Humoral Immune Response to Fibrillar β-Amyloid Peptide†

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ABSTRACT: The β-amyloid peptide (Aβ) is a normal product of the proteolytic processing of its precursor (β-APP). Normally, it elicits a very low humoral immune response; however, the aggregation of monomeric Aβ to form fibrillar Aβ amyloid creates a neo-epitope, to which antibodies are generated. Rabbits were injected with fibrillar human $Aβ_{1-42}$, and the resultant antibodies were purified and their binding properties characterized. The antibodies bound to an epitope in the first eight residues of Aβ and required a free amino terminus. Additional residues did not affect the affinity of the epitope as long as the peptide was unaggregated; the antibody bound Aβ residues 1−8, 1−11, 1−16, 1−28, 1−40, and 1−42 with similar affinities. In contrast, the antibodies bound ∼1000-fold more tightly to fibrillar $Aβ_{1-42}$. Their enhanced affinity did not result from their bivalent nature: monovalent Fab fragments exhibited a similar affinity for the fibrils. Nor did it result from the particulate nature of the epitope: monomeric $Aβ_{1-16}$ immobilized on agarose and soluble $Aβ_{1-16}$ exhibited similar affinities for the antifibrillar antibodies. In addition, antibodies raised to four nonfibrillar peptides corresponding to internal Aβ sequences did not exhibit enhanced affinity for fibrillar $Aβ_{1-42}$. Antibodies directed to the C-terminus of Aβ bound poorly to fibrillar $Aβ_{1-42}$, which is consistent with models where the carboxyl terminus is buried in the interior of the fibril and the amino terminus is on the surface. When used as an immunohistochemical probe, the antifibrillar $Aβ_{1-42}$ IgG exhibited enhanced affinity for amyloid deposits in the cerebrovasculature. We hypothesize either that the antibodies recognize a specific conformation of the eight amino-terminal residues of Aβ, which is at least 1000-fold more favored in the fibril than in monomeric peptides, or that affinity maturation of the antibodies produces an additional binding site for the amino-terminal residues of an adjacent Aβ monomer. *In vivo* this specificity would direct the antibody primarily to fibrillar vascular amyloid deposits even in the presence of a large excess of monomeric Aβ or its precursor. This observation may explain the vascular meningeal inflammation that developed in Alzheimer's disease patients immunized with fibrillar Aβ. Passive immunization with an antibody directed to an epitope hidden in fibrillar Aβ and in the transmembrane region of APP might be a better choice in the search for an intervention to remove Aβ monomers without provoking an inflammatory response.

Amyloid is the generic term applied to poorly soluble proteinaceous deposits, which possess similar tinctorial properties when treated with histochemical stains, and which occur in various tissues in response to disease (*1*). Amyloid is frequently formed from a peptide cleaved from a larger precursor, whose amino acid sequence predisposes it to aggregate in fibrillar β-sheets. As an abnormal substance composed of an endogenous peptide, amyloid presents a difficult problem for the humoral immune system. To eliminate the amyloid, the system must raise antibodies to the neo-epitopes in the aggregated peptide without provoking an autoimmune response, which would possibly destroy tissues that produce the amyloid peptide or its precursor. Such autoimmune processes account for some of the tissue damage occurring in multiple sclerosis, lupus erythematosis, and arthritis. The β-amyloid peptide (Aβ) linked to Alzheimer's disease (AD) forms deposits in the brain, which presents an additional problem in that the location of the deposits is, to a large extent, isolated from the systems of acquired immunity. This may limit their capacity either to generate or to react with a humoral immune response. Aβ is cleaved from a precursor, which is produced by nearly all tissues, and it is found in body fluids. The cell biology of Aβ and its postulated role in Alzheimer's disease have been reviewed (*2*−*4*).

Schenk and colleagues discovered that peripheral immunization with fibrillar human Aβ lowered the level of β-amyloid deposits in transgenic mice expressing the human β-amyloid peptide precursor (β-APP) in their brains (*5*). Although the direct application of this procedure to humans may not be feasible, the result has stimulated interest in the involvement of the immune system in AD and in the utility of immunotherapy in treating the disease (*6*−*10*). Nevertheless, this transgenic mouse AD model, in which human β-APP mRNA expression is driven by the platelet-derived growth factor promoter, may not reproduce the humoral immune response of humans, because the transgenic human β-APP (whose Aβ sequence differs from that of the mouse) is principally produced in the mouse brain, where it and the

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β-amyloid are virtually unseen by mouse antibody-forming cells (*11*).

We decided to characterize the immune response of rabbits to fibrillar human Aβ, because the Aβ sequences of rabbits and humans are identical. Thus, their immune systems should undergo similar processes of Aβ tolerance during development. We found that when immunized with Aβ fibrils, rabbits generate antibodies to the amino-terminal sequence of Aβ, but these antibodies bind to Aβ fibrils with an affinity constant that is more than 1000-fold larger than the affinity constant for their binding to the monomeric sequence. This result may explain why the antibodies fail to react with endogenous β-APP or monomeric Aβ, but may react with cerebral Aβ fibrils and trigger secondary inflammatory processes.

MATERIALS AND METHODS

Human $A\beta_{1-40}$ and $A\beta_{1-42}$ were purchased from California Peptide Co. (Napa, CA) and from Bachem Bioscience, Inc. (King of Prussia, PA). Anaspec (San Jose, CA) supplied the Aβ subsequences of residues 1−11, 1−16, 1−28, 10−20, 17−40, and 25−35. Invitrogen (Grand Island, NY) synthesized $A\beta_{1-8}$ and N_1-Cys-ϵ-aminohexanoyl-$A\beta_{1-8}$ (Cys-ahx-$A\beta_{1-8}$). The following numbering denotes the subsequences: D^{1}AEFRHDSGY^{10}EVHHQKLVFF^{20}AEDVGSNKGA^{30}IIGLMVGGVV^{40}IA. The concentrations of Aβ and of peptides containing tyrosine were measured by UV spectrophotometry using an extinction coefficient at 280 nm of 1280 (*12*).

Preparation and Assay of Fibrillar $A\beta_{1-42}$. Fibrillar $A\beta_{1-42}$ (fAβ) was prepared by two different methods. In the first method, the peptide was dissolved and disaggregated in 0.05 M NH_3 at a concentration of ∼1 mg/mL, and the solution was clarified by centrifugation for 3 min at 12000*g*. The solution was adjusted to pH 7.0 with NaH_2PO_4 to give a final peptide concentration of 0.5 mg/mL in 0.05 M phosphate; to inhibit bacterial growth and oxidation, 3 mM NaN_3 and 5 mM DTT were added. The solution was seeded with 1 μg of fibrillar $A\beta_{1-42}$ and was subjected to slow rotary mixing for 2 days, when aggregation, as assessed by thioflavin T binding, was complete. In the second method, the peptide was dissolved in hexafluoro-2-propanol, dried, redissolved in H_2O, and adjusted to pH 7.0 with 0.05 M sodium phosphate. Aggregation in these and other buffers [Dulbecco's phosphate-buffered saline or 50 mM Tris-HCl (pH 7.5) and 0.15 M NaCl] gave similar extents of thioflavin T binding. Thioflavin T binding was performed by a modification of the method of LeVine (*13*). Approximately 2 μg of fibrillar $A\beta_{1-42}$ was mixed with 2 mL of 5 μM thioflavin T in 50 mM Tris-HCl (pH 7.5) and 0.15 M NaCl. After 1 min, the fluorescence was read (450 nm excitation and 486 nm emission) in a Perkin-Elmer model LS-5 spectrophotofluorometer. The fluorescence yield was a factor of ∼10 greater than the background fluorescence. When this method was applied to the determination of the solubility of fibrillar Aβ, 1 mL of 10 μM thioflavin T was added to 1 mL of fibrillar Aβ 1 min before the fluorescence was measured.

