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Different configurational states of β-amyloid and their distributions relative to plaques and tangles in Alzheimer disease

(molecular pathology/amyloid fibrils/neurodegeneration)

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ABSTRACT **Antibodies have been raised against synthetic peptides corresponding to different parts of the β-amyloid sequence. These antibodies stain different kinds of amyloid distributions in the hippocampal formation in Alzheimer disease, suggesting the existence of different states of aggregation and/or folding of β-amyloid molecules. An antibody directed against the middle region of β-amyloid stained mostly amyloid plaques without cores, whereas an antibody directed against the carboxyl-terminal region of β-amyloid stained only amyloid plaques with cores. An antiserum directed against the amino terminus of β-amyloid stained numerous tangle-bearing cells and bodies, as well as the neuritic component of plaques and neuropil threads. These antibodies, in conjunction with anti-tau antibodies, were used to demonstrate a close spatial relationship between amyloid deposits and neurofibrillary tangles.**

The presence of abundant senile plaques and neurofibrillary tangles in defined brain regions constitutes the major neuropathological characteristic of Alzheimer disease (1, 2). The mature senile plaque is composed of a dense core of extracellular amyloid fibrils surrounded by less condensed amyloid as well as by degenerating neurites, astrocytes, and astrocytic processes (3, 4). The 43-amino acid β-amyloid constitutes the major protein constituent of amyloid fibrils (5–7). It is derived from a family of precursors with the properties of transmembrane proteins; the β-amyloid is located toward the carboxyl-terminal end of these precursors, where part of it is present in the putative transmembrane region (7). Soluble derivatives of the β-amyloid precursor are produced from the membrane-associated forms by proteolytic cleavage thought to occur close to the transmembrane region (8–10). Transcripts encoding the β-amyloid precursors are ubiquitous in central and peripheral tissues (11, 12); in the central nervous system, the cellular localization of β-amyloid precursor mRNAs and proteins is neuronal (12–15).

Neurofibrillary tangles are formed intracellularly in certain classes of nerve cells and it is believed that tangle-bearing cells progressively degenerate, to form extracellular tangles (3). The two classes of tangles can be distinguished immunohistochemically (16–19). The paired helical filament constitutes the major component of the neurofibrillary tangle (20). In addition to its location in nerve cell bodies, it is also found in the neuritic periphery of the amyloid plaque and throughout the neuropil (20, 21). Recent molecular cloning and protein sequencing experiments (22–25), as well as earlier immunohistochemical studies (26–30), have established that the neuron-specific microtubule-associated protein tau is present in the paired helical filament, although the nature of the remainder of the filament remains to be determined.

Despite the progress made in characterizing components of plaques and tangles, only little is known about the pathogenesis of the two lesions and their possible relationships. However, recent work has resulted in the production of sensitive and specific antibody markers for plaques and tangles. This has led to the demonstration of a more widespread amyloid pathology than previously appreciated (31–36) and has shown the presence of various isoforms of human tau protein in neurofibrillary tangles (37).

Our work has been stimulated by a recent study from this laboratory, which has shown that some antisera raised against synthetic peptides from β-amyloid predominantly stain neurofibrillary tangle-bearing cells and not amyloid deposits (19, 38). Faced with this unexpected observation, we decided to examine the question further by using double-labeling immunohistochemistry to investigate the topographical relationships between β-amyloid and tau protein-containing neurofibrillary tangles.

This is the first of two papers reporting the results of this study. As expected, we found that tau proteins were exclusively associated with neurofibrillary tangles, neuritic plaques, and neuropil threads. By contrast, antisera raised against different parts of the β-amyloid sequence predominantly gave exclusive staining of different kinds of amyloid distributions—i.e., either plaque cores, amyloid plaques, neuritic plaques, diffuse amyloid deposits, or, unexpectedly, tangle-bearing bodies or cells, the neuritic component of plaques, and neuropil threads. This indicates that β-amyloid sequences may exist in distinct states of aggregation and/or folding in the different lesions and that the amyloid pathology of Alzheimer disease is not limited to extracellular deposits but is also associated with tangle-bearing cells. This paper is predominantly concerned with describing the different kinds of amyloid deposits observed and with presenting a scheme for the relationship among them. It also demonstrates extracellular β-amyloid deposits closely associated with cells containing neurofibrillary tangles.

The accompanying paper (39) deals specifically with the topographical relationship between cellular β-amyloid staining and neurofibrillary tangles in intact, but affected, nerve cells. It shows that in such tangle-bearing cells the features stained by anti-amyloid and anti-tau antibodies are spatially distinct and that β-amyloid epitopes are invariably peripheral to tau protein. These different observations are then brought together in a conjectural scheme.

