Alzheimer's disease peptide β-amyloid interacts with fibrinogen and induces its oligomerization

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Increasing evidence supports a vascular contribution to Alzheimer's disease (AD), but a direct connection between AD and the circulatory system has not been established. Previous work has shown that blood clots formed in the presence of the β-amyloid peptide (Aβ), which has been implicated in AD, have an abnormal structure and are resistant to degradation in vitro and in vivo. In the present study, we show that Aβ specifically interacts with fibrinogen with a K_d of 26.3 ± 6.7 nM, that the binding site is located near the C terminus of the fibrinogen β-chain, and that the binding causes fibrinogen to oligomerize. These results suggest that the interaction between Aβ and fibrinogen modifies fibrinogen's structure, which may then lead to abnormal fibrin clot formation. Overall, our study indicates that the interaction between Aβ and fibrinogen may be an important contributor to the vascular abnormalities found in AD.

Alzheimer's disease (AD) is a neurodegenerative disorder that leads to progressive cognitive decline and subsequent death. Effective long-term treatments and preventive measures are not available, and new therapeutic targets are needed. Substantial evidence indicates that the β-amyloid (Aβ) peptide, which is derived from the Aβ precursor protein (APP), is involved in AD (1–3). Aβ is soluble in its monomeric or oligomeric states, but can aggregate into fibrils and deposit as extracellular plaques in the brain parenchyma. However, the severity of dementia does not correlate well with the amount of extracellular amyloid plaques and the mechanism by which Aβ causes neurodegeneration is still unclear (4).

Aβ can also accumulate in brain blood vessels, a condition known as cerebral amyloid angiopathy (CAA). CAA is characterized by deposition of Aβ within cerebral vessels, resulting in degenerative vascular changes (5–7). In mouse models of AD, endothelial cells in CAA vessels show early dysfunction, which reduces their response to vasodilators (8) and impairs the regulation of blood flow (9, 10). Many patients with AD present vascular symptoms, including altered cerebral blood flow, damaged cerebral vasculature, and abnormal hemostasis (11). Cerebral blood flow is reduced and many vascular defects are present in patients with AD (12). Vascular diseases such as atherosclerosis correlate in severity with dementia and other symptoms of sporadic AD (13–15). Vascular abnormalities could therefore play an important role in AD, but a direct connection remains unknown.

Fibrinogen is the primary protein component of blood clots. It is 45 nm in length with identical globular domains at each end, which are connected by rod-like strands. It is composed of three pairs of polypeptide chains, designated Aα, Bβ, and γ, which are connected by disulfide bonds (16). When fibrinopeptides A and B of fibrinogen are cleaved by the serine protease thrombin, fibrinogen noncovalently polymerizes to form protofibrils, which then branch to form an insoluble fibrin clot. This clot network forms a mesh around platelets to impede blood flow at sites of vascular injury. Fibrin is naturally degraded by plasmin, which is generated from inactive plasminogen by tissue plasminogen activator.

Increased levels of fibrinogen result in changes in blood rheological properties, such as alterations in vascular reactivity and compromises in endothelial layer integrity (17). Moreover, fibrinogen deposition with or without conversion to fibrin increases inflammation and vascular permeability at the site of deposition (18, 19). Fibrin(ogen) deposition in the CNS after blood–brain barrier disruption or vascular damage increases inflammation via microglial activation and leads to inhibition of neurite outgrowth (20, 21). Thus, increased levels of fibrinogen could contribute to vascular pathology and neuronal dysfunction.

We have previously shown that fibrinogen could be a link between vascular pathology and neurodegeneration in AD (22). Fibrinogen is normally excluded from the brain by the blood–brain barrier, but it accumulates in the damaged vasculature and parenchyma of mice with AD (23). Reducing fibrinogen levels pharmacologically or genetically led to a decrease in the neurovascular pathology and inflammatory response in mice. Moreover, depletion of fibrinogen lessened the incidence of CAA in AD mouse brains and reduced their cognitive impairment. Our in vitro experiments showed that fibrin clots formed in the presence of Aβ have an abnormal structure and are resistant to degradation by fibrinolytic enzymes. Accordingly, intravital brain imaging showed that clot formation and dissolution is abnormal in AD mice (22). These results indicate a potentially critical role for fibrinogen in AD. However, the mechanism underlying how Aβ induces abnormal fibrin clot formation and whether there is physical interaction between Aβ and fibrinogen are still unknown. In this article, we establish a direct interaction between these two molecules as well as a possible mechanism underlying Aβ-induced abnormal fibrin clot formation.