Generation of Antisera. Six rabbits (R261, R262, R285, R286, R333, and R334) were immunized with fibrillar $A\beta_{1-42}$ according to the following schedule: (1) 100 μg of fAβ and complete Freund's adjuvant (1:1), subcutaneously on day 1, (2) 100 μg in incomplete Freund's adjuvant (IFA), subcutaneously on day 14, and (3) 150 μg of fAβ mixed with 4 mg of aluminum ammonium sulfate, intraperitoneally on day 30. Animals were bled at intervals of 3, 5, and 7 weeks after the first injection. The immunization was continued using IFA every 2−3 weeks until antibodies with high affinity were produced. The following additional rabbit antibodies were raised to peptides conjugated to keyhole limpet hemocyanin: R321 and R165, specific for the carboxyl terminus of $A\beta_{1-42}$ (residues 35−42); R287, raised to Aβ residues 27−37; R222, raised to residues 104−118 of presenilin; and R329, raised to the 14 carboxyl-terminal residues of neprilysin.

Other Antibodies. Monoclonal antibodies 4G8 (*14*), specific for Aβ residues 16−24, and 6E10 (*15*), which recognizes an epitope in residues 3−16, are available from Signet Laboratories. Alkaline phosphatase-conjugated secondary antibodies were obtained from Biosource International. The biotinylated secondary antibodies used for immunohistochemistry were products of Amersham Pharmacia Biotech.

AntibodyPurification. Aβ-binding antibodies were purified on the $A\beta_{1-16}$−agarose matrix prepared by the reaction of $A\beta_{1-16}$ modified by the addition of a C-terminal cysteinyl residue with epoxy-activated agarose (Sigma). One milliliter of antiserum was mixed with 0.3 mL of the $A\beta_{1-16}$−agarose matrix for 2 h. The unbound serum proteins were removed, and after the mixture was thoroughly washed with 50 mM Tris-HCl (pH 7.5) and 0.25 M NaCl, the bound antibodies were eluted with 4.5 M $MgCl_2$. The IgG-containing fraction was dialyzed against 50 mM Tris-HCl (pH 7.5) and was stabilized with 1 mg/mL BSA and 3 mM NaN_3. Later work showed that all of the Aβ-specific antibodies could be eluted with 50 mM H_3PO_4.

Fab Fragment Preparation. The IgG fraction of the antiserum was prepared by protein A−agarose chromatography using reagents from Pierce-Endogen. Fab fragments were prepared with the aid of a Pierce-Endogen kit according to the supplier's directions. The papain digestion time was minimized to optimize the recovery of active Fab fragments. Undigested IgG was removed by protein A chromatography, and the preparation was analyzed by PAGE to confirm the absence of undigested IgG. The Fab preparation was further purified on a Pharmacia Superose 12 HR gel filtration column. All of the fibrillar Aβ binding activity emerged in an elution volume expected for a 50 kDa protein, i.e., slightly later than BSA.

Quantitative Immunoblotting Procedures. A previously described method (*16*) was used to measure the titers and specificities of the antisera and to determine the stoichiometry of antibody binding to Aβ fibrils. Two protein A-purified rabbit IgG preparations were quantified by spectrophotometry, using an absorbance value of 1.40 for a 1 mg/mL solution (*17*). To measure the amounts of IgG bound to Aβ fibrils, the samples and standards were dissociated in a sample buffer containing 10 mM dithiothreitol and were subjected to PAGE. The proteins were electroblotted to a nitrocellulose membrane, treated with goat anti-rabbit IgG antibody conjugated to alkaline phosphatase (Biosource), and developed with BCIP/NBT. The 55 kDa IgG heavy chains were quantified by photodensitometry using IPLab Gel software from Scanalytics. The $A\beta_{1-42}$ contents of fibrils

were measured on a duplicate blot developed with affinity-purified antibody R321, which is specific for the C-terminus of Aβ_{1-42}. Known amounts of fibrillar Aβ_{1-42} served as standards. Fibrillar Aβ_{1-42} samples were disaggregated by a brief treatment with 100 μL of 98% formic acid, which was removed by centrifugal vacuum evaporation.

Fibril Binding Assays. The fibrillar Aβ_{1-42} preparation was initially sedimented for 3 min at 12000*g* to remove any unaggregated Aβ and slowly sedimenting aggregates. The antibody preparations were clarified under the same conditions to remove any antibody aggregates. In a 1.5 mL microcentrifuge tube, 0.1−0.5 μg of fibrillar Aβ_{1-42} was incubated at ambient temperature with varying amounts of antibody in 100 μL of binding buffer [25 mM sodium phosphate (pH 7.0), 250 mM NaCl, 3 mg/mL BSA, and 0.04% Tween 20]. After gentle agitation for 1 h on a vortex mixer, the mixtures were diluted with 0.5 mL of wash buffer [100 mM Tris-HCl (pH 7.5), 250 mM NaCl, and 0.04% Tween], and the fibril−antibody complexes were sedimented at 12000*g* for 3 min. Approximately 95% of the supernatant liquid was aspirated, and the process was repeated twice. The sedimented fibrils were then suspended in 100 μL of binding buffer containing 650 ng of goat anti-rabbit Ig coupled to alkaline phosphatase. After a 1 h gentle incubation on the vortex mixer, the fibrils were washed three times as previously described and suspended in 0.5 mL of phosphatase buffer [100 mM Tris-HCl (pH 8.8) and 1 mM $MgCl_2$]. An aliquot of the suspension was transferred to a fresh tube and was incubated with 1 mM nitrophenyl phosphate (NPP) in phosphatase buffer. After ~1 h, a period sufficient to yield an absorbance at 415 nm of ~0.6, the reaction was stopped by the addition of 10 mM EDTA, and the absorbances were measured. Controls lacking fibrils or the primary antibody exhibited absorbances that were less than 5% of those of samples containing immune antisera.

Phage Display Epitope Mapping. The Ph.D.-7 phage display system (New England Biolabs) was used for epitope mapping of antibody R286. The panning was performed in a 96-well polystyrene microtiter plate at room temperature following the protocol suggested by the supplier, with some modifications. Briefly, each well was coated with 100 μL of antibody R286 (10 μg/mL in 0.1 M sodium bicarbonate) for 60 min. The well was then blocked for 60 min before incubation with 100 μL of phage display library (2×10^{11} PFU) for an additional 60 min to allow binding of phage to the coated antibody. After removal of unbound phages with extensive washings, bound phages were then eluted by incubating each well with 100 μL of 0.2 M glycine (pH 2.5) for 10 min and immediately neutralized by mixing with 50 μL of 1 M Tris (pH 8.0). The eluted phage was titered and amplified for the next round of panning. After four rounds of panning, 10 random phage clones were selected for DNA sequencing.

Rabbit Antisera Titer. Wells of a microtiter plate were coated with 100 μL of peptide [10 μL of a 1 mg/mL antigen preparation in 10 mL of 0.05 M carbonate buffer (pH 9.6)] and incubated overnight at 4 °C. The plate was washed with 0.01 M PBS (pH 7.2) with 0.05% Tween 20 (PBST) and blocked with 10% normal sheep serum in PBS for 1 h at room temperature. Rabbit antisera were diluted from 1:1000 with 0.5% BSA in PBST, and 10 serial dilutions were made. The blocked plate was washed; 100 μL of diluted rabbit antisera was added to the wells, and the wells were incubated for 2 h at room temperature. The plate was washed, and 100 μL of goat anti-rabbit IgG conjugated to alkaline phosphatase diluted 1:1000 with 0.5% BSA in PBST, was added and incubated for 2 h at room temperature. The plate was washed and developed using a solution of *p*-nitrophenyl phosphate in 10% diethanolamine (pH 9.8). After 30 min, the absorbance at 405 nm was read using an automated microplate reader. The antibody titer was determined at a dilution showing an absorbance of ~1.0.