MATERIALS AND METHODS

Antibodies. Several polyclonal antisera and one monoclonal antibody raised against synthetic peptides derived from different parts of human β-amyloid or tau protein were used throughout this study (Table 1). 4G8 is a monoclonal antibody that is directed primarily against residues 17–24 of β-amyloid (35); BR88 and BR89 are polyclonal rabbit antisera raised, respectively, against amino acids 1–12 and 28–40 of β-amyloid (Table 1). Each peptide (500 μg), coupled to keyhole limpet hemocyanin using glutaraldehyde, was mixed 1:1 with Freund's complete adjuvant and used to immunize white

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Table 1. Antibodies directed against β-amyloid and tau protein sequences

Amino acid sequence	Amino acids	Antibodies
β-Amyloid protein*		
DAEFRHDSGYEV	1–12	BR88
LVFFAEDV	17–24	4G8
KGAIIGLMVGGVV	28–40	BR89
Tau protein†		
MAEPRQEFEVMEDHAG	1–16	BR133
GSLGNIHHKPGGG	323–335	BR135

Amino acids are designated by the single-letter code.
*The amino acid numbering is taken from Kang *et al.* (7). 4G8 was raised by Kim *et al.* (35).
†The amino acid numbering is taken from Goedert *et al.* (37).

Dutch rabbits. A booster injection was given 2 weeks after the primary immunization using 250 μg of conjugate peptide mixed 1:1 with Freund's incomplete adjuvant. Another booster injection was given after an additional 2 weeks, and the animals were bled 10 days later. BR133 and BR135 are polyclonal antisera raised against amino acid sequences common to known human tau protein isoforms (residues 1–16 and 323–335 in the numbering of ref. 37).

Immunohistochemistry. Tissue blocks containing the hippocampal formation from four patients who had died with a histologically confirmed diagnosis of Alzheimer disease (65, 72, 76, and 79 years old) and from two control patients who had died without neurological or psychiatric disorders (74 and 78 years old) were dissected <6 hr after death and were kept frozen at −70°C until use. They were immersion-fixed in 4% paraformaldehyde for 48 hr and kept in 30% sucrose in phosphate-buffered saline (PBS) at 4°C.

Sections (40 μm thick) were cut on a freezing microtome and were processed free-floating for either single or double labeling. In all experiments described here, the sections were treated with 86% formic acid for 5 min (40). Double labeling was performed as described (41), with minor modifications. For single and double labeling, the tissue sections were incubated overnight at room temperature with the primary antibody in PBS, containing 0.1% Triton X-100 and 1% bovine serum albumin (antibody dilutions: 4G8, 1:30; BR88, 1:500; BR89, 1:500; BR133, 1:200; BR135, 1:200). The sections were then washed in PBS for 1 hr, followed by a 2-hr incubation in biotinylated horse anti-mouse or goat anti-rabbit antiserum (Vector Laboratories) diluted 1:200. After a 1-hr wash in PBS, the sections were incubated for 1 hr with Avidin DH (Vector Laboratories) diluted 1:100. They were washed in PBS for 30 min and incubated for 5 min in 0.09% 3,3-diaminobenzidine containing 0.005% hydrogen peroxide. For double labeling, to avoid nonspecific staining, the sections were then washed in PBS for 30 min, incubated in avidin for 10 min, washed in PBS for 10 min, and incubated with biotin for 10 min (Vector blocking kit). After a 30-min wash in PBS, the sections were incubated overnight at room temperature with the second primary antibody, washed for 1 hr in PBS, and incubated for 2 hr at room temperature with biotinylated horse anti-mouse or goat anti-rabbit antiserum diluted 1:200. After a 1-hr wash in PBS, the sections were incubated for 1 hr with Avidin DH (1:100) and after a 30-min wash in PBS, they were incubated for 5 min with 0.02% 4-chloro-1-naphthol. The sections were washed in PBS for 10 min and mounted onto gelatin-coated microscope slides. Other combinations of chromogens, such as 3,3-diaminobenzidine and 1-naphthol, or substituting in one step peroxidase with alkaline phosphatase and using its red substrate in combination with 3,3-diaminobenzidine or 4-chloro-1-naphthol did not give satisfactory results.