Results

Specific Interaction Between Aβ and Fibrinogen. Fibrin clots formed in the presence of Aβ are structurally abnormal and resistant to degradation, and fibrin(ogen) colocalizes with Aβ in blood vessels of AD mice and human patients (22). We examined if this association could be mediated by a physical interaction between Aβ42 and fibrinogen. We incubated biotinylated Aβ42 with fibrinogen and used streptavidin-Sepharose beads to bind Aβ and any associated proteins. This pull-down assay showed that fibrinogen binds to Aβ42 in vitro and that Aβ40 has less affinity for fibrinogen (Fig. 1*A*). These results indicate that the binding between Aβ and fibrinogen is stronger with the more pathogenic form of Aβ. The binding between fibrinogen and Aβ was specific, as no interaction was observed between Aβ and the ECM protein fibronectin (Fig. S1). By using an ELISA, we observed an Aβ42 dose-dependent increase in binding between Aβ42 and fibrinogen (Fig. 1*B*), and also that fibrinogen has less affinity for Aβ40 than Aβ42 (Fig. S2).

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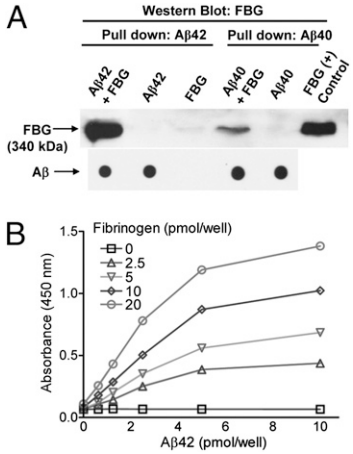

Fig. 1. Aβ peptide and fibrinogen interact in vitro. (*A*) Biotinylated Aβ42 or Aβ40 was incubated with fibrinogen (FBG) and pull-down assays were carried out using streptavidin-Sepharose. All samples were analyzed by Western blot and are representative of multiple experiments. A Western blot was performed under nonreducing conditions by using an anti-fibrinogen antibody, showing that Aβ42 and Aβ40 bind to fibrinogen. Dot blots against Aβ below each pull-down show that comparable amounts of biotinylated Aβ were pulled down. (*B*) The binding curve of biotin-labeled Aβ42 and fibrinogen was determined by ELISA. When no Aβ42 was added, the level of bound fibrinogen was negligible, regardless of fibrinogen concentration. Therefore, there is no nonspecific binding between fibrinogen and streptavidin in the well.

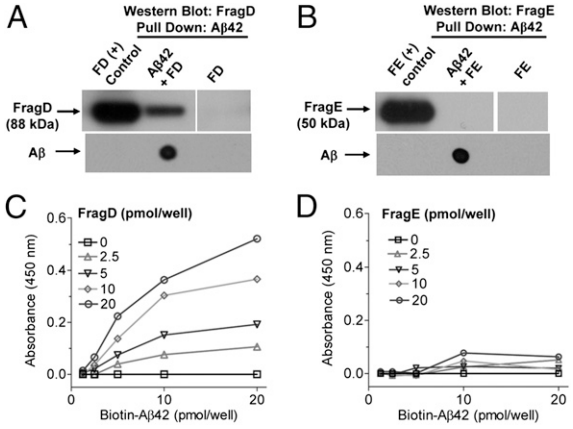

Fig. 2. Aβ42 binds to FragD of fibrinogen. Biotinylated Aβ42 was incubated with (*A*) FragD (FD) or (*B*) FragE (FE), and pull-down assays were carried out using streptavidin-Sepharose. A Western blot was performed using an anti-fibrinogen antibody. Dot blots against Aβ showed that comparable amounts of Aβ were pulled down. Binding curve of biotin-labeled Aβ42 and FragD or FragE was determined by ELISA. We observed an Aβ42-dependent increase in binding of FragD (*C*), but no binding between Aβ42 and FragE (*D*). These results represent multiple experiments.