Assay of Inhibition with an ELISA. Wells of a microtiter plate were coated with peptide and blocked as described above. The peptide used as an inhibitor was serially diluted, and the antibody was diluted in 0.5% BSA in PBST. The antibody control was a 1:2 dilution of the antibody in buffer. Equal volumes of inhibitor and antibody solutions were combined in a test tube, mixed, and incubated at room temperature for 2 h and then overnight at 4 °C. The plate was washed, and 100 μL of the different antigen/antibody solutions was added to the wells and incubated for 2 h at room temperature. After the mixture had been washed, 100 μL of goat anti-rabbit IgG conjugated to alkaline phosphatase diluted 1:1000 with 0.5% BSA in PBST was added and the mixture incubated for 2 h at room temperature. The plate was washed and developed as described above. When the antibody control wells reached an OD of approximately 1.0 at 405 nm, the plate was read using an automated microplate reader.

Electron Microscopy. A drop of a solution containing 9 volumes of fibrillar Aβ_{42} (0.2−1 μM as monomers) and 1 volume of antiserum in PBS was applied to a 400-mesh Formvar-coated grid. The grid was drained, washed with glass-distilled deionized water, and treated with a solution of 1% BSA and 0.05% Tween 20 in PBS for 10 min. If the sample contained bound IgG, it was incubated with 1% protein A labeled with 10 nm colloidal gold (Sigma) at a concentration of 0.05 A_{520} unit/mL in BSA and PBS for 1 h. All grids were rinsed with ~20 drops of BSA and PBS and 20 drops of distilled water. The grid was then negatively stained with 1% uranyl acetate, washed with four drops of water, drained, and air-dried. Samples were examined and photographed at a 10000× or 40000× magnification in a Hitachi 7000 electron microscope.

Immunohistochemistry. The methodology of this study was approved by the New York State Institute for Basic Research Institutional Review Board. Brain sections obtained from individuals who succumbed to AD showed the characteristic neuropathology of late-stage AD. The sections were fixed in 10% formalin for more than 1 month, dehydrated in ethanol, embedded in paraffin, and cut into 8 μm thick serial sections. The endogenous peroxidase in the sections was blocked with 0.2% hydrogen peroxide in methanol. Some of the sections were treated with 90% formic acid for 30 min. The sections were then treated with 10% fetal bovine serum in PBS for 30 min to block nonspecific binding. The antibodies were diluted in 10% fetal bovine serum in PBS and were incubated with the sections overnight at 4 °C. The sections were washed and treated for 30 min with either biotinylated sheep anti-mouse IgG antibody or biotinylated donkey anti-rabbit IgG antibody (each at a dilution of 1:200). The sections were treated with an extravidin peroxidase conjugate (1:200) for 1 h followed by diaminobenzidine (0.5

mg/mL) and 1.5% hydrogen peroxide in PBS. The sections were counterstained with cresyl violet.

RESULTS

Fibril Binding Assay. Our initial objective was to determine whether the antibodies generated by immunization with fibrillar Aβ_{1-42} specifically bound to the fibrils. Because of the conceptual and experimental limitations of filtration and fibril immobilization methods in detecting and quantifying these antibodies, a sedimentation procedure was developed. Its advantages include the negligible level of nonspecific binding and the certainty that the observed binding is not due to a low level of monomeric Aβ. In addition, the antibody−fibril complexes readily can be characterized by other techniques, such as EM and Western blotting. Although the 0.1 μg aliquot of fibrillar amyloid used in the assay is invisible, ~80% of it can be recovered at the end of the assay. Loss of fibrillar Aβ_{1-42} due to dissolution is not a problem. Using the thioflavin T binding assay to follow the time course, we detected a 17% loss of fibrillar Aβ_{1-42} kept for 70 h at ambient temperature at a concentration of 2 μg/mL in 50 mM Tris-HCl (pH 7.5) and 0.15 M NaCl.

The assay conditions were optimized for the amounts and incubation times of the primary and secondary antibodies. The amount of antifibrillar Aβ_{1-42} antibody was generally 10−20% of the binding capacity of the fibrils, and the amount of secondary antibody conjugate was ~5−10-fold greater than the amount of bound primary antibody. The half-times of the antibody binding reactions were 10−15 min. The 1 h incubation periods allow both binding reactions to proceed nearly to completion. At subsaturating concentrations of the primary antibody, the rate of nitrophenolate production directly increases with the concentration of primary antibody (Figure 1A). At higher primary antibody concentrations, the reaction rate reaches a plateau, where all of the fibril binding sites are filled. Similarly, at a fixed primary antibody concentration, the amount of sedimentable antibody increases as the amount of fibrillar Aβ_{1-42} is increased (Figure 1B) until the entire amount of the fibril-specific antibody was bound.

Identification of Fibril-Binding Antibodies. After two injections of fibrillar Aβ_{1-42}, antibodies appeared which could be detected with an ELISA on Aβ_{1-42}-coated plates and with the fibril binding assay (Table 1). The preimmune antiserum or heterologous antisera to neprilysin (R329) or presenilin (not shown) did not produce a positive response on an ELISA or in the fibril binding assay. These antisera did not react with Aβ_{1-42} on Western blots, at which concentrations other anti-Aβ antisera reacted (result not shown). This apparent lack of reactivity with monomeric Aβ on Western blots suggested the possibility that the antifibrillar Aβ antisera recognized an epitope that was unique to the fibrillar form of Aβ.

To confirm that the antifibrillar Aβ antibodies bound to Aβ fibrils, we examined the antibody−fibril complexes by electron microscopy after labeling with immunogold particles. The gold particles were predominantly associated the fibrils (Figure 2), which confirmed that the fibrils contained an epitope that can be recognized by the antibody. R222, a heterologous anti-presenilin antiserum, showed only background labeling.

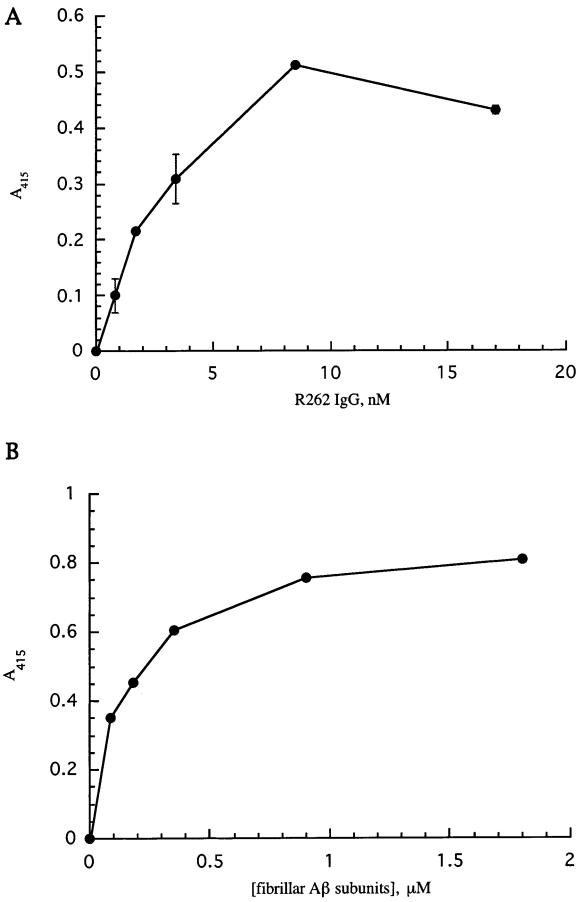

FIGURE 1: Sedimentation of IgG with fibrillar Aβ_{1-42}. (A) Fibrillar Aβ_{1-42} (0.08 μg, 18 pmol of monomer) was incubated with increasing amounts of immunopurified R262 IgG in a volume of 100 μL, and the mixture was processed with the anti-rabbit IgG−alkaline phosphatase conjugate as described in the text. One-tenth of the sedimented complex was assayed for nitrophenyl phosphatase activity. The absorbance at 415 nm was measured after 30 min. Data points represent averages of two independent measurements. (B) Immunopurified R262 IgG (0.17 pmol) was incubated with increasing concentrations of fibrillar Aβ_{1-42} in a volume of 100 μL and was processed as described for panel A. The abscissa units indicate concentrations of fibrillar Aβ subunits. Error bars represent the average deviation from the mean of two independent measurements. Error bars that are not visible lie within the areas of the data points.

