Chromosome 13 dementias, familial British dementia (FBD) and familial Danish dementia (FDD), are associated with neurodegeneration and cerebrovascular amyloidosis, with striking neuropathological similarities to Alzheimer’s disease (AD). Despite the structural differences among the amyloid subunits (ABri in FBD, ADan in FDD, and Aβ in AD), these disorders are all characterized by the presence of neurofibrillary tangles and parenchymal and vascular amyloid deposits co-localizing with markers of glial activation, suggestive of local inflammation. Proteins of the complement system and their pro-inflammatory activation products are among the inflammation markers associated with AD lesions. Immunohistochemistry of FBD and FDD brain sections demonstrated the presence of complement activation components of the classical and alternative pathways as well as the neo-epitope of the membrane attack complex. Hemolytic experiments and enzyme-linked immunosorbent assays specific for the activation products iC3b, C4d, Bb, and C5b-9 indicated that ABri and ADan are able to fully activate the complement cascade at levels comparable to those generated by Aβ1–42. ABri and ADan specifically bound C1q with high affinity and formed stable complexes in physiological conditions. Activation proceeds ~70–75% through the classical pathway while only ~25–30% seems to occur through the alternative pathway. The data suggest that the chronic inflammatory response generated by the amyloid peptides in vivo might be a contributing factor for the pathogenesis of FBD and FDD and, in more general terms, to other neurodegenerative conditions.

The classic hallmark lesions of Alzheimer’s disease (AD), cerebral senile plaques and neurofibrillary tangles (NFTs), have been known for nearly a century. During the past two decades, a wide range of inflammatory markers, typically absent or significantly reduced in the normal elderly population, were reported in AD brains (1), and accumulating evidence suggests that sustained brain inflammation might be an essential cofactor in AD pathogenesis (2, 3). In this sense, immunological factors and inflammation mediators, including complement proteins and pro-inflammatory peptides generated at different stages of complement activation (4) as well as various cytokines (5), have been implicated in accelerating the progression of AD.

The complement system is a highly regulated, powerful effector mechanism of the immune system that destroys and clears deleterious substances. It is composed of more than twenty proteins that become sequentially activated in a proteolytic cascade. Originally, activation of the complement system was thought to occur only by binding of immune complexes to C1q, the recognition component of the classical pathway. However, it became then evident that the complement system can directly be activated, in the absence of antibody, by interaction of certain foreign molecules with C3 (alternative activation pathway), C1q (antibody-independent classical activation pathway), or by specific lectins on the surface of certain microorganisms (lectin activation pathway) (3, 6, 7).

The first step in the classical complement pathway involves the binding of an activator to C1q resulting in the subsequent conversion of the serine proteases C1r and C1s to their active forms and, in turn, in the activation of C4, C2, and then C3. The alternative pathway differs from the classic pathway in that activation begins at the level of C3 and involves factors B and D and Properdin. Proteolytic modification of C3 by either pathway leads to the cleavage of C5 and the incorporation of C6, C7, C8, and multiple molecules of C9 resulting in the formation of the membrane attack complex (MAC), C5b-9, a transmembrane channel capable to produce cell lysis (8).

Activation-derived proteins of both the classical and alternative pathways have been demonstrated in association with AD lesions by numerous groups (3). We investigated the complement activation cascade in chromosome 13 dementias, two hereditary conditions, familial British dementia (FBD) and familial Danish dementia (FDD), that are also associated with performance liquid chromatography; MAC, membrane attack complex; NFTs, neurofibrillary tangles; NHS, normal human serum; PBS, phosphate-buffered saline; Tricine, N,N,N’-pentaethylene tetramine N,N’-bis(carboxymethyl)ethylglycine; PVDF, polyvinylidene difluoride; CAPS, 3-(cyclohexylamino)propanesulfonic acid; CNS, central nervous system; Ab, antibody.
neurodegeneration and amyloid deposition in the central nervous system. FBD has been described in members of three British pedigrees and is characterized clinically by dementia, cerebellar ataxia, and spastic paraparesis with the disease onset typically in the fourth to fifth decade of life and death occurring some ten years later (9). FDD is a disease associated with a single Danish family with the onset of cataracts in patients before the age of thirty. Affected family members subsequently develop sensory hearing loss, cerebellar ataxia, psychosis, and dementia leading to death between the ages of fifty and sixty years (10). The neuropathology in both diseases is remarkably similar to that seen in AD, including cerebral amyloid angiopathy, pre-amyloid lesions, amyloid plaques of various types, and NFTs, ultrastructurally composed of paired helical filaments with an electrondens aromatic pattern of abnormal hyperphosphorylated tau indistinguishable from that observed in AD. Activated microglia, expressing the major histocompatibility class II antigens that are characteristic of inflammatory processes, can be demonstrated around amyloid plaques and dystrophic neurites but not in pre-amyloid lesions in both FBD and FDD (10, 11) in a topographical distribution similar to that found in AD. In these disorders the deposited amyloid proteins, ABri in FBD and ADan in FDD, are proteolytic fragments of a larger precursor molecule BriPP codified by a multixenic gene BRI2 (also known as ITM2B) located on the long arm of chromosome 13 (12–14). The amyloid peptides originate as a result of two different genetic defects, namely a Stop-to-Arg mutation before the stop codon in FDD. Regardless of the nucleotide changes, the final outcome is common to both diseases: the neurodegeneration and amyloid deposition in the central nervous system.

