**Bi-specific diabodies targeting β-amyloid to microglial phagocytic proteins for treating neurodegenerative diseases.**

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**Introduction**. Bi-specific diabodies are composed of two short chain variable fragments (scFv) with the variable domains (VH-VL) bonded together (Hollinger et al., 1993). They can join two different proteins together and are currently used therapeutically to treat acute myeloid leukaemia and haemophilia. We hypothesised that diabodies could clear toxic β-amyloid peptides (Aβ) from the brain by directly coupling Aβ to microglial proteins involved in Aβ phagocytosis.

**Aims**. To develop high affinity bi-specific diabodies against Aβ and microglial proteins involved in Aβ phagocytosis. Then to test these diabodies in Aβ phagocytosis and neuroinflammation assays and for blood-brain-barrier permeability.

**Methods**. Bi-specific diabodies (δ) were designed to bind to Aβ and either “triggering receptor expressed on myeloid cells 2” (TREM2) or “cluster of differentiation 33” (CD33). Diabodies were expressed in Expi293F cells and purified using standard protein chromatography. Binding affinities (pKD) for the diabodies were determined for Aβ and microglial proteins by microscale thermophoresis (MST) or surface plasmon resonance (SPR). pHrodo-red-Aβ phagocytosis was measured by flow cytometry. Neuroinflammation was measured by qPCR, Western blots and ELISA. Diabodies were administered to mice by tail vein injection (10mg/kg) and the amount crossing into the brain was determined by Western blots.

**Results**. The bi-specific diabodies bound to Aβ with micromolar affinity (pKD = 5.9±0.1; n=3), to TREM2 (pKD = 8.8; n=2) and CD33 (pKD = 8.8; n=2) with nanomolar affinities. δTREM2 (ΔMFI = 195) and δCD33 (ΔMFI = 9764) diabodies increased Aβ phagocytosis. δTREM2 triggered neuroinflammation but δCD33 did not. δCD33 successfully crossed into the brain.

**Discussion.** Bi-specific diabodies can directly couple Aβ to microglial proteins involved Aβ phagocytosis. Our results suggest that δCD33 increases microglial phagocytosis of Aβ without triggering neuroinflammation. δCD33 successfully crossed the blood-brain-barrier in mice. Together these results suggest that directly coupling Aβ to microglial proteins involved Aβ phagocytosis is a feasible strategy for novel neurodegenerative disease treatments.

**Reference**: Hollinger et al., 1993, PNAS 90(14):6444–6448