Table 1: ELISA Titers and Fibril Binding Activities of Antifibrillar Aβ Antisera

antiserum	ELISA titer[a] ($\times 10^{-3}$)	fibril binding[b]
R262	45 (100)	0.63 (100)
R286	32 ± 1 (71)	0.49 (78)
R333	11 (24)	0.29 (46)
R334	38 (84)	0.76 (120)
R286 preimmune	<0.1 (<0.3)	0.02 (<3)
R329 control	<0.1 (<0.3)	0.02 (<3)

[a] The ELISA was performed on a plate coated with Aβ_{1-42} as described in the text. [b] Each antiserum (0.5 μL) was incubated with 0.5 μg of fibrillar Aβ_{1-42} and processed by the sedimentation assay as described in the text. Results are ΔA_{415}. Numbers in parentheses are the percentage of the value for antiserum R262. ELISA results are averages of two measurements ± the average error; fibril binding results are single measurements.

Further ELISA experiments (see Figure 3) revealed that the antisera bound to wells coated with Aβ_{1-28}, a fragment with a weak tendency to form fibrils. This result suggested

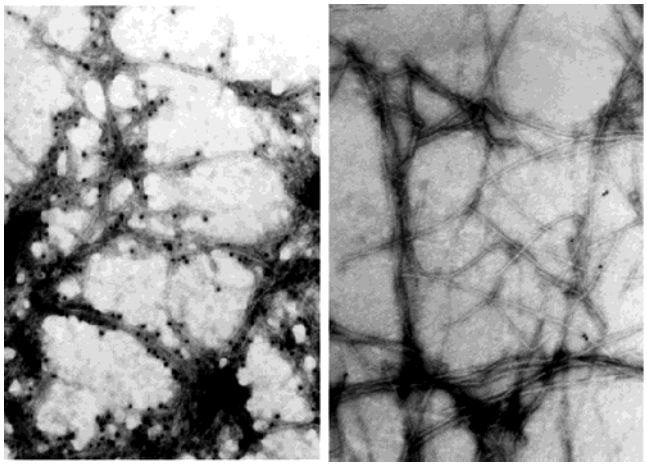

FIGURE 2: Electron micrograph of antibody R262 bound to fibrillar Aβ_{1-42} revealed by gold-labeled protein A: (left) R262 antiserum and (right) the same concentration of R222 antiserum used as a control. The experimental details are described in Materials and Methods. The diameter of the gold particles is 10 ± 1.5 nm. The magnification is 112000×.

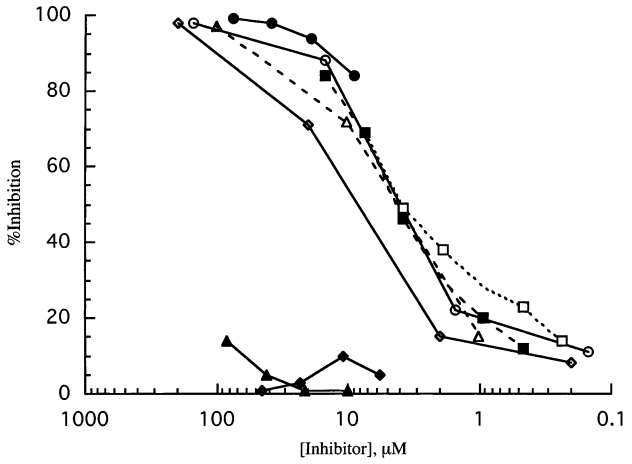

FIGURE 3: Inhibition of antibody R286 binding by Aβs determined with an ELISA: (○, ●, □, and ■) Aβ_{1-11}, (△) Aβ_{1-16}, (◇) Aβ_{1-8}, (▲) Aβ_{1-8}, amino-terminal Cys-aminohexanoyl derivative, and (◆) Aβ_{3-11}. ELISA plates were coated with Aβ_{1-28}.

that the antiserum might also bind to a linear epitope; however, the antiserum did not bind to wells coated with Aβ_{1-16}, Aβ_{10-20}, or Aβ_{17-42}. To resolve this apparent paradox, we performed inhibition assays with the peptides (Figure 3). In this assay, Aβ_{1-16}, Aβ_{1-11}, and Aβ_{1-8} completely blocked antibody binding to wells coated with Aβ_{1-42}, whereas Cys-ahx-Aβ_{1-8} and Aβ_{3-11} did not inhibit antibody binding. We interpret these results to indicate that the antibodies are monospecific for an epitope within residues 1−8 and that binding requires an unmodified amino-terminal aspartyl residue. The lack of immunoreactivity with Aβ_{1-16} in the direct ELISA suggests that the epitope is masked when the peptide binds to the polystyrene plate. By an M13 phage display system, the epitope also was determined to be located within residues 1−7 (Table 2). Although the first three residues, aspartyl-alanyl-glutamyl, and arginyl-5 occur in all of the recognized sequences, there is some variability in the subsequent residues, which suggests that the antibodies are less specific for these residues.

To determine whether the antibodies that bound to Aβ_{1-8} also bound to fibrillar Aβ_{1-42}, we purified R262 and R286

Table 2: Antibody R286 Epitope Identified by Phage Display[a]

Residue number	1	2	3	4	5	6	7
Aβ sequence	**D**	**A**	**E**	**F**	**R**	**H**	**D**
Phage sequence A	**D**	**A**	**E**	**F**	**R**	N	L
B	**D**	**A**	**E**	**F**	**R**	T	A
C	**D**	**A**	**E**	S	**R**	T	V
D	**D**	**A**	**E**	I	**R**	N	H
E	**D**	**A**	**E**	P	**R**	R	L

[a] Identical residues are denoted by bold letters.

Table 3: ELISA and Fibril Binding Activity of Affinity-Purified Antibody R286

	ELISA titer[a]	fibril binding activity[c]
crude antiserum	34000	0.3 ± 0.03
unbound antiserum	70	0.020 ± 0.001
4.5 M $MgCl_2$ eluate[b]	7500	0.13 ± 0.01

[a] Assays were performed on a plate coated with Aβ_{1-28} as described in the text. [b] Results were corrected by a factor of 2.5 for dilution during dialysis. [c] Fibril bindng activities were determined as described in the text. The results are averages of two independent measurements of ΔA_{412} ± the average error.