**Chromosome 13 Dementia and Complement Activation**

**TABLE I**

| Antibody       | Dilution | Species        | Paraffin/frozen sections and pretreatment | Source |
|----------------|----------|----------------|------------------------------------------|--------|
| 5282 (anti-ADan) | 1:1000   | Rabbit anti-human | Paraffin, formic acid 99% for 10 min     | Ref. 14 |
| 338 (anti-ABri)  | 1:2000   | Rabbit anti-human | Paraffin, formic acid 99% for 10 min     | Ref. 12 |
| Anti-C1q         | 1:150    | Rabbit anti-human | Paraffin, trypsin, 15 min                | Dako   |
| Anti-C4d         | 1:1000   | Mouse anti-human  | Frozen section fixed in acetone          | Quidel |
| Anti-C5b-9       | 1:50     | Rabbit anti-human | Frozen section fixed in acetone          | Quidel |
| Anti-Bb          | 1:50     | Mouse anti-human  | Frozen section fixed in acetone          | Quidel |

**Complement Reagents**—Pooled normal human serum (NHS), C1q-depleted serum, purified C1q protein, polyclonal goat anti-C1q anti-serum, and ELISA kits for the quantitation of the activation products C4d, Bb, iC3b, and SC5b-9 were purchased from Quidel, Inc., Mountain View, CA. An EZ diagnostic kit for the assay of total complement activation (CH50) was obtained from Diamedex Corp., Miami, FL.

**Immunohistochemistry Reagents**—Antibodies immuno-reactive with ABri (Ab 338) and ADan (Ab 5282) molecules were raised in New Zealand White rabbits by immunization with synthetic peptides comprising positions 22–34 of the respective amyloid molecules, as previously described (12, 14). Polyclonal rabbit anti-C1q was purchased from Dako (Carpenteria, CA), polyclonal anti-C5b-9 (neo-epitope), and monoclonal antibodies anti-C4d and anti-Bb were obtained from Quidel, Inc.

**Immunohistochemical Studies**

Sequential paraffin sections from the hippocampus, including the dentate fascia were used for C1q immunostaining in five cases with FBD (mean age at death, 62.4 years; range, 59–68 years) and three cases with FDD (mean age at death, 53.7 years; range, 43–60 years). For the immunohistochemical detection of C4d, C5b-9, and Bb, sequential frozen sections, fixed in acetone, were used. Sections from four controls (mean age at death, 64.3 years; range, 33–88 years) without a neurological disease and seven cases with Alzheimer’s disease (mean age at death, 78.2 years; range, 63–92 years) were also stained in a similar manner. Depending on the antibody, a number of different pretreatments were employed for the paraffin-embedded tissues, which are detailed in Table 1. After pretreatment, the tissue sections were incubated with the pertinent primary antibodies, followed by sequential incubations with either biotinylated anti-mouse or anti-rabbit secondary antibodies, as appropriate, and the corresponding ABC complex (Dako, Denmark). Color was developed using diaminobenzidine/H₂O₂ followed by hematoxylin counterstaining.

**Characterization of Amyloid Peptides**

**Structural Analysis**—Secondary structure was assessed by circular dichroism spectrometry in the far-UV range (190–250 nm) at 24 °C using a Jasco J-720 spectropolarimeter (Jasco, Tokyo, Japan) as previously described (19).

**Peptide Solubilization and Aggregation**—The different ABri and ADan peptides were solubilized in 10 mM NaHCO₃, pH 9.6, aliquoted, and lyophilized. Before use, each aliquot was dissolved in distilled water at a concentration of 1 mg/ml and immediately used in the complement activation assays. ABri–42 was dissolved in distilled water, and aliquots of concentrated PBS to reach a final concentration of 150 mM NaCl. Solutions of 1 mg/ml were allowed to aggregate for 7 days at 37 °C, as described (19).