It is important to note that an antibody in the double-labeling experiments is not always associated with the same color. This is because the 4-chloro-1-naphthol blue precipitate is water soluble; therefore, 3,3-diaminobenzidine that results in a brown precipitate had to be used first, even when the order of the primary antibodies was changed. Specificity of staining was confirmed by concurrent processing of tissue sections in which one or the other primary antibody was either omitted or else adsorbed for 6–12 hr at room temperature with 10 μg of the synthetic peptide per ml. Omission of the second primary antibody allowed us to determine whether the second secondary antibody cross-reacted with any of the first step antibodies or whether any residual peroxidase activity had remained from the first step and reacted with the chromogen of the second staining. The staining pattern for each antibody used in the double labeling was also tested in parallel in serial sections by single labeling.

RESULTS

Immunohistochemical Localization of Various β-Amyloid Epitopes. The antibodies used are listed in Table 1. Antibody 4G8 (raised against a central sequence of β-amyloid) (35) stained numerous amyloid deposits, many with plaque shapes, throughout the hippocampal formation from Alzheimer disease patients (Fig. 1*a*). These were mostly diffuse, with only a small number showing dense amyloid cores surrounded by more diffuse staining. Antiserum BR89 (raised against a carboxyl-terminal region of β-amyloid) also stained amyloid plaques but, in contrast to 4G8, predominantly those plaques with cores, as well as isolated cores (Fig. 1*b*). Antiserum BR88 (raised against the amino-terminal region of β-amyloid) stained a small number of diffuse amyloid deposits (Fig. 1*c*), as well as a large number of pyramidal cells and processes and objects with the characteristic appearance of extracellular tangles (Fig. 1*d*); the results presented in the accompanying paper (39) demonstrate that these BR88-positive cells invariably contain neurofibrillary tangles. Immunoreactive bodies were present throughout the hippocampal formation, with the largest number in layer CA1, subiculum, and entorhinal cortex. They were of all sizes ranging from small compact to large distended objects. Tissue sections were treated with formic acid prior to immunostaining in all the experiments described here. As reported before for other anti-β-amyloid antibodies (40), a substantial increase in the number of stained structures and the intensity of staining was observed with 4G8, BR89, and BR88.

In age-matched control brains, 4G8 and BR89 stained occasional amyloid deposits and BR88 labeled occasional cells, in accordance with the age-related appearance of plaques and tangles. In the hippocampal formation from Alzheimer disease patients, no staining was observed when the primary antibody was either omitted or else followed preadsorption of the primary antibody with the corresponding synthetic peptide.

Double-labeling Immunohistochemistry. The anti-β-amyloid antibodies used in these experiments are those just described. As markers for neurofibrillary tangles we have used anti-tau protein antibodies (Table 1). BR133 and BR135 are antisera directed, respectively, against the amino terminus and the repeat region of tau protein (37); they both stain intracellular tangles, neuropil threads, and neuritic plaques (37). BR133 labels mostly intracellular tangles, whereas BR135 labels both intra- and extracellular tangles (19). The failure of BR133 to label extracellular tangles is explained by the likely proteolytic digestion of the amino terminus of tau when exposed in the extracellular space (19).

Anti-β-amyloid and anti-tau protein antibodies were used in various combinations. For the reasons explained above, the reaction product from the first primary antibody always resulted in a brown color and the reaction product from the second primary antibody resulted in a blue color.