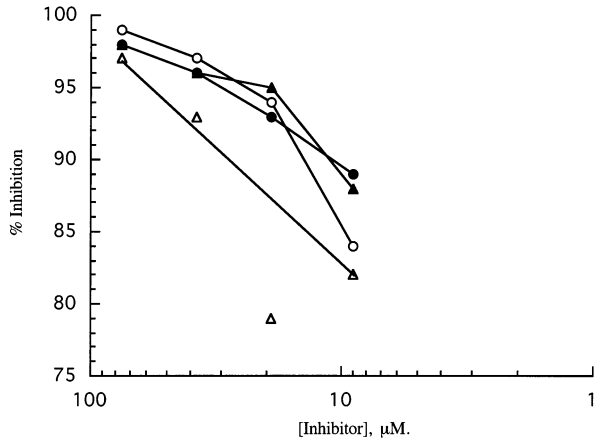

FIGURE 4: ELISA of inhibition of antibody R286 binding to Aβ_{1-42} or Aβ_{1-28} by Aβ_{1-11}: (filled symbols) crude R286 antiserum, (empty symbols) affinity-purified R286, (circles) Aβ_{1-42}-coated plate, and (triangles) Aβ_{1-28}-coated plate.

by adsorption to an Aβ_{1-16}−agarose affinity matrix. The antibodies adsorbed and recovered from the affinity matrix amounted to ∼3% of the total IgG in the antisera. Both the purified antibodies and the depleted antisera were analyzed with an ELISA and the fibril binding assay (Table 3). The ELISA revealed that virtually the entire amount of the Aβ_{1-8} binding antibody had been removed from the antiserum. Nearly all of the fibril binding activity was also adsorbed onto the affinity matrix, which indicated that the fibril-binding antibody did not necessarily recognize a topological epitope in the fibrils. The absolute magnitudes of the values from the two assays cannot be directly compared, because of the differences in the way each assay was performed. Regardless of whether the wells were coated with Aβ_{1-28} or the full-length peptide, the affinity-purified antibody exhibited the same Aβ_{1-11} binding properties as the crude antiserum (Figure 4), which confirmed that the purified antibody contained the same Aβ binding activity as the crude antiserum.

Another affinity matrix was constructed with Aβ_{1-16} linked to epoxyagarose through an amino-terminal cysteine. When

Table 4: Exposure of Epitopes in Fibrillar $A\beta_{1-42}$

antibody	epitope, $A\beta$ residues	binding activity[a]
R286	1−8	67 ± 4
6E10	3−16	44 ± 9
4G8	16−24	55 ± 4
R287	27−37	46 ± 16
R165	35−42	5 ± 1

[a] Binding activities were measured by the sedimentation assay. Similar amounts of antibodies (based upon ELISA titers) were used. The activities were expressed as the nitrophenolate absorbance of the sample relative to that of the nonimmune control serum. Results are averages of two independent determinations ± the average error.

R286 was equilibrated with this matrix, no fibril binding activity or peptide binding activity was depleted from the antiserum and no activity could be eluted from it with 4.5 M $MgCl_2$. This finding indicated that the fibril-binding antibody did not bind nonspecifically to the matrix and that the antibody required the free amino-terminal aspartyl residue to bind to $A\beta$.

Detection and Quantification of Fibril Epitopes. The stoichiometry of antibody molecules bound per $A\beta_{1-42}$ monomer was determined by measuring the amounts of antibody cosedimenting with $A\beta_{1-42}$ fibrils at saturating antibody concentrations. The ratio of antibody molecules to $A\beta_{1-42}$ monomers was calculated to be 0.3 ± 10% ($n = 6$). This ratio is ~6-fold higher than what we expected from the presumed sizes of the IgG molecule and the fibril epitope (see Discussion).

Other exposed peptide sequences in $A\beta_{1-42}$ fibrils can be identified by the fibril binding assay (Table 4). Antibodies 6E10, 4G8, and R287 bound to the fibrils much more extensively than did nonimmune control antibodies, but R165, which recognizes the C-terminal sequence of $A\beta_{1-42}$, bound scarcely more than the control. The determination of the binding stoichiometries of these antibodies would provide an accurate measure of the relative exposure of the epitopes in the fibrils.

Relative Affinities of the R286 and R262 Epitopes in the $A\beta$ Monomer and in the Fibril. The affinity of R262 or R286 for the epitope in monomeric $A\beta$ relative to that in the $A\beta$ fibril was studied by measuring the extent to which $A\beta$s inhibited the binding of the affinity-purified antibody to $A\beta_{1-42}$ fibrils. Two versions of the fibril binding assay were performed. In the first version, the antibody was preincubated with the monomer before the fibrils were added, and in the second version, the peptide was added to the preformed antibody−fibril complex. In both protocols, the extents of inhibition were similar (Table 5), which indicated that during the 1 h incubation the ternary mixture reached equilibrium.

The R262 and R286 antibodies bound much more strongly to the epitope in fibrils than in the monomeric peptides (Table 5). The ratio of the dissociation constant of the fibril−antibody complex (K_B) to that of the peptide−antibody complex (K_I) could be calculated from the expression

$$K_{rel} = K_B/K_I = ([B]/[I])([AI]/[AB])$$

where [B], [I], [AI], and [AB] are the concentrations of the fibrillar amyloid (expressed as monomers), inhibitor, antibody−inhibitor complex, and antibody−fibril complex, respectively. The ratio K_{rel} is much less than unity, which indicates that K_B is much smaller than K_I. The K_{rel} values in Table 5 were calculated using the concentrations of $A\beta$ monomers in fibrillar $A\beta$. Since only a fraction of the $A\beta$ monomers can bind the antibody, the concentration of fibrillar amyloid should be proportionately lowered, which would lower K_{rel} (see Discussion). The value of K_{rel} was not constant, but decreased as the concentration of the inhibitor was increased. This trend may result from heterogeneity in the fibril binding sites such that the stronger binding sites are less readily displaced.

Table 5: Inhibition of R262 Antibody Binding to Fibrillar $A\beta_{1-42}$ ($fA\beta$)[a]

[inhibitor] (μM)	[$fA\beta$] (μM)	protocol[b]	% inhibition	K_{rel}[c]
$A\beta_{1-16}$				
25	0.16	1	36 ± 2	0.0045
82	0.16	1	55 ± 2	0.0033
82	0.16	2	49 ± 3	0.0026
250	0.16	1	64 ± 2	0.0017
$A\beta_{1-40}$[d]				
27	0.18	1	69 ± 2	0.0014
$A\beta_{1-42}$[d]				
1.7	0.18	1	6.6 ± 1.3	0.006

[a] Measurements were performed by the sedimentation assay using 1.7 nM affinity-purified R262 antibody. [b] In protocol 1, the antibody and inhibitor were preincubated for 30 min at 37 °C; in protocol 2, the antibody and fibrils were preincubated for 30 min at 37 °C. [c] $K_{rel} = K_B/K_I$, as defined in the text. [d] Monomeric species. Results are averages of two independent measurements ± the average error.

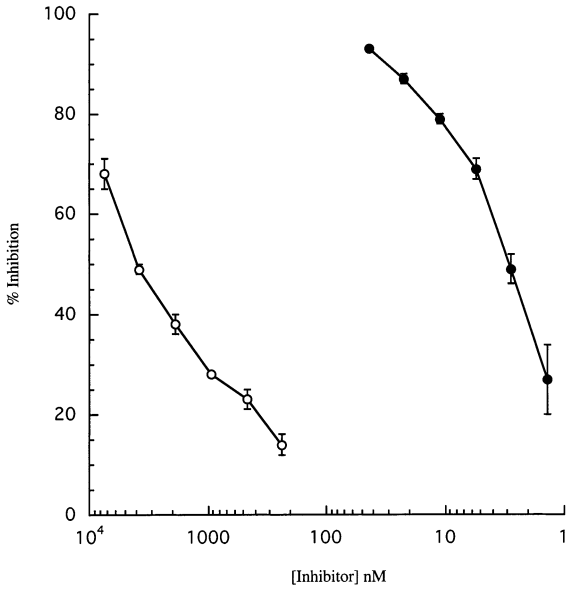

FIGURE 5: ELISA of the inhibition of antibody R286 binding to an $A\beta_{1-28}$-coated plate by fibrillar $A\beta_{1-42}$ (●) and $A\beta_{1-11}$ (○); averages of three independent measurements. Error bars indicate average errors.

The higher affinity of the $A\beta$ fibrils for these antisera also could be demonstrated with an ELISA (Figure 5). Fibrillar $A\beta_{1-42}$ produced a 50% inhibition of the binding of R286 antibody to coated $A\beta_{1-28}$ at a concentration more than 1000-fold lower than the $A\beta_{1-11}$ concentration needed to produce the same degree of inhibition. As in Table 5, the fibrillar $A\beta$ concentration was expressed as monomers.