**HPLC Purification**—Fractions enriched in ABri and ADan monomers were obtained via reverse-phase HPLC (Appli Biosystems, 130A Separation system) on a 0.21 × 25-cm Vydac C4 T502 micro bore column (Vydac, Hesperia, CA), using isocratic conditions at 25% acetonitrile in 0.1% trifluoroacetic acid for 10 min followed by a 30-min linear gradient of 25 to 40% acetonitrile in 0.1% trifluoroacetic acid at a flow rate of 200 μl/min. Fractions were collected in accordance to the absorbance at 220 nm.
Western Blot Analysis—The degree of oligomerization of various peptides was assessed by Western blot. Aliquots of 100–150 ng of each peptide freshly dissolved or after 1-h incubation in either PBS, pH 7.4, or Tris-HCl, pH 7.4, containing 2.5 mM Ca²⁺, 1 mM Mg²⁺, 75 mM NaCl were separated in 16% Tris-Tricine SDS-PAGE gels and transferred for 45 min at 400 mA to polyvinylidene difluoride (PVDF) membranes (Immobilon-P, Millipore, Bedford, MA) using CAPS (3-cycloexylaminopropanesulfonic acid, Sigma, St. Louis, MO) buffer, pH 11, containing 10% methanol. After transference, membranes were blocked with 5% nonfat milk in PBS, pH 7.4, containing 0.1% Tween 20 and incubated with the corresponding primary antibody (anti-ABri, antibody 338 (1 μg/ml IgG); anti-ADan, antibody 5282 (2 μg/ml IgG)) for 3 h at room temperature followed by horseradish peroxidase-conjugated anti-rabbit immunoglobulins (Amer sham Biosciences, Piscataway, NJ) at a dilution of 1:100 for the quantitation of C4d and Bb, 1:300 for iC3b, and 1:200 for SC5b-9 prior to the analysis by ELISA. The formation of the ABri/ADan-C1q complexes was also analyzed in 1% agarose gels in 75 mM veronal buffer, pH 8.6, containing 2 mM sodium lactate and contact-transferred to PVDF membranes. Transferred proteins were stained for 1 min with 0.125% Coomassie Blue R-250 in 47% methanol, membranes were destained and extensively washed with water, and the bands of interest were excised and subjected to N-terminal sequencing on a Procise 494 protein sequencer (Applied Biosystems, Foster City, CA).

Immunohistochemistry—Fig. 1A shows that antibody 338 specific for the C-terminal region of ABri strongly labels amyloid plaques and amyloid-laden blood vessels, including affected small arteries, arterioles, and capillaries in FBD. Both the vascular and parenchymal amyloid lesions were strongly labeled with anti-C1q (B), anti-C4d (C), anti-CS5b-9 (neoe- epitope) (D), and anti-Bb (E) in a staining pattern similar to that seen for ABri immunohistochemistry. Diffuse deposits, defined as Congo Red and Thioflavin S-negative or weakly positive ABri parenchymal lesions (11), were only faintly stained (not shown). The FDD lesions, mainly vascular amyloid and parenchymal amyloid plaques, were highlighted by antibody 5282 recognizing the C-terminal end of ADan (Fig. 1F). The anti-complement antibodies (anti-C1q (G), anti-C4d (H), anti-CS5b-9 (neoe-epitope) (I), and anti-Bb (J)) labeled amyloid fibrillar arteriolar and capillary walls and capillaries. The immunoreactivity with these antibodies was weak or absent in the parenchymal lesions, which have been shown to be composed primarily of protein in pre-amyloid conformation (Congo Red and Thioflavin S-negative or weakly positive deposits) (10). Immunohistochemical analysis of AD cases, which were used as positive controls, showed labeling of both vascular Aβ amyloid deposits and parenchymal Aβ-positive plaques by anti-C1q, anti-C4d, and anti-CS5b-9 antibodies. Smaller numbers of the Aβ-positive lesions were also stained with anti-Bb antibody (not shown). Two of the normal controls were entirely negative for complement proteins, whereas in the two normal control cases with ages of 81 and 88 years, occasional Aβ-positive plaques immunoreacted only with the anti-C1q antibody (not shown).

RESULTS

Chromosome 13 Dementia and Complement Activation

The formation of the ABri/ADan-C1q complexes was assessed via amino acid sequence analysis. 10 μg of C1q in TBS was incubated with 20 μg of either ABri or ADan peptides for 1 h at 37 °C. The resulting complexes were separated by electrophoresis on 1% agarose gels in 75 mM veronal buffer, pH 8.6, containing 2 mM sodium lactate and contact-transferred to PVDF membranes. Transferred proteins were stained for 1 min with 0.125% Coomassie Blue R-250 in 47% methanol, membranes were destained and extensively washed with water, and the bands of interest were excised and subjected to N-terminal sequencing on a Procise 494 protein sequencer (Applied Biosystems, Foster City, CA).

