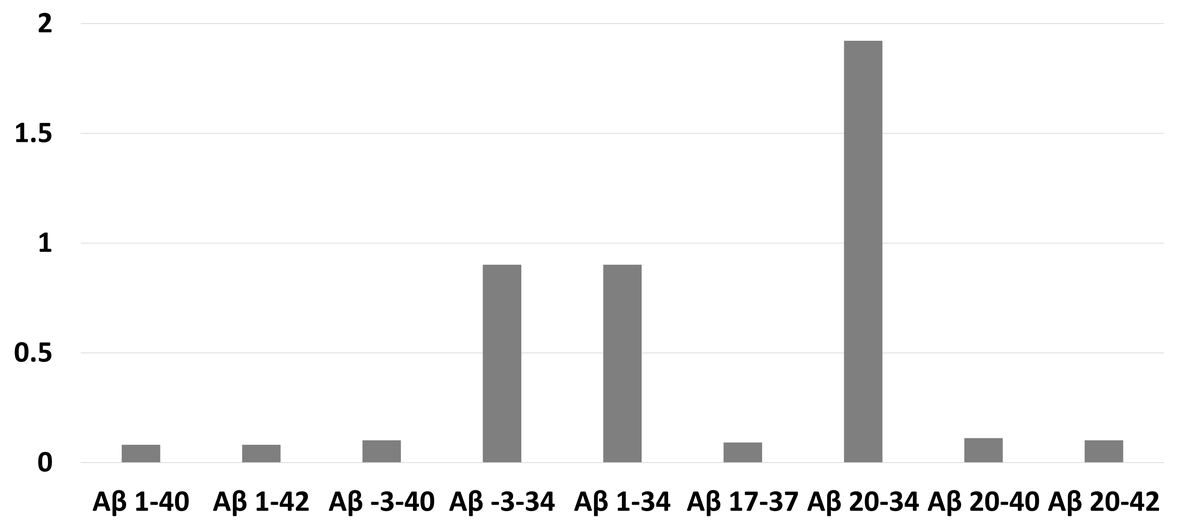

Figure 2. Sandwich immunoassay response using capture mAb 2A9 and detector antibody HuCal H3. The samples are solutions with 1 ng/mL of each of the Aβ peptides annotated by the x-axis, while the y-axis gives signal intensity responses.

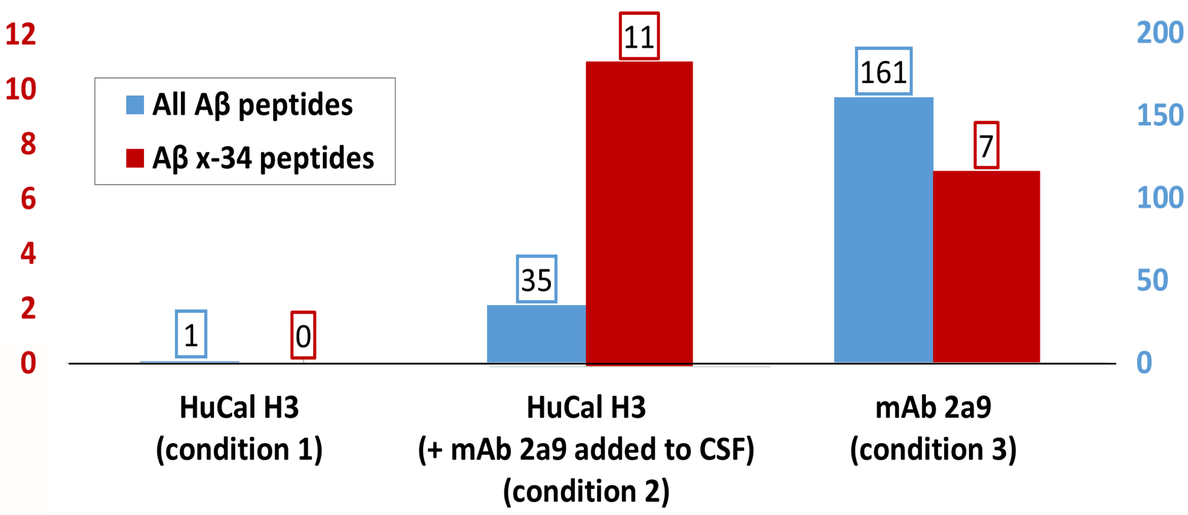

Figure 3. Number of IP nLCMS Aβ peptides identified in CSF pool sample aliquots. The x-axis gives the IP conditions. In condition 1; IP with HuCal antibody H3 in CSF, in condition 2; IP with HuCal antibody H3 in CSF pre-incubated with mAb 2A9, and in condition 3; IP with mAb 2A9 in CSF. The left y-axis gives the number of identified Aβ x-34 peptides (in red), while the right y-axis gives the total number of identified Aβ peptides (in blue). The respective count of identified peptides for each condition are represented in with blue and red bars and their number in blue and red frames.

Conclusion

We present an antibody pair that forms a highly selective anti-complex immuno-sandwich for mid-domain Aβ x-34 peptides. Cleavage at the 34 bond may be relevant for intracellular Aβ detoxification, and measurement of intracellular Aβ x-34 levels may be useful to assess clearance. The blood immunoassay application of this antibody pair is shown an accompanying CTAD contribution (Wettergreen et al, 2019).

References

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