The longer peptides ($A\beta_{1-28}$, $A\beta_{1-40}$, and $A\beta_{1-42}$) appear to be better inhibitors (Table 5). It is possible that the secondary or tertiary structures of these peptides affect the conformation of the amino-terminal epitope. Alternatively,

Table 6: Inhibition of R286 Antibody Binding to Agarose-Linked $A\beta_{1-16}$[a]

[$A\beta_{1-16}$ inhibitor] (μM)	[matrix-bound $A\beta_{1-16}$] (μM)	% inhibition
6	1	61 ± 2
17	1	80 ± 1
75	1	92 ± 1

[a] Measurements were performed as described in the text. The mixtures contained 23 nM affinity-purified R286 antibody. Results are averages of two measurements ± the average error.

Table 7: Inhibition of Antibody Binding to Fibrillar $A\beta_{1-42}$ by $A\beta_{1-28}$[a]

antibody, concn (nM)	[$A\beta_{1-28}$] (μM)	% inhibition
mAb 6E10, 9	6.1	78 ± 1
mAb 4G8, nd	6.1	89 ± 2

[a] Measurements were performed by the sedimentation assay using fibrillar $A\beta_{1-42}$ (2.2 μM monomers). The concentration of mAb 4G8 was not determined; its ELISA titer was similar to that of mAb 6E10. Results are averages of two measurements ± the average error.

there might be an unrecognized effect of anchoring the epitope to a larger structure, somewhat analogous to the equilibrium isotope effects observed in chemical reactions. To test this hypothesis, $A\beta_{1-16}$ was anchored to epoxy-activated agarose through a C-terminal cysteinyl residue, and its affinity for the R262 antibody relative to that of soluble $A\beta_{1-16}$ was determined (Table 6). In this case, the soluble peptide extensively displaced the antibody from the matrix, which indicated that the immobilization of the epitope on a large structure does not account for the higher affinity of fibrillar Aβ.

If the antibodies raised to fibrillar Aβ were specifically selected for fibril binding, then antibodies raised to linear Aβ epitopes might not selectively bind to fibrillar Aβ. To test this hypothesis, we measured the relative affinities of fibrillar $A\beta_{1-42}$ for mAb 6E10 (specific for residues 3−12) and mAb 4G8 (specific for residues 16−24). In contrast to its effect on antibodies R286 and R262, the monomeric peptide $A\beta_{1-28}$ strongly inhibited the binding of antibodies 6E10 and 4G8 to the fibrils (Table 7). Similarly, with an ELISA, $A\beta_{1-16}$ was about as effective as fibrillar $A\beta_{1-42}$ as an inhibitor of the binding of mAb 6E10 to $A\beta_{1-28}$-coated plates (Figure 6). Thus, some antibodies directed to Aβ epitopes bind about as well to monomeric peptides as to fibrils.

Relative Affinities of IgG and the Fab Fragment. A possible explanation for the enhanced affinity of R262 and R286 is that their bivalent character allowed them simultaneously to bind two epitopes on the fibril, which would decrease the unfavorable entropy component of complex formation. To test this hypothesis, we prepared the monovalent R286 Fab fragment and tested its relative affinity for fibrillar $A\beta_{1-42}$. Contrary to this hypothesis, in the presence of excess $A\beta_{1-28}$, the R286 Fab fragment preferentially bound to fibrillar Aβ (Table 8). At similar concentrations of the inhibitor peptide, the binding of R286 Fab (Table 8) and the binding of R262 IgG (Table 5) are inhibited to similar extents. Previous experiments had shown that the binding properties of R262 and R286 antibodies are indistinguishable.

Immunoreactivity of R286 IgG with Brain Amyloid Deposits. $A\beta_{1-42}$ fibrils generated *in vitro* may not have the same structure as β-amyloid fibrils that form in the AD brain parenchyma and vessels. Using AD brain sections, we compared the immunoreactivities of affinity-purified R286 IgG and mAb 6E10 with and without $A\beta_{1-16}$ added as a blocking peptide. R286 did not react well with parenchymal amyloid unless the slides were pretreated with formic acid (Figure 7a), an enhancer of brain amyloid immunoreactivity (*18*). In contrast, under our staining conditions, 6E10 reacted with parenchymal amyloid even without formic acid pretreatment (Figure 7b). The binding of each antibody was blocked by added $A\beta_{1-16}$ (Figure 7c,d). These findings suggested that parenchymal amyloid does not possess the same structure as fibrillar Aβ generated *in vitro*; however, it might also be argued that formic acid treatment disaggregated the fibrillar structure, which allowed R286 IgG to bind to amino-terminal sequences. A strikingly different result was obtained from the immunostaining of cerebellar vascular amyloid. In this tissue, both antibodies stained the amyloid without formic acid pretreatment (Figure 7e,f); however, the binding of mAb 6E10 was almost completely blocked by the addition of $A\beta_{1-16}$ (Figure 7h), whereas the level of binding of R286 IgG was scarcely diminished (Figure 7g). We concluded that cerebellar vascular amyloid contains fibrils with binding properties similar to those of fibrillar amyloid generated *in vitro*.

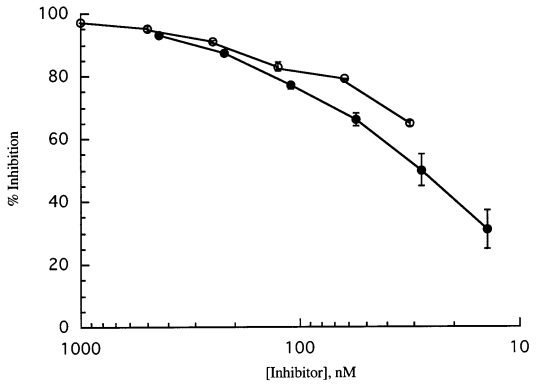

FIGURE 6: ELISA of the inhibition of mAb 6E10 binding to an $A\beta_{1-28}$-coated plate by fibrillar $A\beta_{1-42}$ (●) and $A\beta_{1-16}$ (○); averages of three independent measurements. Error bars represent average errors. Error bars that are not visible lie within the areas of the data points.

Table 8: Inhibition by $A\beta_{1-28}$ of R286 Fab Fragment Binding to Fibrillar $A\beta_{1-42}$[a]

[$A\beta_{1-28}$] (μM)	% inhibition
25	42 ± 7
82	61 ± 4
250	71 ± 6

[a] Measurements were performed by the sedimentation assay using a Fab preparation estimated to contain 7 nM Aβ-specific fragments. The fibrillar Aβ concentration was 0.17 μM (expressed as monomeric units). The results are averages of two independent experiments ± the average error.