Solid-phase Binding Assays: Binding of C1q to Immobilized Amyloid Peptides

ELISA microtiter wells were coated for 2 h at 37 °C with ABri1–34, ADan1–34, ABr1–23, and Aβ1–42 peptides at a concentration of 400 ng/0.1 ml of 0.1 mM NaHCO₃, pH 8.6. After blocking with 1% bovine serum albumin for 1 h at room temperature, variable concentrations (0–20 mM in TBS) of C1q were incubated with the peptide-coated wells for 1 h at room temperature. Bound C1q was detected with goat polyclonal anti-C1q antisera (1:1000 in TBS containing 0.1% Tween 20 and 0.1% bovine serum albumin (TBST)) followed by alkaline phosphatase-labeled swine anti-goat IgG (1:5000 in TBST, BioSource International, Camarillo, CA). The reaction was developed with p-nitrophenyl phosphate in diethanolamine buffer (BioRad, Richmond, CA) stopped with 0.4 M NaOH, and the absorbance at 405 nm was quantitated in a Spectracon ELISA microplate photometer (Packard, Meriden, CT). Binding data were analyzed by non-linear regression using GraphPad Prism (GraphPad Software, Inc., San Diego, CA).
values that reached a minimum of 18% for ABri1–34 and 23% for ADan1–34 at the maximal concentration tested (final concentration: 500 µg/ml, −120 nmol/ml). The consumption of complement induced by the ABri and ADan peptides was also similar to that of 7-day-aggregated Aβ1–42 that, under the conditions tested, reduced the complement activity to 23% of the values obtained in the absence of peptide, in agreement with previously published data (23). As a positive control for the classical pathway activation, Fig. 2A also depicts the decrease in complement activity induced by incubating NHS with aggregated IgG, a known activator of the classical pathway. As it can be deduced from the data, aggregated IgG is a more potent activator of the complement system than ABri and ADan, achieving similar levels (∼30% of the original complement activity) at a much lower molar ratio (500 µg/ml, 3.3 nmol/ml), as described (24). No differences in activation were observed among ABri or ADan peptides bearing different post-translational modifications, i.e. N-terminal glutamate or pyroglutamate, oxidized cysteines 5 and 22, or peptides containing serine residues replacing cysteines 5 and 22 (not shown).

In view of the values obtained in the CH50 hemolytic assay, we quantitated the in vitro formation of the activation products C4d, iC3b, and SC5b-9 via specific capture ELISAs employing specific monoclonal antibodies directed against neo-epitopes originated in the activation-derived fragments (8). The C4d levels generated by incubation of NHS with ABri and ADan peptides are shown in Fig. 2B. C4d, together with C4c, are the physiological degradation products of C4b as a result of proteolytic cleavage by the complement regulatory protein Factor I in the presence of either C4-binding protein or complement receptor 1 (CR1) (25, 26). The ability of both amyloid peptides to generate C4d in a dose-response manner indicates that activation of the complement system occurred through C1 activation, because the conversion of the proenzyme C1s is solely needed to produce the C4 cleavage. Proteolytic fragments of C3 (Fig. 2C) and the soluble terminal complex SC5b-9 (Fig. 2D), on the
other hand, may originate by activation of both the classical and the alternative pathways. Quantitation of iC3b was used to estimate C3b generation, because, once produced, C3b is rapidly inactivated by Factor I in conjunction with either Factor H or CR1 as cofactors (26, 27). As indicated in Fig. 2C, both ABri and ADan were able to generate iC3b in a dose-dependent manner. The assembling of SC5b-9 shown in Fig. 2D, determined by a widely used standard method to assess complement activation (24, 28), confirmed the ability of ABri and ADan to in vitro trigger the complement cascade to full completion, including the terminal stages. The terminal cytolytic C5b-9 complex generated by the assembly of C5b, C6, C7, C8, and multiple C9 molecules in the absence of a target membrane (as in the case of these experiments) binds to the naturally occurring serum S protein (vitronectin) resulting in the formation of the soluble, non-lytic form of the MAC, SC5b-9. The data in Fig. 2 also indicate that incubation of NHS with ABri and ADan peptides results in the production of activation-generated fragments of the complement proteins C4 and C3 as well as the complex SC5b-9 to levels comparable to those induced by incubation with aggregated Ap1–42, in agreement with the CH50 findings (Fig. 2A). In addition, the levels of SC5b-9 produced by incubation of Ap1–42 with NHS confirm previously reported data acquired under similar experimental conditions (29). Similarly to the results obtained using the hemolytic assay, aggregated IgG produced a comparable level of activation-generated fragments at a lower molar ratio. In the presence of EDTA, a chelator of both Ca\(^{2+}\) and Mg\(^{2+}\) ions essential for the activation of the complement cascade, none of the activation products C4d, iC3b, and SC5b-9 were generated, as expected (not shown).