Characterization of the Aβ–Fibrinogen Interaction. To refine the region of fibrinogen responsible for Aβ binding, we examined Aβ42 binding to two subregions of fibrinogen: fragment D (FragD) and fragment E (FragE). Fibrinogen has a symmetrical structure, with a central domain (FragE) connecting two identical nodules at each end (FragD). During clotting, thrombin releases fibrinopeptide A and B from the central region of fibrinogen (FragE), exposing two knobs, A and B. These exposed knobs interact with β or γ holes in the terminal regions (FragDs) of other fibrinogen molecules (16), which induces the polymerization of fibrinogen into a fibrin network. When the fibrinolytic enzyme plasmin degrades fibrin clot, plasmin cleaves specific sites between the central domain and the two identical nodules at each end, producing two FragDs and one FragE.

Pull-down assays with FragD and FragE were performed using biotinylated Aβ42. Results indicated that FragD interacts with Aβ42, but FragE does not (Fig. 2 *A* and *B*). By using ELISA, we showed that the Aβ42–FragD interaction increased in a dose-dependent manner, whereas FragE did not bind Aβ42 (Fig. 2 *C* and *D*). Many physiological proteins interact with fibrin(ogen) via the FragD region, such as αIIbβ3 integrin in platelets, leukocyte integrin αMβ2/Mac-1, and fibulin-1 (24–26). Therefore, FragD might be structurally more accessible to other proteins than FragE.

To further characterize the binding between Aβ42 and fibrinogen, equilibrium dissociation constants (K_d) were determined by using fluorescence polarization (FP). TAMRA (5-carboxy-tetramethylrhodamine)-labeled Aβ42 was mixed with increasing concentrations of fibrinogen or FragD. The K_d for the Aβ42 and fibrinogen interaction was 26.3 ± 6.7 nM (Fig. 3*A*), and that for Aβ42 and FragD was 54.2 ± 5.0 nM (Fig. 3*B*). As a control, we showed that there was no binding between TAMRA-labeled Aβ42 and fibronectin (Fig. S3).

Aβ42 Binding Site on Fibrinogen. The identification of the specific regions of fibrinogen that interact with Aβ is critical in defining the nature of the complex. We therefore investigated the binding site for Aβ within fibrinogen. For these experiments, fibrinogen was partially digested using plasmin, generating fibrinogen degradation products (FDPs). These FDPs were incubated with biotinylated Aβ42. The Aβ-interacting peptides were pulled down with streptavidin-Sepharose beads, eluted, and analyzed using SDS/PAGE. To identify nonspecific binding fragments, we incubated FDPs and streptavidin-Sepharose beads without biotinylated Aβ42.

The pull-down experiment yielded a approximately 7 kDa fragment interacting with Aβ42 (Fig. 4*A*). This fragment did not appear in the negative control lane, indicating that it binds specifically to Aβ42. The fragment was excised, further digested by trypsin, and analyzed by liquid chromatography/tandem MS (LC-MS/MS). This analysis identified a 10-aa fragment that matches a portion of the C-terminal region of the fibrinogen β-chain (β396–β407). Consistent with this result, N-terminal sequencing of the 7-kDa fragment also placed it within the C-terminal region of the β-chain (β366–β370; Fig. S4). To identify the exact size of this fragment, we analyzed it with a MALDI-TOF mass spectrometer, and the mass of the fragment corresponded to 49 aa (Fig. S4). We could therefore place the interacting fragment between β366 and β414. These results indicate that Aβ42 interacts with fibrinogen

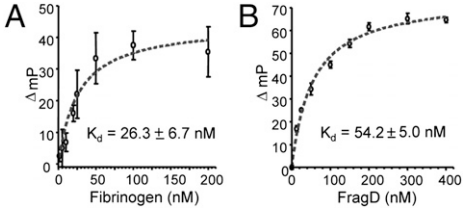

Fig. 3. The interaction of Aβ42 with fibrinogen or FragD is concentration-dependent. The binding kinetics between Aβ42 and fibrinogen (*A*) or FragD (*B*) were monitored by using FP. Increasing concentrations of fibrinogen or FragD were incubated with TAMRA-labeled Aβ42. After the reaction reached equilibrium, polarization was measured, and K_d was calculated by fitting the data to a single-site binding equation (n = 3). The K_d of Aβ42 binding to fibrinogen (two sites) was less than that to FragD (one site), and the data fit well to a one-site binding model. When the data are fitted to a two-site binding model, the dissociation constants for the two sites are virtually identical. It is likely that the distance between the two binding sites on fibrinogen may allow them to bind Aβ independently of each other.