DISCUSSION

This study was initially motivated by the remarkable finding of Schenk et al. (*5*) that transgenic mice bearing amyloid plaques could be relieved of their plaque burden by immunization with aggregated Aβ. In our own experience

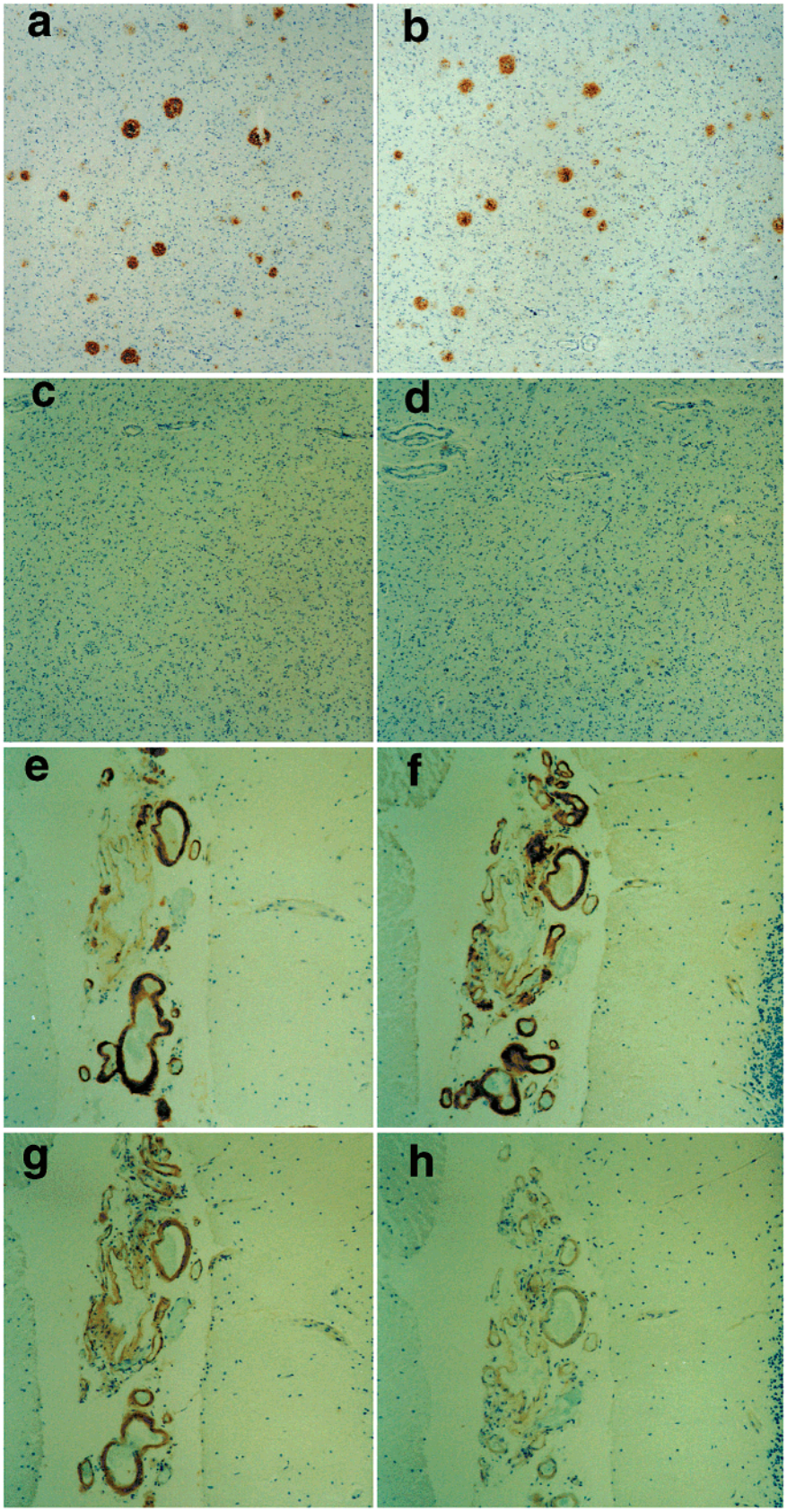

FIGURE 7: Comparative immunostaining of AD brain sections by affinity-purified R286 and mAb 6E10 with or without the Aβ_{1-16} blocking peptide: (a−d) amygdala and (e−h) cerebellum. Sections a and c were pretreated with formic acid. Sections a, c, e, and g were stained with R286. Sections b, d, f, and h were stained with mAb 6E10. Sections c, d, g, and h contained added 25 μM Aβ_{1-16}.

with generating antibodies to Aβ and its subsequences, we had found that it was difficult to breach the barrier against producing autoantibodies. Accordingly, we expected that the mouse antibodies raised to aggregated human Aβ would be directed toward the nonhomologous sequence encompassed by residues 5−13, and hence that a similar response would

not be generated in a species immunized with a peptide corresponding to its own Aβ sequence. We chose to conduct the experiment in rabbits, whose Aβ sequence is identical to that of humans. Surprisingly, rabbits immunized with aggregated Aβ_{1-42} produced moderate titers of antibodies against Aβ_{1-42} according to an ELISA. However, when assayed by immunoblotting, the antisera did not detectably react with Aβ_{1-42} at peptide loadings of 0.1 pmol, which could be readily detected by other anti-Aβ antibodies, such as mAb 6E10 and 4G8, and rabbit antibody R165 (*16*).

In electron micrographs of mixtures of the antiserum with fibrillar Aβ_{1-42}, antibody molecules were found to be bound to Aβ fibrils. The antisera were found not to be absolutely specific for the fibrillar form of Aβ. Antibodies affinity-purified from the antisera on an Aβ_{1-16} matrix possessed all of the fibril binding activity of the crude antisera. These antibodies were specific for an epitope within residues 1−8 of Aβ, and they required a free amino group on Asp-1. To further study the interaction, a simple sedimentation assay was developed, which permitted the estimation of the binding constants of the antibody−fibril interaction, as well as of those for the interactions of the antibody with Aβ fragments.

The specificity of the fibril binding assay was confirmed by negative controls. Preimmune serum and heterologous antisera gave nearly background levels of nitrophenolate production (Table 1). Further confirmation of the specificity was provided by the result that the fibril binding activity was removed by adsorption to an agarose−Aβ_{1-16} matrix if the peptide were linked through its carboxyl terminus, but it could not be adsorbed if the peptide were linked through its amino terminus. The antibodies eluted from the matrix were 30-fold enriched in their fibril binding activity. In addition, not all anti-Aβ antibodies bound to the fibrils; antibodies directed to the carboxyl terminus of Aβ showed little on no affinity for the fibrils (Table 4).

Antibodies raised to many proteins bind to neuritic amyloid plaques. It usually has not been possible to demonstrate that binding activities are specific. The sedimentation procedure could be used to eliminate β-amyloid fibril binding as a possible source of nonspecific binding.

Although all of the antibodies generated to fibrillar Aβ bound to the Aβ_{1-8} sequence, they exhibited a much greater affinity for fibrillar Aβ. The results from the fibril binding assay and the ELISA agreed that the peptides in the fibrils were ~500 times more effective at binding the antibodies than free peptides, even without correcting for steric hindrance, which limits the number of antibody molecules that can bind to the fibrils. How many IgG molecules could bind to the fibril depends on the size of the epitope and its distribution on the fibril as well as the size of the IgG molecule. In recent models of the Aβ fibril, the molecules are oriented in register in β-sheets with their amino termini extended outward in the medium (*19*, *20*). Our antibody binding results agree with these models in that the antifibrillar Aβ antibodies bind to the amino termini, whereas the antibodies directed to the carboxyl-terminal epitope, e.g., R165, do not bind to fibrillar Aβ. In one model, the fibril consists of a laminate of six of these sheets separated by 1.0 nm, with a molecular spacing of 0.5 nm (*19*). If the epitope size were similar to that of lysozyme (2.0 nm × 3.0 nm) (*21*), the antibody would cover ~20 Aβ monomers. Thus, the microscopic binding constant of the antibody might be at least 20-fold larger than the constant calculated using the total Aβ concentration.

Hypothetically, the antifibrillar IgGs might have bound more tightly to Aβ fibrils than to monomeric Aβ because of the propinquity of a second site for the bivalent IgG. This phenomenon, termed "monogamous bivalency" (*22*), has been observed (*23*); nevertheless, three lines of evidence do not support this explanation. First, the antibodies did not bind more tightly to agarose-bound Aβ_{1-16} than to the free peptide; therefore, their enhanced affinity for the fibril did not result from the incorporation of multiple epitopes into a macromolecular structure. Second, antibodies to two other Aβ epitopes, those of mAb 6E10 and 4G8, did not bind more strongly to fibrillar Aβ; therefore, the tighter binding was not a property common to Aβ fibrillar epitopes. Finally, the monovalent Fab fragments of the antifibrillar antibodies also preferentially bound to fibrillar Aβ; therefore, the tighter binding did not result from the antibody simultaneously binding to two epitopes.