**Alternative Pathway of Complement Activation**—The ability of the ABri and ADan peptides to trigger the alternative pathway was assessed by measuring the generation of Bb by ELISA, a method that provides a direct, specific indication of alternative pathway activation (30). As shown in Fig. 3A both ABri and ADan are able to induce the production of Bb to similar levels as Ap1–42 following incubation with NHS. These values are significantly different from those originated spontaneously by incubation of NHS with buffer in the absence of amyloid peptides, which are also shown in the figure for comparison purposes. Although the Bb levels that result from incubation with the amyloid peptides are similar to those reported in certain infectious pathological conditions that take place with activation of the alternative pathway (31), they are significantly lower than those induced by incubation of NHS with CVF, a potent activator of the pathway. CVF, a functional analog of human C3b, forms stable C3/C5 convertases that are not inactivated by the plasma regulatory proteins factor H and factor I. It binds factor B rendering it available for cleavage by factor D and initiating in this way a potent alternative pathway activation.

To estimate the contribution of the alternative pathway to the total activation of the complement system, we quantitated the levels of SC5b generated by incubation of the peptides with NHS under conditions in which both pathways are activated (presence of Ca\(^{2+}\) and Mg\(^{2+}\) ions) and compared them with those originated by activation of the AP only (presence of Mg\(^{2+}\) EGTA or substitution of NHS by C1q-depleted serum). As shown in Fig. 3B, whereas addition of EDTA resulted in complete absence of SC5b-9 corroborating that activation of the system is required to produce SC5b-9, the incubation with Mg\(^{2+}\)/EGTA reduced the levels to an average of 25%, 32%, and 31% of the total values of SC5b-9 for ABri, ADan, and Ap1–42, respectively. Corroborating these results, incubation with C1q-depleted serum reduced SC5b-9 levels to similar values (an average of 35% for ABri, 24% for ADan, and 28% for Ap1–42). Therefore, the classical pathway appears to be the major route of activation of the complement system for all the amyloid peptides tested here, representing 70–75% of the total activation, whereas the alternative pathway accounts for the remaining 25–30%.

**Complex Formation with C1q**—All the above data strongly suggested that the trigger of the complement cascade by ABri and ADan mainly proceeds through the classical activation pathway, most likely through a direct binding interaction of the peptides to C1q. In a set of solid-phase binding experiments, incubation of either ABri-, ADan-, or Ap1–42-coated microtiter wells with increasing concentrations of C1q (0–20 nM) resulted in a dose-response relationship that reached saturation (Fig.

