Human Plasma Contains Cross-Reactive Aβ Conformer-Specific IgG Antibodies[†]

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ABSTRACT: Two conformers of aggregated Aβ, i.e., fibrils and oligomers, have been deemed important in the pathogenesis of Alzheimer's disease. We now report that intravenous immune globulin (IVIG) derived from pools of human plasma contains IgGs that recognize conformational epitopes present on fibrils and oligomers, but not their soluble monomeric precursor. We have used affinity chromatography to isolate these antibodies and have shown that they cross-reacted with comparable nanomolar avidity with both types of Aβ aggregates; notably, binding was not inhibited by soluble Aβ monomers. Our studies provide further support for investigating the therapeutic use of IVIG in Alzheimer's disease.

Alzheimer's disease (AD) is characterized by the presence within the brain of amyloid fibril-containing neuritic plaques comprised of proteolytically derived 40−42-residue fragments of the amyloid precursor protein (*1*). Historically, Aβ fibrils have been deemed primarily responsible for neuronal dysfunction and death; however, more recent evidence indicates that oligomers (*2*), which include soluble cross-linked β-amyloid protein species (CAPS) (*3*), are the most pathogenic Aβ conformers.

The demonstration that IVIG products prepared from the plasma of presumably healthy donors contain anti-Aβ IgG antibodies (*4*−*6*), and that the sera of patients with AD may have reduced concentrations of these Igs as compared to age-matched controls (*7, 8*), has provided the rationale for exploring the therapeutic use of IVIG in these individuals; notably, promising results have been reported in early clinical trials (*9, 10*). However, the following questions have not been answered: which of the Aβ conformers (monomer, oligomers, or fibrils) these naturally occurring antibodies recognize, how they modulate AD progression, and if reactivity with circulating Aβ monomer (which is implicated in lipid homeostasis) (*11*) may have adverse effects.

To determine definitively which of the three Aβ species are recognized by IgGs contained in IVIG, we used affinity columns to isolate conformer-specific antibodies. On the basis of the amount of anti-fibril, -CAPS, and -monomer IgG recovered, it was determined that IVIG contains ~0.10, 0.04, and 0.02% of each, respectively. As shown in Figure 1, anti-fibril and -CAPS-enriched preparations bound ~30-fold more to the plate-immobilized Aβ conformer used for isolation (EC_{50} ~ 40 nM) than did unfractionated IVIG, as demonstrated in a highly sensitive fluoroimmunoassay (*5, 12*), while monomer-isolated antibodies exhibited only moderate binding to their target (EC_{50} ~ 350 nM). Further, the antibody-containing eluates had similar reactivity against homologous Aβ42 conformers.

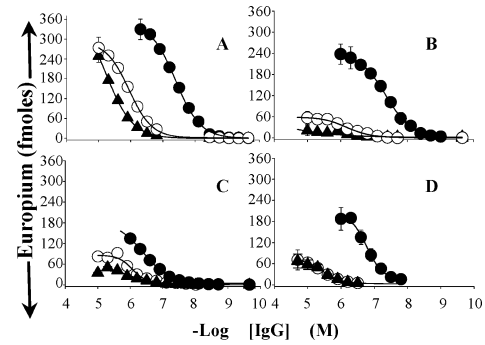

FIGURE 1: Aβ40 conformer binding by Aβ affinity-fractionated and -unfractionated IVIG. Antibody titration curves of affinity-purified (●), residual (flow-through) (▲), and unfractionated (○) IVIG binding to plate-immobilized Aβ conformers used to fractionate IVIG: (A) fibrils, (B) CAPS, (C) wild-type monomer, and (D) equimolar mix of N- and C-terminal cysteinylated F19P monomeric peptides.

To ensure that all Aβ monomer-reactive antibodies were isolated, the IVIG preparation was passed through a column consisting of an equimolar mixture of thiol-immobilized N- and C-terminal cysteinylated mutant F19P Aβ monomers, which are less prone to aggregation and β-sheet formation than the native protein (*13*). In these studies, although the purified antibodies had an ~2-fold greater binding capacity (EC_{50} ~ 157 nM) than did those from the wild-type Aβ40 column, the amounts recovered were essentially the same.