Two other hypotheses might explain the preferential binding of the antibodies to the fibrils. First, the fibrillar antibodies might preferentially bind to a conformation of Aβ_{1-8} that is stabilized in fibrillar Aβ. Second, epitopes on adjacent peptides might contribute to the binding energy by interacting with other sites in the antibody complementarity-determining region. It has long been known that antibodies raised to intact proteins may relatively weakly bind to linear peptides derived from the protein. For instance, an antibody to native staphylococcal nuclease bound to a cyanogen bromide-generated fragment comprising the carboxyl-terminal one-third of the protein; however, in this case, the antibody's affinity for the peptide was only 1/5000 of its affinity for the intact protein (*24*). Other examples of antibodies preferentially binding to peptide sequences in proteins have been reported; for instance, antibodies raised to myoglobin recognize a specific myoglobin peptide, but they preferentially bind to the intact protein (*25*, *26*). Antibodies against intact proteins generally bind to a linear epitope as well as to additional residues near the epitope. These additional interactions supposedly are created by somatic mutations in the light and heavy chain genes as the initially selected B cell clones mature to produce high-affinity antibodies (*27*). Fibrillar β-amyloid might resemble an intact protein in the antigenic response it produces. Thus, antibodies directed to residues 1−8 might be matured to high-affinity antibodies that bind to secondary epitopes in the amino-terminal sequences of adjacent Aβ molecules.

Various studies indicate that, in the fibrils, the amino-terminal domain of Aβ is relatively disorganized (*28*). In a nuclear magnetic resonance study of ^{13}C-labeled Aβ_{1-40}, the ^{13}C line widths of labeled atoms in Ala-2, Phe-4, and Gly-9 were greater than those of labeled atoms in residues between Val-12 and Val-39 and resemble the line widths observed in unfibrillized Aβ (*29*). This conclusion appears to conflict with our first hypothesis that the antifibrillar antibodies bind to a specific conformation of the amino-terminal domain. However, the antibodies bound to only to a few percent of the Aβ monomers in the fibril; therefore, they would select epitopes that were stabilized in high-affinity conformations. If these structures comprised a minority of the amino-terminal sequences, they would contribute to the observed ^{13}C line width increase by placing the atoms in different environments

but would not be abundant enough to be resolved as separate peaks. A protease sensitivity study found that ~20% of the amino-terminal domain of fibrillar β-amyloid resisted proteolysis (*30*). This finding is not inconsistent with the hypothesis that a minor fraction of the Aβ amino-terminal domain exists in a fixed conformation. The results of the competition study (Table 5) indicated that the antibodies had a range of affinities for the fibrillar epitopes. This result would be consistent with a disordered structure where the epitope adopted several conformations (although it may also indicate heterogeneity in the antibody population).

Both parenchymal plaques and cerebrovascular amyloid deposits bound the antifibrillar Aβ antibodies, but only the latter displayed affinity for the antibody in the presence of a blocking peptide. This observation may relate to the differences between the peptide compositions of the two types of amyloid. The Aβs of neuritic amyloid cores predominantly terminate at Ala-42, whereas cerebrovascular amyloid Aβs end at Val-40 (*31*). Of more relevance to this study is the finding that very few neuritic core Aβ molecules begin at Asp-1, which is essential for high-affinity binding of the antifibrillar Aβ antibodies. The great majority of these peptides begin with Glu-3, pyroGlu-3, Phe-4, Glu-11, and pyroGlu-11 (*31*−*33*). It is not known whether these peptides are incorporated randomly into fibrils or are located in specific regions of the plaque. Two hypotheses might explain the relatively weak affinity of neuritic amyloid for the antifibrillar Aβ antibody. First, fibrils formed from truncated amyloid peptides may possess weak affinity for the antibody. Alternatively, since the enhanced affinity of fibrillar $A\beta_{1-40}$ or $A\beta_{1-42}$ is a colligative property of the peptide, it may be that only in homogeneous aggregates is the epitope constrained to the high-affinity conformation or are determinants on adjacent molecules available. Thus, the weak and reversible staining of neuritic plaques would result from the binding of the antibody to isolated full-length Aβ molecules, with an affinity similar to that of the monomeric peptide. If this hypothesis withstands further tests, the antifibrillar Aβ antibodies may prove to be useful for the selective identification of "full-length" fibrillar β-amyloid deposits.

When transgenic mice that express human β-APP are immunized with fibrillar human $A\beta_{1-42}$, they generate antibodies, which, *in vivo*, mediate the dissolution of amyloid plaques (*5*, *34*). These antibodies bind to an epitope containing human Aβ residues 4−10 (*35*), a sequence that differs from the mouse Aβ sequence at residues 5 and 10. It is the epitope recognized by mAb 6E10 used in the study presented here (our unpublished results). Mice generate antibodies to this epitope regardless of whether the injected peptide is fibrillar or monomeric. Whether the mouse antibodies generated to fibrillar human Aβ preferentially bind to fibrillar Aβ has not been reported. In an *in vitro* experiment, antisera generated to fibrillar $A\beta_{1-42}$ convert fibrillar Aβ to an amorphous aggregate (*36*). Another study reported that monoclonal antibodies directed to human Aβ residues 3−6 could disaggregate fibrillar $A\beta_{1-42}$ (*37*). Our antibodies to fibrillar $A\beta_{1-42}$ did not disaggregate the fibrils but, rather, thoroughly coated them, as might be expected from thermodynamic considerations. True antifibrillar human Aβ monoclonal antibodies also have been isolated from mice (*38*). These antibodies recognize not only fibrillar Aβ but also several other types of amyloid.

Passive immunization with antibodies generated to monomeric Aβ lowered the brain amyloid burden in transgenic APP mice (*7*). Although the mechanism of this process is unknown, the clearance occurs within a few days. The dissociation of amyloid fibrils is exceedingly slow under physiological conditions; therefore, the simple displacement of the equilibrium between the monomeric and fibrillar states cannot be involved in this clearance process.

After the completion of this study, a description of the immune response of humans to fibrillar β-amyloid appeared (*39*). Like the rabbit antibodies described in our study, the human antisera did not detect Aβ on immunoblots but did bind to amyloid deposits in tissue sections. Several of the immunized AD patients developed severe meningeal inflammation. This result might be explained by our finding that antifibrillar Aβ antibodies possess enhanced affinity for vascular amyloid deposits, since vascular amyloid deposits frequently occur in meningeal vessels (*40*), and antibody−amyloid complexes might be expected to initiate an inflammatory response (*41*).

As a therapy for AD, passive immunization with humanized monoclonal antibodies might be preferable to direct immunization with fibrillar Aβ, if an epitope that did not trigger the inflammatory response could be found. Some studies suggest that Aβ oligomers are more neurotoxic than fibrillar Aβ (*42*−*44*). Furthermore, the Aβ oligomers may be in dynamic equilibrium with Aβ monomers; therefore, passive immunization with an antibody directed to an epitope in the Aβ monomer might eliminate Aβ oligomers. Most of the anti-human Aβ antibodies thus far generated in mice bind to a sequence (residues 4−10), which is shared with cell-associated β-APP, circulating β-APP_s, and the cell-associated β-secretase-generated β-APP carboxyl-terminal fragment. The consequences of treating humans with these antibodies are unknown. A better choice of epitope might be the carboxyl-terminal sequence of $A\beta_{1-42}$ (MVGGVVIA). This sequence is buried within Aβ fibrils; consequently, antibodies directed to it will not bind to Aβ fibrils and so are less likely to initiate a complement-mediated inflammatory response.

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