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**FIG. 3. Activation of the alternative pathway by ABri and ADan peptides.** A. generation of Bb. To determine activation of the alternative pathway, NHS was incubated for 45 min at 37 °C with ABri, ADan, and Ap1–42 peptides at a concentration that resulted in maximum classical pathway activation (final concentration, 500 μg/ml). The concentration of Bb was subsequently assessed by ELISA employing Bb quantitation kit (Quidel, Inc.) as described under “Experimental Procedures.” For comparison purposes the levels of Bb, produced by incubation of NHS with CVF and in the absence of any activators, are also indicated. Results show mean ± S.D. of three independent experiments. B. generation of SC5b-9. To estimate the contribution of the classical and alternative pathways to the total complement activation, ABri, ADan, and Ap1–42 peptides (500 μg/ml) were incubated with NHS: (i) under conditions in which both pathways are active (presence of Ca\(^{2+}\) and Mg\(^{2+}\) ions), (ii) under conditions in which only the alternative pathway may be activated (presence of Mg\(^{2+}\) ions/EGTA), and (iii) in the presence of EDTA in which no complement activation takes place. Additionally the peptides were incubated in the presence Ca\(^{2+}\) and Mg\(^{2+}\) ions but substituting NHS with C1q-depleted serum to corroborate the activation induced by the alternative pathway. In all cases the concentration of SC5b-9 produced was analyzed by ELISA. Results represent mean ± S.D. of two independent experiments. Open bar, EDTA; dotted bar, Ca\(^{2+}\)Mg\(^{2+}\); checked bar, Mg\(^{2+}\)/EGTA; black bar, C1q-depleted serum.
The ADan peptides in the presence of Ca\(^{2+}\) at 405 nm was quantitated. Data represent the means and S.D. of 3 independent experiments. The dissociation constants for ABri and ADan are 1.9 ± 0.4 nM and 1.2 ± 0.3 nM, respectively, within the same range to that of Aβ1–42 (2.4 ± 0.5 nM). These high affinity interactions correlated well with the pronounced capability of the amyloid peptides to activate the classical complement cascade in hemolytic assays (Fig. 2A). In addition, these high affinity values suggested that the ABri or ADan peptides likely form complexes with C1q. In vitro complex formation was performed in a 50-fold molar excess of the peptides, to assure that most (if not all) the C1q was part of the complex. To visualize the final product, we took advantage of the characteristic cathodic electrophoretic mobility of C1q in agarose gels and the mostly anodic migration of the peptides in the same system. As indicated in Fig. 4B, Coomassie blue staining of the PVDF-transferred material shows C1q (lane 2) with its characteristic cathodic mobility, matching the very end of the gamma region in the human serum profile shown for comparison purposes (lane 1). When complexed with ABri or ADan, the electrophoretic mobility shifted noticeable toward more anodic positions (lanes 3 and 4, respectively). To corroborate the formation of the complexes, the bands were excised and subjected to limited N-terminal sequence analysis. Three identifiable sequences were recovered in each case (see Fig. 4B for details); two sequences corresponded to the A and C chains of C1q while the other matched the N terminus of the ABri or the ADan peptides. No sequence was retrieved for the B chain of C1q known to contain a blocked N-terminal glutamine residue (pyrrolidone carboxylic acid) (protein ID no. P02746, Swiss-Prot database of the Swiss Institute of Bioinformatics, available at www.expasy.org).

**Mapping the Complement Activation Activity of ABri and ADan**—To map the complement activating activity to a specific region of the ABri and ADan molecules, we employed synthetic peptides representing different regions of the amyloid subunits in the CH50 hemolytic assay. The peptides tested consisted of the common N-terminal region of the amyloid subunits (ABri/ADan1–23), as well as the C-terminal fragments from both molecules ABri24–34 and ADan24–34. Because the molecular mass of the peptides corresponding to different regions of the molecules differ significantly, identical molar concentrations of the peptides were used in the experiments. For comparison purposes, the molar concentrations of the full-length ABri and ADan peptides used were equivalent to the peptide concentrations displayed in Fig. 2 (e.g., 12, 48, and 120 nmol/ml, corresponding to 50, 200, and 500 μg/ml, respectively). As indicated...
in Fig. 5, both C-terminal fragments lack the ability to activate and consume complement proteins, whereas the common N-terminal region of the amyloid molecules retains the functional activity (27% at a concentration of 120 nmol/ml for ABri/ADan1–23 compared with 18% for ABri and 23% for ADan).

The localization of the complement-activating site to the common N-terminal fragment correlates with the similar behavior of both ABri and ADan peptides in inducing almost identical levels of complement activation. In addition, the mapping of the complement-activating activity to the ABri/ADan1–23 region correlates with the capacity of the N-terminal peptide to bind C1q with almost identical affinity as the full-length peptides (ABri1–23 Kₐ, 1.45 ± 0.3 nM).

Peptide Oligomerization and Complement Activation—An important element in the activation of the complement system by amyloid peptides seems to be directly related to their degree of oligomerization. In our experimental conditions, the activation of the classical pathway takes place with freshly solubilized peptides, reaching complement activation values similar to those obtained with 7-day-aggregated Aβ1–42. To clarify this issue, we examined the secondary structure and the oligomerization state of ABri and ADan peptides immediately after solubilization and after 45-min incubation at 37 °C, the incubation time required for the hemolytic assays and the ELISA experiments. As shown in Fig. 6A, full-length ABri and ADan rendered circular dichroism spectra compatible with high β-sheet content, whereas the respective C-terminal fragments (ABri24–34 and ADan23–34) showed a random coil configuration suggesting a role for β-sheet structure in the complement activating ability of the peptides. Both peptides were already heavily aggregated at the starting conditions (Fig. 6, B and C, lanes 1), although the oligomerization was even more evident after the incubation required for all the complement assays (B and C, lanes 2). Similar results were observed with the N-terminal peptide ABri/ADan1–23 (not shown). These findings indicate that the ABri/ADan peptides have a higher tendency to form oligomers than Aβ1–42 and suggest a faster aggregation kinetics. Of note, although both peptides share a similarly high β-sheet content, ABri (B) seems to aggregate even faster than ADan (C) as indicated by the presence of higher molecular mass oligomers under the same experimental conditions. Despite this apparently different aggregation kinetics, both full-length peptides trigger complement activation to practically the same extent suggesting that other factors besides the degree of oligomerization play a role in the activation mechanism.