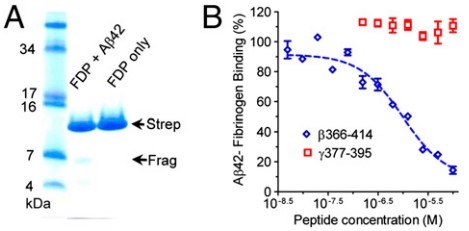

Fig. 4. Aβ42 specifically interacts with fibrinogen near the C terminus of the fibrinogen β-chain. (*A*) FDPs were incubated with biotinylated Aβ42 followed by streptavidin-Sepharose beads. Fragments interacting with Aβ42 were eluted from the beads and analyzed using SDS/PAGE. Reaction mixtures lacking biotinylated Aβ42 served as a control for nonspecific binding ("FDP only" lane). The major band below 16 kDa is streptavidin (Strep). A fragment interacting with Aβ42 (Frag) was identified around the 7 kDa protein size marker. (*B*) The inhibition curve of the fibrinogen-derived 49-mer peptide (β366–β414) for the Aβ42–fibrinogen interaction was measured using AlphaLISA. The 49-mer peptide had an IC_{50} of 0.99 ± 00.1μM, whereas fibrinogen peptide γ377–γ395 did not show any inhibition of the Aβ42–fibrinogen interaction (n = 3).

near the C terminus of the fibrinogen β-chain, a region that encompasses the β hole.

To further explore the interaction of this fragment with Aβ42, we synthesized the 49-mer peptide (β366–β414) corresponding to the identified fragment and tested its ability to inhibit the interaction between Aβ42 and fibrinogen using AlphaLISA. Biotinylated Aβ42 and fibrinogen were incubated with increasing concentrations of the fibrinogen-derived 49-mer peptide. Inhibition of binding was observed with an IC_{50} of 0.99 ± 0.1 μM (Fig. 4*B*). As a control, we tested whether a random peptide derived from fibrinogen (γ377–γ395) could inhibit the Aβ42–fibrinogen interaction. This peptide is known to inhibit the interaction between fibrinogen and integrin receptor Mac-1 (27), but it did not inhibit the Aβ42–fibrinogen interaction (Fig. 4*B*).

Fibrinogen Oligomerization Induced by Aβ42. Fibrin clots formed in the presence of Aβ are structurally abnormal and resistant to degradation (22). It is possible that the interaction between Aβ42 and fibrinogen can induce a structural change in fibrinogen, and this structurally altered fibrinogen could polymerize into an abnormal fibrin clot upon fibrinopeptide cleavage by thrombin. We therefore investigated whether Aβ42 could affect the intermolecular association of fibrinogen molecules before polymerization by thrombin.

To examine this possibility, fibrinogen was incubated with Aβ42 and the samples were separated by size-exclusion chromatography. Although fibrinogen without Aβ42 was eluted only in a monomeric state, oligomers of fibrinogen were present when fibrinogen was incubated with Aβ42 (Fig. 5*A*). Moreover, when eluates from the Aβ42–fibrinogen mixture were analyzed by Western blot using an anti-Aβ42 antibody (4G8), Aβ42 was detected only with oligomeric states of fibrinogen, but not in the monomeric state (Fig. 5*B*). Our results indicate that Aβ42 binds fibrinogen monomers and causes fibrinogen oligomerization, which in turn may alter the structural properties of fibrin clots.

Because Aβ42 also binds FragD, it may induce FragD oligomerization. To test this possibility, size-exclusion chromatography was repeated with an Aβ42-FragD mixture. Although we confirmed Aβ42 binding with FragD by Western blot using an anti-Aβ42 antibody (4G8), Aβ42 binding did not induce FragD oligomerization (Fig. S5). This result suggested that Aβ42 binding is not enough to cause protein oligomerization and that Aβ42-induced oligomerization is specific to fibrinogen. To test whether other types of amyloid peptides can induce fibrinogen oligomerization, the experiment was repeated in the presence of amylin and calcitonin, which are amyloid peptides known to be associated with human disease (28, 29). Neither amylin nor calcitonin affected the oligomeric state of fibrinogen (Fig. S6), indicating that peptide-induced fibrinogen oligomerization is specific to Aβ.

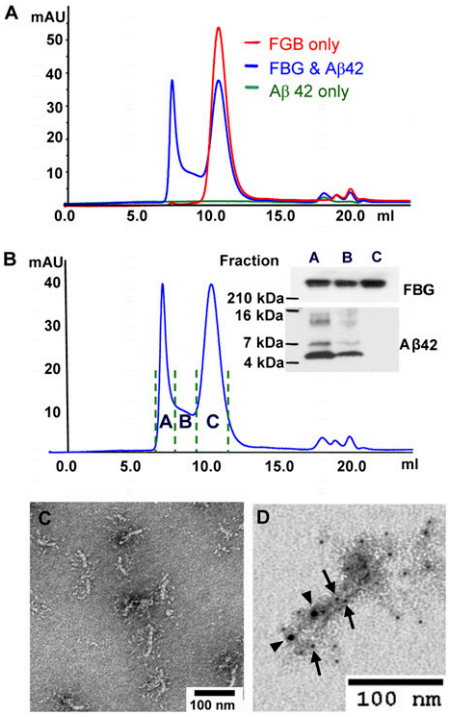

Fig. 5. Aβ42 binding induces oligomerization of fibrinogen. Fibrinogen (FBG; 1 μM) plus Aβ42 (blue; 5 μM), FBG alone (red; 1 μM), or Aβ42 alone (green; 5 μM) were incubated for 2 h at 37 °C. (*A*) Samples were run on a Superose 6 gel filtration column on FPLC. (*B*) Eluates from the Aβ42–fibrinogen mixture were analyzed by Western blot using an antibody against FBG or Aβ42. (*C* and *D*) TEM images were obtained from fibrinogen oligomers purified using size-exclusion chromatography. Immunogold labeling shows that oligomer is composed of both Aβ and fibrinogen (*D*). Aβ42-fibrinogen oligomers were labeled with anti-fibrinogen antibody (6 nm gold; arrow) and anti-Aβ antibody (12 nm gold; arrowhead). This result represents multiple experiments.

Because fibrinogen oligomers were eluted in the void volume from size-exclusion chromatography, it was possible that there were several species of fibrinogen oligomer or even nonspecific aggregation of fibrinogen. We therefore analyzed the size distribution of Aβ-induced fibrinogen oligomers using dynamic light scattering (DLS). DLS is a nondestructive method to estimate the hydrodynamic radius and theoretical size of particles in solution by analyzing the Brownian motion of the scattering particles (30). Fig. S7 *A* and *B* show the size distribution histogram of the fibrinogen monomer and Aβ-induced fibrinogen oligomer, respectively, determined by DLS. The fibrinogen oligomer exhibited a monodisperse profile with a hydrodynamic radius of approximately 31.3 nm and a theoretical size of an approximately 10,653-kDa sphere (Fig. S7*B*), whereas the fibrinogen monomer exhibited a monodisperse profile with a radius of approximately 11.0 nm and a size of an approximately 917-kDa sphere (Fig. S7*A*). As DLS assumes a spherical particle, the theoretical size of the fibrinogen monomer estimated from DLS does not accurately estimate the size of rod-shaped fibrinogen. However, when we compare the oligomer with the monomer, we can estimate the Aβ-induced fibrinogen oligomer contains approximately 12 molecules.

A transmission EM (TEM) image of Aβ-induced fibrinogen oligomers obtained from size-exclusion chromatography (fraction A of Fig. 5*B*) also shows that fibrinogen oligomers have uniform size and structure (Fig. 5*C*). When Aβ-induced fibrinogen oligomers

were labeled with gold-conjugated antibodies against fibrinogen and Aβ, the TEM image with immunogold labeling showed that oligomers are composed of both fibrinogen (6 nm gold; arrow) and Aβ (12 nm gold; arrowhead, Fig. 5*D*). When the oligomers were labeled with each antibody separately, the specific location of fibrinogen or Aβ within the oligomer was more clearly seen (Fig. S8 *A* and *B*). However, when purified fibrinogen monomer (red peak of Fig. 5*A*) was labeled with both anti-fibrinogen and anti-Aβ antibodies (Fig. S8*C*), no oligomeric structures were observed and the anti-Aβ antibody did not bind. These results suggest that the structures observed are Aβ42-induced fibrinogen oligomers, and that the anti-Aβ antibody specifically binds to the Aβ-induced fibrinogen oligomer.