Further characterization of the three enriched antibody preparations demonstrated that both Aβ fibril and CAPS-purified IgGs selectively bound to the Aβ species used for isolation, with ~2−5-fold weaker binding to the other type of aggregate and 30- and 100-fold less reactivity with wild-type and mutant monomeric peptides, respectively (Figure 2) (the weaker binding to the latter indicates the importance of phenylalanine at position 19 for antibody−Aβ interactions). Preferential antibody reactivity with the conformer used for isolation presumably reflected subtle molecular differences (i.e., H-bond donor/acceptor pairings) between epitopes exposed on fibrils and oligomers. In contrast, Igs

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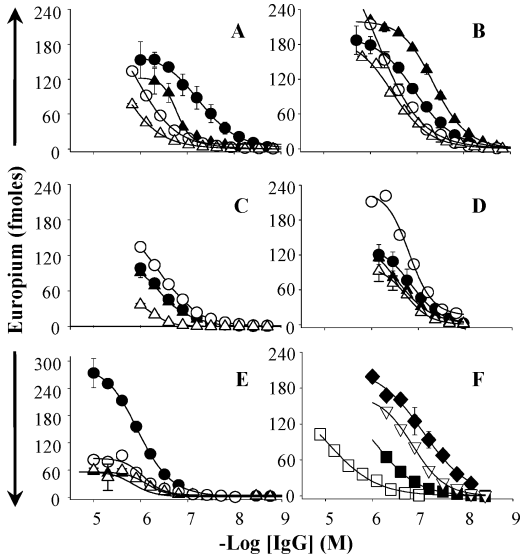

FIGURE 2: Aβ40 conformer and AD plaque binding by Aβ affinity-fractionated and unfractionated IVIG. Antibody titration curves against plate-immobilized Aβ conformers: fibrils (●), CAPS (▲), wild-type (○), and F19P monomer (△). The IgGs tested were isolated from affinity columns that contained (A) Aβ fibrils, (B) CAPS, (C) wild-type monomer, or (D) an equimolar mix of N- and C-terminal cysteinylated F19P monomers. (E) Binding curves for unfractionated IVIG. (F) Antibody titration curves against AD plaque cores: fibril-enriched (◆), CAPS-enriched (▽), and wild-type-isolated antibodies (■), as well as unfractionated IVIG (□).

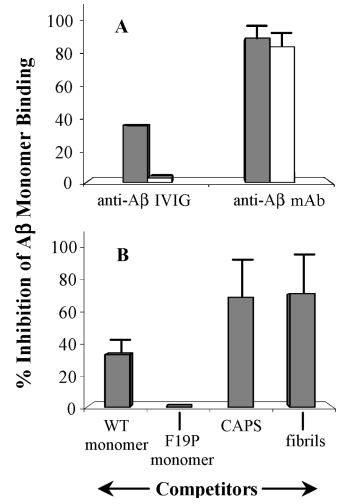

FIGURE 3: Aβ monomer binding by affinity-fractionated IVIG. (A) Comparison of the inhibition of Aβ monomer binding by affinity-isolated IgGs and an anti-Aβ N-terminally reactive mAb, MAB1560, by a 100-fold weight excess of wild-type (gray bars) or F19P (white bars) Aβ monomer. Fractionated IVIG and MAB1560 were used at concentrations equivalent to twice their EC_{50} values (600 and 3 nM, respectively). (B) Binding of the F(ab′) fragment (3.8 μM) of Aβ monomer affinity-purified IVIG to plate-immobilized Aβ monomer in the presence of a 100-fold weight excess of Aβ conformer competitors.

isolated from wild-type and mutant Aβ monomer columns, as well as unfractionated IVIG ($EC_{50} \sim 200$ nM), lacked specificity for any one of the Aβ species.

To elucidate further the binding potential of the anti-Aβ conformer-enriched IgG preparations, antibody titration curves were generated against congophilic neuritic plaque cores isolated from the brain of a patient with AD. As also shown in Figure 2, each of the affinity-purified reagents had at least 10-fold more avidity for the amyloid than did unfractionated IVIG, though the fibril-purified antibodies bound to a somewhat greater extent ($EC_{50} \sim 80$ nM) than did the Aβ CAPS- and monomer-isolated IgGs ($EC_{50} \sim 140$ and 400 nM, respectively).