DISCUSSION

Although viewed for many years as an immune-privileged organ, the CNS contains many immune system components among them proteins of the complement system that are synthesized by astrocytes, microglia, and neurons. Whereas the pathogenic role of complement is well recognized in systemic disorders, its contribution to neurodegenerative diseases has only recently emerged. One of these pathologic entities in which the activation of the complement system was studied in more detail is Alzheimer’s disease, the most common form of human dementia. Proteins of the classical pathway and their activation fragments, namely C1q, C3b, and C4b, as well as the terminal MAC have all been identified in senile plaques, cerebrovascular amyloid deposits, and in association with dystrophic neurites and neurofibrillary tangles, indicating that the complete cascade can be fully triggered in vivo (4, 5, 23, 32–35).

Of note, the presence of C5b-9 was demonstrated in AD but not in non-demented elderly control cortices (36), suggesting that complement-induced injury and the chronic inflammation resulting from the system activation may be at least partially responsible for the progression of the disease. Although originally not described, both mRNAs and proteins of the alternative pathway have more recently been demonstrated in AD brains together with their specific activation fragments (37).

Our data demonstrate that in other unrelated neurodegenerative disorders resulting in dementia, namely FBD and FDD, complement proteins of both the classical and alternative pathways co-localize with parenchymal plaques and cerebrovascular amyloid deposits, closely resembling the findings in AD. Complement immunoreactivity in FBD and FDD was mainly associated with Congo Red/Thioflavin-positive parenchymal pre-amyloid lesions.
Activation of the classical complement pathway in an antibody-independent manner was demonstrated for various non-immune substances such as C-reactive protein, serum amyloid P component, DNA (38–40), amyloid Aβ (23), and neurofibrillary tangles (24). Aggregated Aβ peptides in vitro are able to directly activate the classical complement system both in fluid phase and immobilized onto solid matrices by binding to the recognition component C1q (23, 28, 29, 41). Our results indicate that both ABri and ADan peptides are also able to induce activation of the classical complement system through a similar mechanism. The data from the hemolytic assays, the quantitation of activation fragments by ELISA, and the immunohistochemical analysis demonstrate that both amyloid molecules can trigger the activation of the classical pathway and proceed to the terminal stages with the in vitro and in vivo generation of the terminal MAC. Their direct high affinity binding to C1q, and the corresponding formation of complexes achieved under physiological conditions strongly suggest that both peptides trigger the classical pathway of complement activation mainly through direct interaction with the recognition protein C1q.

Activation of the alternative pathway may be initiated by a variety of elements or cellular surfaces, including pathogenic bacteria, parasites, viruses, and virus-infected cells. Different studies demonstrated some degree of in vitro activation of this pathway by Aβ aggregates leading to the production of the activation fragments C3b, which remained covalently bound to the fibrillar Aβ, as well as of the alternative pathway-specific Bb fragment (29, 42, 43). The results presented here demonstrate that ABri and ADan are also able to trigger the alternative pathway resulting in the production of Bb in comparable levels to Aβ1–42. However, the classical pathway appears to be the major route of activation of the complement system accounting for ∼70–75% of the total activation as indicated by the concentration of SC5b-9 generated under specific conditions for alternative pathway activation. These findings coincide with previous reports for Aβ peptides in which 70% of the C3 convertase activity formed by incubation of aggregated Aβ1–42 with NHS originated from the classical pathway (42).