Discussion

Our results show that Aβ42 specifically interacts with fibrinogen near the C terminus of the fibrinogen β-chain and induces fibrinogen oligomerization. These results support our previous study, which showed that fibrin clots formed in the presence of Aβ42 have abnormal structure and are resistant to degradation by fibrinolytic enzymes (22). Moreover, fibrinogen deposits often closely colocalize with CAA deposits around blood vessel walls in the postmortem brain tissue of patients with AD, and depletion of fibrinogen improved cognitive performance of AD mice (22). Taken together, these results suggest that the interaction between Aβ42 and fibrinogen may induce abnormal fibrinogen structures before fibrin clot formation and that this oligomeric fibrinogen plays an important role in AD.

We have developed a model that places the interaction between Aβ42 and fibrinogen in the context of AD pathology (Fig. 6). In the nondisease state, fibrinogen is converted to fibrin by thrombin cleavage, and this normal fibrin clot is degraded efficiently and completely by fibrinolytic enzymes. In AD, the Aβ peptide interacts with fibrinogen and induces fibrinogen oligomerization. These structurally altered fibrinogen oligomers may be converted into abnormal fibrin clots, which are more resistant to degradation by fibrinolytic enzymes. Persistent fibrin clots could lead to vascular deficiencies, decreased blood flow, and neuroinflammation, all contributing to AD pathogenesis.

In the absence of its conversion to fibrin, the Aβ42-induced fibrinogen oligomer may also codeposit with Aβ and could increase neurovascular damage in blood vessels. In vivo and in vitro evidence suggests the Aβ-fibrinogen interaction plays a role in CAA. When fibrinogen levels were genetically or chemically reduced in AD mice, the CAA area in AD mice was significantly decreased (22). In addition, when Aβ42 and the β-sheet detecting dye thioflavin T were incubated with or without fibrinogen, fibrillization of Aβ42 was highly increased in the presence of fibrinogen (Fig. S9). Both results suggest that the Aβ42–fibrinogen interaction increases amyloid fibril deposits in blood vessels and exacerbates CAA pathology. Moreover, the Aβ-induced fibrinogen oligomer is less stable than the fibrinogen monomer. For example, the size distribution of fibrinogen oligomers immediately after gel filtration was narrow, and the average hydrodynamic radius was approximately 31 nm (Fig. S7*B*). However, 48 h later, the size distribution of the oligomers became wider, with an average hydrodynamic radius of approximately 70 nm, whereas the fibrinogen monomer did not change. These results indicate that the fibrinogen oligomers are unstable. Therefore, the fibrinogen oligomer might be an intermediate state during the Aβ-induced fibrinogen aggregation process. Fibrinogen deposition with or without conversion to fibrin increases inflammation and vascular permeability where it is deposited (18, 19). Increased levels of fibrinogen alter vascular reactivity and impair endothelial cell layer integrity by binding to endothelial cell membrane receptors (17, 31). Taken together, the Aβ–fibrinogen interaction could reduce fibrin degradation and induce fibrinogen deposition in blood vessels. Both persistent fibrin and fibrinogen oligomer deposits in blood vessels may increase inflammation and cause neurovascular damage.

Protein misfolding and aggregation are hallmarks of several neurodegenerative disorders (32, 33), exemplified by Aβ in AD (1). Our study shows that Aβ can alter the physical properties and induce the aggregation of an entirely different protein (fibrinogen) as well as induce its own fibrillization. Although Aβ42 binds both fibrinogen and FragD, Aβ42 induced oligomerization of only fibrinogen, not FragD. This result indicates that Aβ42 binding itself is not enough to induce oligomerization of a binding partner and that fibrinogen is a specific target for Aβ-induced oligomerization. Fibrinogen has two identical ends (FragDs), and Aβ42 may bind at each. The symmetrical shape of fibrinogen with two binding sites could be the reason why Aβ42 induces oligomerization of only fibrinogen and not FragD.