Because plate immobilization of CAPS may have induced a neoepitope, we tested the reactivity of isolated anti-CAPS antibodies with the three Aβ species in a fluid-phase assay. Here, it was revealed that binding was effectively blocked by homologous aggregates, but not by noncovalent SDS-stable Aβ42 oligomers, monomers, or non-amyloid elastin fibrils (Figure S1A of the Supporting Information), thus indicating that these antibodies recognized unique epitopes present on both plate-immobilized and solution-phase CAPS. Notably, CAPS and noncovalent SDS-stable Aβ42 oligomers (*14*) were equally effective at inhibiting the interaction of Aβ fibril affinity-purified antibodies with immobilized CAPS (Figure S1B of the Supporting Information). These antibodies also bound to plate-immobilized lysozyme oligomers (*15*) (Figure 2 of the Supporting Information) which demonstrated that, although both the fibril- and CAPS-enriched antibody preparations contained cross-reactive IgGs against higher-order Aβ aggregates, only the former was capable of discriminating among different oligomer conformers.

Competition studies involving Aβ monomers and anti-Aβ monomer-enriched intact IgG and its F(ab′) fragments showed that an ~100-fold weight excess of wild-type or F19P Aβ monomers weakly inhibited their interaction with plate-immobilized native monomeric peptide (in contrast, both were potent inhibitors of the commercially available Aβ N-terminally reactive mAb, MAB1560) (Figure 3). The lack of antibody interaction with the solution-phase Aβ peptides indicated that binding to the immobilized peptides was not directed against a linear sequence epitope. Neither was it due to low avidity, since these molecules did not effectively compete with the F(ab′) fragments binding to the immobilized wild-type monomeric peptide. In contrast, CAPS and Aβ fibrils strongly blocked such reactivity.

Since the binding of monomer-enriched IgG to plate-immobilized Aβ monomers was strongly inhibited by Aβ fibrils and CAPS (but not the monomer), we posit that the column-linked peptide adopted an amyloid-like configuration. Presumably, the cryptic binding site(s) required surface-induced β-sheet structure (*16*) since reactivity with the wild-type Aβ40 monomer was stronger than with the F19P mutant peptide which is less prone to β-sheet and fibril formation (*13*). Although the structural changes in the Aβ monomer that occurred upon conjugation with the column matrix, as well as when it was adsorbed to the surface, were artifacts of the experimental system, a similar transition may occur in vivo, given that the deposition of this peptide in brain tissue could initiate Aβ aggregation by serving as a fibril nucleus (*17*).

The fact that the anti-fibril and -CAPS IgGs cross-reacted with non-native conformational, i.e., nonlinear, epitopes on amyloidogenic assemblies reflects the fact that profound structural changes occur upon formation of β-sheet-rich amyloid fibrils (*18, 19*). We previously have reported that human plasma contains pan amyloid fibril-reactive IgGs directed against conformational epitopes that are only partially reliant on primary sequence (*5*). Presumably, these neoepitopes have a unique peptide backbone conformation,

e.g., chain reversal, which requires H-bond donor/acceptor pairing not present or exposed in the native state (*20*). These antibodies, which specifically bind pathologic Aβ aggregates, may have been generated in response to non-Aβ amyloid fibrils, e.g., those associated with aging (*21*), food products (*22*), or bacteria (*23*). Moreover, the Igs may selectively sequester or clear toxic Aβ aggregates without binding the presumed physiologically important monomer (*11*). In this regard, the cognitive improvement observed when IVIG was given to AD patients (*9, 10*) could be due, in part, to boosting their senescent immune systems with a more diverse anti-Aβ antibody population.

In summary, we have provided definitive evidence that normal human plasma contains anti-fibril and anti-CAPS IgGs, both of which recognize a cross-reactive conformational, i.e., nonlinear, epitope found on aggregated Aβ conformers, but not expressed by the native monomer. These studies provide further rationale for investigating the use of IVIG in the treatment of patients with AD.

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SUPPORTING INFORMATION AVAILABLE

Supplementary figures as well as Materials and Methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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