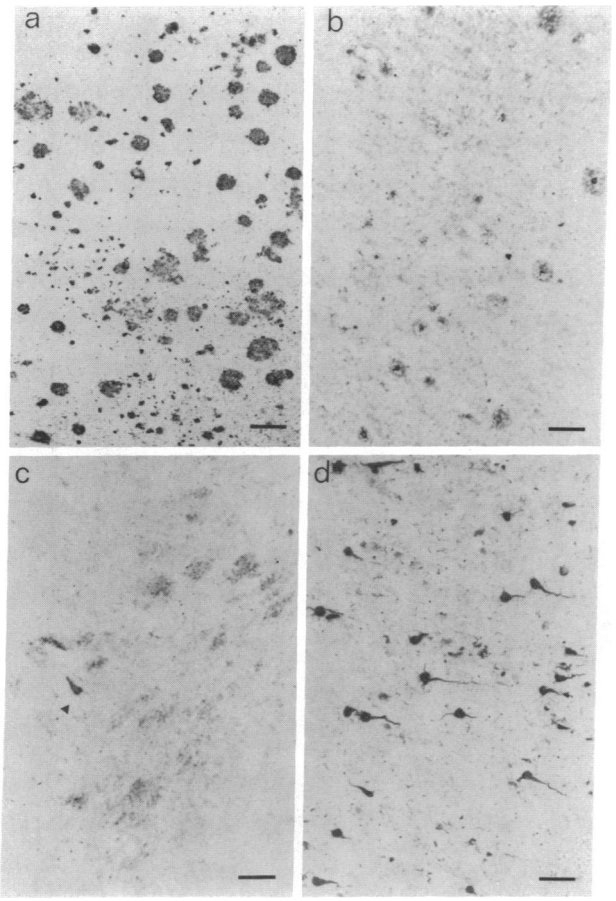

FIG. 1. Labeling of subiculum/entorhinal cortex from Alzheimer disease patients with antibodies raised against different parts of the β-amyloid sequence. (*a*) 4G8 stains amyloid plaques and diffuse amyloid deposits. (*b*) BR89 stains plaque cores strongly and the rest of the plaques more weakly, but not diffuse amyloid deposits. (*c*) BR88 stains diffuse amyloid deposits weakly and a pyramidal cell (arrowhead). (*d*) BR88 stains numerous nerve cells throughout the entorhinal cortex. These are tangle-bearing cells, as demonstrated by BR135 staining of serial sections from this area (data not shown). (Bars = 80 μm.)

Double labeling using 4G8 followed by BR133 or BR135 showed that about half of the amyloid deposits stained only with 4G8 (Fig. 2*a*, 1), whereas the other half also contained tau protein staining material; most often tangle fragments in various stages of degeneration were surrounded by diffuse amyloid deposits (Fig. 2*a*, 2). However, ≈10% of double-labeled structures consisted of tangle-bearing cells with small amyloid patches apposed to the cell body (Fig. 2*a*, 3) or with wisps of amyloid at the apical dendrite (Fig. 2*b*, 1).

Double labeling using BR88 followed by 4G8, which are directed, respectively, against the amino-terminal region and the middle part of β-amyloid, showed two immunoreactive structures: (*i*) tangle-bearing nerve cells, which reacted with BR88, and (*ii*) amyloid plaques, which reacted with 4G8 (Fig. 2 *c* and *d*). In layer II of the entorhinal cortex from Alzheimer disease patients, many stellate cells were BR88 positive (Fig. 2 *d*, 1), as were degenerating tangle-bearing cells surrounded by largely diffuse 4G8-positive amyloid deposits (Fig. 2*d*, 2). BR88-positive cells closely associated with small 4G8-positive patches were also observed (Fig. 2*d*, 3).

Double labeling using BR89, which is directed against the carboxyl-terminal region of β-amyloid, followed by 4G8 demonstrated that the same plaque can be labeled by both antibodies (Fig. 2*e*, 1) and confirmed that 4G8 predominantly recognizes diffuse amyloid deposits or plaques (Fig. 2*e*, 1 and 2), with BR89 staining primarily plaque cores (Fig. 2*e*, 1).

Double labeling using BR89 followed by the anti-tau protein antiserum BR133 showed tangle-bearing nerve cells labeled by BR133 (Fig. 2*f*, 1), neuritic plaques with a core labeled by BR133 and BR89 (Fig. 2*f*, 3), and "burnt-out plaques" labeled by BR89 (Fig. 2*f*, 4). Control experiments showed the double labeling to be specific.

DISCUSSION

The present study demonstrates that antisera against peptides in β-amyloid can be obtained that react predominantly with one of a number of different objects—namely, amyloid plaques without cores, amyloid plaques with cores, or tangle-bearing bodies and cells. This suggests that the β-amyloid sequence exists in different configurations and/or in different states of aggregation in amyloid plaques and neurofibrillary tangle-bearing cells. These cytological findings, together with the knowledge that has been gained on plaque and tangle constituents, suggest a molecular explanation, which it might be helpful to outline in the next paragraph, before proceeding to the more detailed discussion of the observations.