Overall, the interaction between Aβ and fibrinogen caused fibrinogen oligomerization, promoted fibrin(ogen) deposition, as well as increased Aβ fibrillization. This study, combined with our

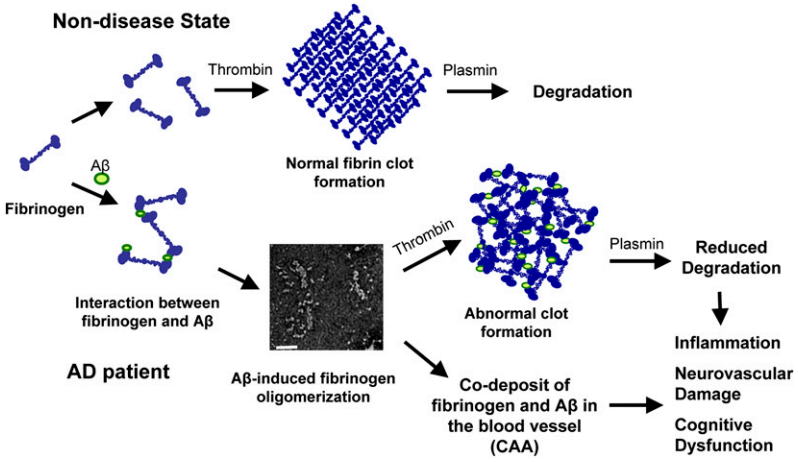

Fig. 6. Proposed model for the role of Aβ42 and fibrinogen binding in AD pathology. The Aβ peptide interacts with fibrinogen and modifies the formation of fibrin clots. These altered clots have an abnormal structure and are resistant to degradation by fibrinolytic enzymes. In patients with AD, persistent fibrin (ogen) could lead to vascular deficiencies, decreased blood flow, cognitive dysfunction, and neuroinflammation. (Scale bar: 50 nm in TEM image of fibrinogen oligomer.)

previous work, suggests that the interaction between Aβ and fibrinogen may be an important contributor to AD pathogenesis. The development of AD therapies has been hampered thus far by the lack of a defined pathological mechanism in AD. Our discovery suggests a unique mechanism for AD and a unique target (fibrinogen) for AD treatment. Molecules that block this pathological interaction could restore the normal structure of the fibrin clot and could be used as therapeutic agents for AD without affecting normal blood clotting.

Materials and Methods

Pull-Down Assay for Interaction Between Aβ and Fibrinogen/Fibrinogen Fragments. FragD and FragE were purified by peptide affinity chromatography (34). For more information, see *SI Materials and Methods*. Aβ peptides were prepared by dissolving in NH_4OH (4% final volume), then adding PBS solution (pH 7.4) to make a 1-mg/mL solution. Aliquots were immediately stored at −80 °C. Before use, aliquots were centrifuged for 15 min at 17,000 × *g* to remove preaggregated material. The concentration of biotinylated Aβ42 or 40 (Anaspec) was determined by using a bicinchoninic acid assay. Biotinylated Aβ42 or 40 (0.5 μg) was incubated with fibrinogen (0.5 μg; Calbiochem) for 1 h at room temperature (RT) in 500 μL of binding buffer (50 mM Tris·HCl, pH 7.4, 500 mM NaCl, 0.5% Nonidet P-40, 0.1% BSA, and protease inhibitor mixture). The samples were gently rotated 1 h at RT with 40 μL streptavidin-Sepharose high performance beads (GE Healthcare). After incubation, the beads were washed five times with binding buffer, and nonreducing sample buffer was added to the beads to elute any Aβ–fibrinogen complex that formed. Sample were incubated at 70 °C for 5 min, loaded onto a 6% polyacrylamide gel, transferred onto PVDF membrane, and incubated with a peroxidase-conjugated anti-fibrinogen antibody (Dako). In parallel, pull-down assays were carried out under the same conditions with fibronectin (Calbiochem), FragD, or FragE. Dot blots were carried out using anti-Aβ antibody 4G8 (Covance) to show comparable amounts of Aβ were also being pulled down.

ELISA for Aβ–Fibrinogen or Aβ–FragD Binding. Biotinylated Aβ42 or 40 (0.2–10 pmol per well; Anaspec) was incubated in a Reacti-Bind streptavidin-coated plate (Pierce) that was preblocked with 0.1% BSA in 25 mM TBS (pH 7.4)–0.05% Tween 20 for 1 h at RT. After washing, the plate was incubated with increasing amounts of fibrinogen for 1 h at RT, washed, and incubated with HRP conjugated anti-fibrinogen antibody (Dako). After washing, the plate was incubated with TMB One solution (Promega) for 30 min, and the absorbance was read at 450 nm.

Kinetic Study of the Aβ–Fibrinogen Interaction with FP. To determine the K_d for the interaction between Aβ42 and fibrinogen or FragD, 2 nM TAMRA-labeled Aβ42 was mixed with increasing concentrations of fibrinogen or FragD in 50 mM PBS solution, pH 7.4, 0.001% Tween 20, and 0.001% BSA in black 384-well plates (Greiner) at RT. After binding reached equilibrium, polarization measurements were recorded with a PerkinElmer EnVision plate reader with excitation at 490 nm and emission at 535 nm. The FP response was monitored and plotted as milli-Polarization (mP) unit. The data were fitted to a single-site binding equation [$\Delta mP = \Delta mP_{max} \cdot (protein) / (K_d + [protein])$] using GraphPad Prism 4 to calculate K_d.

Identification of the Regions of Fibrinogen that Interact with Aβ. Fibrinogen was digested with plasmin (5 mg/mL fibrinogen in 50 mM Tris, pH 7.4, 150 mM NaCl, 5 mM iodoacetamide, 0.6 μM plasminogen, 0.37 μM tissue plasminogen activator), generating FDPs. FDPs were incubated for 3 h at RT with biotinylated Aβ42 (200 nM), the Aβ-interacting peptides pulled down with streptavidin-Sepharose beads, and the peptides eluted with sample loading buffer and analyzed using SDS/PAGE. Incubations that did not contain biotinylated Aβ42 served as a control for nonspecific binding to the streptavidin-Sepharose beads. The protein gel bands were excised from the SDS/PAGE. Gel bands were reduced with 10 mM of DTT and alkylated with 55 mM iodoacetamide, then digested with Sequence Grade Modified Trypsin (Promega) in ammonium bicarbonate buffer at 37 °C overnight. Digestion products were extracted twice with 0.1% TFA, 50% acetonitrile, and 1.0% TFA, respectively. The extracted mixture was dried by Speed-Vac, redissolved in 10 mL of 0.1% TFA, and then analyzed using LC-MS/MS. *SI Materials and Methods* provides further information.

AlphaLISA Assay. Biotinylated Aβ42 (20 nM) and fibrinogen (1 nM) were incubated with increasing concentrations of fibrinogen-derived peptides for 30 min at RT in assay buffer (25 mM Tris·HCl, pH 7.4, 150 mM NaCl, 0.05% Tween 20, 0.1% BSA). The mixture was incubated with anti-fibrinogen antibody (Dako), 20 μg/mL streptavidin-conjugated donor, and protein A-conjugated acceptor beads (PerkinElmer) for 90 min at RT. Samples were read by a PerkinElmer EnVision plate reader.

Size-Exclusion Chromatography to Identify Aβ-Induced Fibrinogen Oligomer. Fibrinogen (1 μM) or FragD (1 μM) were incubated with 5 μM Aβ42 in 50 mM PBS solution (pH 7.4) and 0.001% Tween 20 for 2 h at 37 °C. The Aβ42-fibrinogen mixture was separated by size-exclusion chromatography on a Superose 6 10/300 GL column (GE Healthcare) in 50 mM PBS solution (pH 7.4) and 0.001% Tween 20 at 4 °C. Elution was monitored by measuring absorbance at 280 nm. For control experiments, 5 μM amylin or 5 μM calcitonin was incubated with 1 μM fibrinogen under the same conditions and separated on a Superose 6 10/300 GL column. Aβ binding to fibrinogen or FragD was confirmed by Western blotting using anti-Aβ antibody 4G8 (Covance).

TEM. Purified Aβ42-induced oligomers were applied to a glow-discharged carbon grid for 5 min, washed three times with ddH_2O, blotted dry, and then stained with 2% uranyl acetate. For immunogold labeling, fibrinogen was labeled using anti-fibrinogen antibody (Dako) and anti-rabbit IgG 12-nm gold-conjugated. Aβ was labeled using anti-Aβ antibody 6E10 (Covance) and anti-mouse IgG 6-nm gold-conjugated. Images were obtained using a FEI TECNAI G2 Spirit BioTwin transmission electron microscope at The Rockefeller University EM Resource Center.

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