The degree of oligomerization of the amyloid peptides represents an important element in the activation of the complement system. In the case of AD, fibrillar or aggregated Aβ species activate complement in vitro, whereas non-aggregated peptides do not (28, 43). Although both Aβ1–40 and Aβ1–42 are able to trigger the activation, on a molar basis Aβ1–42 was found to be a more potent activator (41, 43), a difference that most likely reflects the ability of Aβ1–42 to aggregate more readily and at lower concentrations than Aβ1–40 (44). In vivo, complement activation components co-localize with parenchymal and vascular Aβ amyloid deposits and are almost absent in the non-fibrillar (pre-amyloid) lesions (3, 45) and in “cotton wool” plaques seen in a variant form of Alzheimer’s disease due to a deletion of exon 9 of presenilin 1 (46). In the case of FBD and FDD, components of the complement activation cascade also co-localize with fibrillar but not with non-fibrillar deposits in vivo. In vitro, ABri and ADan peptides form spontaneous β-sheet-rich structures that exhibit very fast aggregation kinetics, modifying their degree of oligomerization even after the short incubation time required for the various assays. Under these conditions, both peptides achieve complement activation values similar to those obtained with 7-day-aggregated Aβ1–42. Although both ABri and ADan are able to activate complement in vitro to practically the same extent, the in vivo accumulation of activation components in FDD parenchymal lesions is significantly lower than in FBD parenchymal deposits. This most likely reflects the fact that FDD parenchymal lesions are mainly of a pre-amyloid, non-fibrillar nature (Congo Red-negative) and thus unable to achieve high levels of complement activation, as demonstrated by the in vitro studies. Despite their similarities FBD and FDD present striking differences in their respective CNS pathology, including the more severe neocortical involvement in FDD and the nature of most of the hippocampal and neocortical lesions showing features of amyloid in FBD (11) and of pre-amyloid in FDD (10). The difference in the aggregation/fibrillization state between these two types of lesions with the concomitant difference in their ability to activate the complement system most likely accounts for the different topographical distribution of associated complement proteins. Aggregated/fibrillar deposits translate in the presence of activation-derived components in association with vascular amyloid in both diseases and with FBD parenchymal amyloid plaques, and in their absence in non-fibrillar lesions. However, the paucity of complement-derived proteins observed in the parenchymal, pre-amyloid lesions in FDD suggests that activation of the complement system may not be the only critical factor in neurodegeneration. This is also supported by similar observations in the cotton wool variant of familial AD, in which the characteristic morphological feature is the presence of plaques largely composed of Congo Red-negative pre-amyloid Aβ species unassociated with complement activation (46–48).

The importance of inflammation in neurodegenerative processes, in particular in Alzheimer’s disease, has become clear over recent years. The presence of dementia invariably correlates with the detection of inflammatory markers, activated microglia, and reactive astrocytes and increased levels of cytokines and complement products around amyloid plaques and dystrophic neurites (3). Epidemiological studies have shown that anti-inflammatory drugs reduce the risk of AD (49), whereas animal models of sustained CNS inflammation lose cholinergic nerve cells in the hippocampus and exhibit memory and learning impairments (50, 51). These findings, together with the in vitro demonstration that complement activation can lead to cell death in both rat hippocampal and neuronal cell lines (52, 53), point to the importance of chronic inflammation in the pathogenesis of AD dementia. Supporting this notion, complement activation products and inflammatory mediators have also been found in Down’s syndrome in association with Aβ amyloid deposits (54) and in animal models of AD (55). Immune and inflammatory responses in the CNS are also observed in other chronic and acute neurological conditions, including multiple sclerosis, myasthenia gravis, head trauma and stroke, as well as in animal models of some of these disorders (3, 7, 56, 57). Complement activation proteins have been also demonstrated in association with Lewy and Pick bodies in Parkinson and Pick’s disease, respectively (58, 59), with dystrophic neurites and early stage extracellular neurofibrillary tangles in the Parkinsonism dementia complex of Guam (60), with clusters of degenerating axons in amyotrophic lateral sclerosis (61) as well as around immunoglobulin light chain (AL) and transthyretin (TTR) amyloid deposits in peripheral nerves in both acquired and hereditary neuropathy (51, 62). In Creutzfeldt-Jacob and Gerstmann-Strassler-Scheinker diseases (63), complement activation products have been found associated with amyloid plaques, and it has been recently demonstrated that the complement system plays a role in the early prion pathogenesis in transmissible spongiform encephalopathies (64, 65). In this case, follicular dendritic cells participate in the prion replication before the infective agent moves through the nerves into the spinal cord or brain stem, and finally into the brain. Mice bearing deficiencies of either one of the early complement factors or complement receptors present significant delays in both the onset of disease symptoms and...
The splicing accumulation of the pathological prion protein following injection of infective scrapie strains indicates that activation of complement is most likely involved in the initial trapping of prions in lymphoreticular organs.

The activation of the complement system demonstrated in all these neurological diseases and the concomitant generation of opsonins and anaphylatoxins that drive numerous inflammatory mechanisms, including scavenger cell activation, chemotaxis, cytokine release, and the secretion of cytokines, chemokines, and reactive oxygen and nitrogen species (1), may contribute to some of the neuropathological features of the different disorders. Our studies suggest that the chronic inflammatory response generated by the amyloid peptides might be a contributing (although not the solely responsible) factor to the pathogenesis of FBD and FDD and, in more general terms, to other neurodegenerative disorders. Thera-...