As illustrated in Fig. 3*a*, in the central nervous system intact β-amyloid precursors are nonamyloidogenic neuronal transmembrane proteins (14, 15, 42) that are probably not recognized by any of the antibodies used here, as no staining of normal nerve cells was observed. In the accompanying paper, we show that, at the level of the light microscope, BR88 epitopes, which are chemically formed of the first 12 amino acids of the β-amyloid molecule, are close to the surface of the affected cell (39). It appears, therefore, that, in tangle-bearing cells that react with BR88, these precursors have been cleaved to expose the amino-terminal sequence of β-amyloid itself but are not yet completely processed to β-amyloid and, moreover, are still anchored in, and distributed over, the cell surface, in keeping with their origin as transmembrane proteins (Fig. 3*b*). Further proteolysis of the precursors would cut at the point corresponding to the carboxyl terminus of β-amyloid, freeing β-amyloid protein and leading to its deposition and aggregation in the extracellular space (Fig. 3 *c*, and *d*). Thus, there would be a shift from the tangle-predominant configuration reacting with BR88 (Fig. 3*b*) to the plaque-predominant configuration reacting with 4G8 (Fig. 3*c*), and finally to plaque cores that react with BR89, representing the highest degree of amyloid condensation (Fig. 3*d*). Amyloid deposition would thus be at least a three-step process, consisting of the production of an amyloidogenic protein, which is at first still located at the cell surface, and which after further proteolytic processing is released as β-amyloid that aggregates to form amyloid fibers.

This view is also supported by the demonstration of local processing of β-amyloid precursors in Alzheimer disease (43–45) and by the evidence indicating that β-amyloid deposits derive directly from precursor proteins that are proteolyzed at the cell surface (46). Single-labeling studies showed that antibody 4G8 stained a large number of diffuse amyloid deposits and plaques, in agreement with the previously observed widespread diffuse amyloid pathology (33–36). Antiserum BR89 stained predominantly the plaque cores and surrounding amyloid deposits that are characteristic of mature senile plaques; it also labeled the cores of a number of burnt-out plaques. By contrast, antiserum BR88 stained numerous neurofibrillary tangle-bearing objects and some neuropil thread-containing processes throughout the hippocampal formation; only a small number of diffuse amyloid deposits are observed.

These observations suggest an interpretation based mostly on sequential antigenic determinants. 4G8 is directed against amino acids 17–24 of β-amyloid, whereas BR88 and BR89

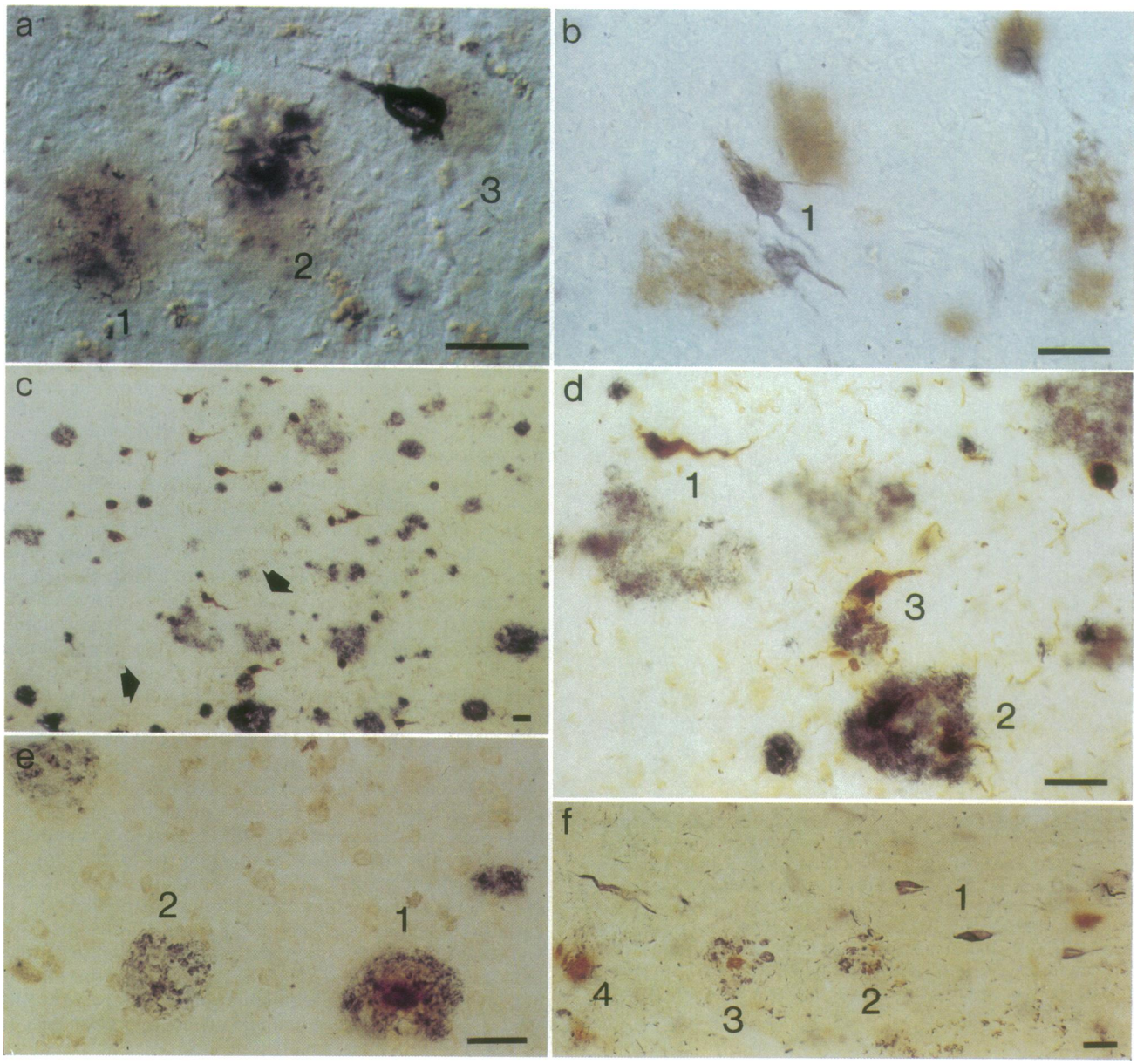

FIG. 2. Double labeling of subiculum/entorhinal cortex from Alzheimer disease patients with 4G8 and other antisera. *(a) 4G8 (brown) followed by BR133 (blue).* 1, Amyloid plaque; 2, amyloid plaque containing tangle fragments; 3, tangle-bearing nerve cell with 4G8-positive amyloid patch elaborated around one end of it. *(b) 4G8 (brown) followed by BR135 (blue).* 1, Tangle-bearing nerve cell with 4G8-positive amyloid accumulated in the region of the apical dendrite. *(c and d) BR88 (brown) followed by 4G8 (blue).* (*c*) Two nonoverlapping populations of BR88-positive tangle-bearing cells and 4G8-positive amyloid plaques. (*d*) Higher power view of the region between the arrows in *c*. 1, BR88-positive stellate cell (layer II entorhinal cortex); 2, large plaque surrounding BR88-positive nerve cells, showing signs of breakdown; 3, nerve cell with BR88-positive staining over most of the cell body and a 4G8-positive amyloid patch distinct from it (cf. *a*, 3). *(e) BR89 (brown) followed by 4G8 (blue).* 1, BR89- and 4G8-positive amyloid plaque, containing a strongly stained BR89-positive core. 2, 4G8-positive amyloid plaque without a core. *(f) BR89 (brown) followed by BR133 (blue).* 1, Tangle-bearing pyramidal cells; 2, neuritic plaque without a core; 3, neuritic plaque with a core; 4, plaque core alone (burnt-out plaque). (Bar = 35 μm.)

were raised, respectively, against amino acids 1–12 and 28–40. It has been demonstrated that amino acids 14–28 of β-amyloid are sufficient for amyloid fibril formation *in vitro* (47, 48); moreover, it has been reported that β-amyloid is ragged at the amino terminus (7, 49). It follows that the 4G8 epitope is hidden in plaque cores, which probably represent the highest degree of amyloid condensation, whereas it is accessible in diffuse amyloid deposits and amyloid plaques. Conversely, the BR89 epitope, which is not required for amyloid fibril formation *in vitro*, is accessible to the antibody in plaque cores and the surrounding amyloid. The BR88 epitopes are masked in the classical amyloid deposits. This could be due to lack of accessibility or to the requirement of an intact, nonragged amino terminus of β-amyloid; as a consequence, BR88 labels a large number of tangle-bearing cells,but only a small number of diffuse amyloid deposits, with no staining of amyloid plaques or plaque cores. We note that masking of epitopes could occur not only by changes in configuration and/or accessibility, as indicated in Fig. 3, but also by modifications such as glycosylation (50) and by the possible interaction of β-amyloid with proteoglycans (51).

The use of the double-labeling technique has allowed us to determine some precise topographical relationships between β-amyloid deposits and neurofibrillary tangles, using antisera directed against microtubule-associated protein tau as tangle markers. Double labeling with 4G8 followed by BR133 or BR135 showed that $\approx$50% of amyloid deposits were also tau protein positive; most cells with tangles were in various

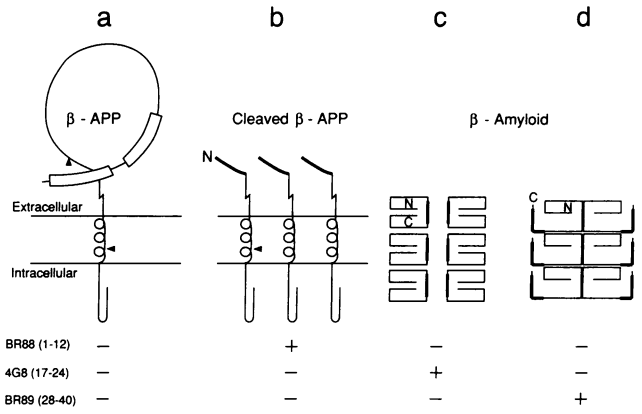

FIG. 3. Diagram of proposed stages leading to the deposition of β-amyloid in the hippocampal formation of Alzheimer disease patients, based on the staining pattern observed with anti-β-amyloid antibodies BR88, 4G8, and BR89. (*a*) Schematic drawing (not to scale) of the intact β-amyloid precursor protein (beta-APP) as a nonamyloidogenic transmembrane protein (from ref. 7). (*b*) The first proteolytic cleavage produces the amino terminus of β-amyloid, leading to the formation of an amyloidogenic protein that is BR88-immunoreactive, but still membrane-bound and dispersed. (*c*) The second proteolytic cleavage frees the carboxyl terminus of the β-amyloid, leading to the formation and aggregation of β-amyloid that is 4G8 immunoreactive; the BR88 and BR89 epitopes are hidden. (*d*) More highly condensed aggregates of β-amyloid protein that are BR89 immunoreactive; the BR88 and 4G8 epitopes are hidden. The drawings in *c* and *d* are highly schematic and are only meant to illustrate different epitope availabilities in condensing and condensed β-amyloid. The detailed configuration(s) of β-amyloid is not known. Positions of positive epitopes are marked by thickened lines.

states of degradation. However, some tangle-bearing cells looked intact and yet had β-amyloid in direct apposition in the form of wisps at the apical dendrite or of patches apparently being elaborated from the cell body (Fig. 2*a*, 3 and Fig. 2*b*, 1); a similar staining pattern was observed when BR88 was used instead of BR133 or BR135 (Fig. 2*d*, 3). These amyloid deposits were BR88 and BR89 negative, suggesting that they represent an intermediate between dispersed and fully condensed amyloid. The frequent association between BR135-positive tangle fragments and 4G8-immunoreactive amyloid deposits may indicate that the tangle-bearing cells and their processes progressively degenerate, leading to plaque formation and to the formation of plaque cores identified by antiserum BR89.

Taken together, these findings indicate that much is to be gained by using different anti-β-amyloid antisera in trying to assess the full amyloid pathology of Alzheimer disease. The results of this and the accompanying paper (39) lead us to outline a conjectural relationship between plaques and tangles that is described in the following paper.

1. Alzheimer, A. (1907) *Allg. Z. Psychiat.* **64**, 146–148.
2. Perusini, G. (1910) *Histol. Histopathol. Arb.* **3**, 297–351.
3. Kraepelin, E. (1910) *Psychiatrie: Ein Lehrbuch für Studierende und Ärzte* (Barth, Leipzig).
4. Wisniewski, H. M. & Terry, R. D. (1973) *Prog. Neuropathol.* **2**, 101–142.
5. Glenner, G. G. & Wong, C. W. (1984) *Biochem. Biophys. Res. Commun.* **12**, 885–890.
6. Masters, C. L., Simms, G., Weinman, N. A., Multhaup, G., McDonald, B. L. & Beyreuther, K. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4245–4249.
7. Kang, J., Lemaire, H. G., Unterbeck, A., Salbaum, J. M., Masters, J. L., Grzeschik, K. H., Multhaup, G., Beyreuther, K. & Müller-Hill, B. (1987) *Nature (London)* **325**, 733–736.
8. Weidemann, A., König, G., Bunke, D., Fischer, P., Salbaum, J. M., Master, C. L. & Beyreuther, K. (1989) *Cell* **57**, 115–126.
9. Palmert, M. R., Siedlak, S. L., Podlisny, M. B., Greenberg, B., Shelton, E. R., Chan, H. W., Usiak, M., Selkoe, D. J., Perry, G. & Younkin, S. G. (1989) *Biochem. Biophys. Res. Commun.* **165**, 182–188.
10. Ghiso, J., Tagliavini, F., Timmers, W. F. & Frangione, B. (1989) *Biochem. Biophys. Res. Commun.* **163**, 430–437.
11. Tanzi, R. E., Gusella, J. F., Watkins, P. C., Bruns, G. A. P., St. George-Hyslop, P., Van Keuren, M. L., Patterson, D., Pagan, S., Kurnit, D. M. & Neve, R. L. (1987) *Science* **235**, 880–884.
12. Goedert, M. (1987) *EMBO J.* **6**, 3627–3632.
13. Bahmanyar, S., Higgins, G. A., Goldgaber, D., Lewis, D. A., Morrison, J. H., Wilson, M. C., Shankar, S. K. & Gajdusek, D. C. (1987) *Science* **237**, 77–80.
14. Shivers, B. D., Hilbich, C., Multhaup, G., Salbaum, M., Beyreuther, K. & Seeburg, P. (1988) *EMBO J.* **7**, 1365–1370.
15. Card, J. P., Meade, R. P. & Davis, L. G. (1988) *Neuron* **1**, 835–846.
16. Probst, A., Ulrich, J. & Heitz, P. U. (1982) *Acta Neuropathol.* **57**, 75–79.
17. Okamoto, K., Hirano, A., Yamaguchi, H. & Hirai, S. (1983) *J. Clin. Electron Microsc.* **16**, 77–82.
18. Schmidt, M. L., Gur, R. E., Gur, R. C. & Trojanowski, J. Q. (1988) *Ann. Neurol.* **23**, 184–189.
19. Bondareff, W., Wischik, C. M., Novak, M., Amos, W. B., Klug, A. & Roth, M. (1990) *Am. J. Pathol.*, in press.
20. Kidd, M. (1963) *Nature (London)* **197**, 192–193.
21. Braak, H., Braak, E., Grundke-Iqbal, I. & Iqbal, K. (1986) *Neurosci. Lett.* **65**, 351–355.
22. Goedert, M., Wischik, C. M., Crowther, R. A., Walker, J. E. & Klug, A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4051–4055.
23. Wischik, C. M., Novak, M., Edwards, P. C., Klug, A., Tichelaar, W. & Crowther, R. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4884–4888.
24. Wischik, C. M., Novak, M., Thøgersen, H. C., Edwards, P. C., Runswick, M. J., Jakes, R., Walker, J. E., Milstein, C., Roth, M. & Klug, A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4506–4510.
25. Goedert, M., Spillantini, M. G., Potier, M. C., Ulrich, J. & Crowther, R. A. (1989) *EMBO J.* **8**, 393–399.
26. Brion, J. P., Passareiro, J. P., Nunez, J. & Flament-Durand, J. (1985) *Arch. Biol.* **95**, 229–235.
27. Delacourte, A. & Defossez, A. (1986) *J. Neurol. Sci.* **76**, 173–186.
28. Grundke-Iqbal, I., Iqbal, K., Quinlan, M., Tung, Y. C., Zaidi, M. S. & Wisniewski, H. M. (1986) *J. Biol. Chem.* **261**, 6084–6089.
29. Kosik, K. S., Joachim, C. L. & Selkoe, D. J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4044–4048.
30. Wood, J. G., Mirra, S. S., Pollock, N. J. & Binder, L. I. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4040–4043.
31. Wong, C. W., Quaranta, V. & Glenner, G. G. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8729–8732.
32. Allsop, D., Landon, M., Kidd, M., Lowe, J. S., Reynolds, G. P. & Gardner, A. (1986) *Neurosci. Lett.* **68**, 252–256.
33. Yamaguchi, H., Hirai, S., Morimatsu, M., Shooji, M. & Ihara, Y. (1988) *Acta Neuropathol.* **76**, 541–549.
34. Tagliavini, F., Giaccone, G., Frangione, B. & Bugiani, O. (1988) *Neurosci. Lett.* **93**, 191–196.
35. Kim, K. W., Miller, D. L., Sapienza, V. J., Chen, C. M. J., Bai, C., Grundke-Iqbal, I., Currie, J. R. & Wisniewski, H. M. (1988) *Neurosci. Res. Commun.* **2**, 121–130.
36. Davies, L., Wolska, B., Hilbich, C., Multhaup, G., Martins, R., Simms, G., Beyreuther, K. & Masters, C. L. (1988) *Neurology* **38**, 1688–1693.
37. Goedert, M., Spillantini, M. G., Jakes, R., Rutherford, D. & Crowther, R. A. (1989) *Neuron* **3**, 519–526.
38. Wischik, C. M. (1989) Ph.D. Thesis, University of Cambridge.
39. Spillantini, M. G., Goedert, M., Jakes, R. & Klug, A. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 3952–3956.
40. