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A bifunctional peptide, “peptope”, for pre-targeting antibody 7D8 to systemic amyloid deposits

Jonathan S. Wall^{1,2}, Angela Williams¹, James S. Foster¹, Emily B. Martin¹, Tina Richey¹, Alan Stuckey¹, Sallie Macy¹, Wagner Zago³, Gene G. Kinney³, and Stephen J. Kennel^{1,2}

¹Department of Medicine, University of Tennessee Medical Center, Knoxville, TN, USA

²Department of Radiology, University of Tennessee Medical Center, Knoxville, TN, USA

³Prothena Biosciences, San Francisco, CA, USA

Background

At present, there are two amyloid fibril-reactive antibodies in clinical trial for the treatment of patients with systemic light chain-associated (AL) amyloidosis [1,2]. In an effort to potentially enhance the utility of these antibodies, we have developed a system that would allow these reagents to be used as immunotherapies for patients with other kinds of systemic amyloidosis, notably patients with transthyretin-associated (ATTR) amyloidosis. We have synthesized a bi-functional peptide (peptope) that comprises the pan-amyloid reactive peptide, p5, with a linear epitope recognized by the 7D8 monoclonal antibody (mAb), which specifically targets AA and light-chain amyloid (Figure 1(A)). The peptope was designed to facilitate the binding of mAb 7D8 with amyloid deposits for which it did not display a natural reactivity, e.g., ATTR. The use of a bifunctional peptope may provide enhanced binding and expand the utility of mAbs already present in the clinical space for the treatment and removal of diverse amyloid deposits, regardless of the precursor protein from which they are comprised.

Materials and methods

A panel of peptopes was synthesized using peptide p5 as the amyloid-reactive sequence. The peptope incorporated one of the four linker sequences at the C-terminus followed by the –HEDT–COO– tetrapeptide, which represents the epitope sequence recognized by mAb 7D8. The binding of each peptope to amyloid and mAb 7D8 was measured separately by ELISA and surface plasmon resonance, to ensure that both functions were preserved in the context of peptope. The peptope-mediated binding of ¹²⁵I-labeled 7D8 mAb to synthetic fibrils composed of Aβ(1–40) or human IAPP, as well as human ATTR amyloid extracts (non-natural targets of the mAb) was performed in the presence and absence of peptope using a solution-phase “pulldown” assay and by ELISA. Reactivity with ATTR amyloid deposits in formalin-fixed paraffin-embedded (FFPE) tissue sections was assayed using biotinylated-7D8 mAb with or without peptope pretreatment. Finally, *ex vivo* phagocytosis

assays were performed using pHrodo green-conjugated human ATTR amyloid extracts in the presence of Raw 246.7 macrophage cells.

Results

The 7D8 mAb bound the peptide in the ELISA with ~0.5– 3 nM affinity (data not shown). In the absence of peptide, the mAb 7D8 did not bind A β (1–40), IAPP fibrils, or ATTR amyloid extracts; however, immunoreactivity (~5 nM EC₅₀) was observed following peptide pretreatment. The mAb 7D8 did not bind ATTR amyloid in FFPE tissue sections when applied alone or in the presence of the control peptide (Figure 1(B)). However, when the tissue was pretreated with peptide, there was dramatic and specific reactivity with the amyloid (Figure 1(C)). Finally, when pHrodo green-labeled human ATTR amyloid extract was incubated in the presence of cultured RAW 246.7 macrophages, there was little uptake by the cells, as evidenced by the scarcity of green fluorescence (indicating the presence of ATTR in the phagolysosome - Figure 1(D)). In contrast, when the amyloid was opsonized by the addition of peptide followed by mAb 7D8, ATTR human amyloid extracts were efficiently phagocytosed by cultured macrophages (Figure 1(E)).

Discussion

Bifunctional peptides that comprise an amyloid-reactive moiety, such as peptide p5, as well as a high-affinity linear peptide epitope can be used to target existing mAbs to amyloid and thereby opsonize the deposits. The peptide construct developed for mAb 7D8 can be used to enhance the reactivity of this antibody and extend the binding to amyloid deposits for which it has no natural affinity, such as ATTR. Based on these promising data, we hypothesize that by using the peptide technology, it may be possible to extend the immunoreactivity and utility of mAbs as immunotherapeutics in patients with amyloidosis.

Acknowledgments

Declaration of interest

JSW receives support from Prothena Biosciences. SJK, JSF and JSW are inventors on a patent describing the use of peptides for amyloid-targeted immunotherapy.

References

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Figure 1. Peptide-mediated antibody binding to amyloid. (A) Schematic representation of bifunctional peptide-facilitated binding of mAb 7D8 to amyloid. Immunostaining (Brown, uppermost right) of human ATTR amyloid in FFPE tissue by mAb 7D8 in the absence (B) or presence (C) of the peptide. Original mag. 160×. Phagocytosis of pHrodo green-labeled human ATTR amyloid extract was low in the presence of mAb 7D8 alone (D), but enhanced in the presence of peptide plus mAb 7D8 (E). Original mag. 80×, cell nuclei stained with Hoechst dye appear blue.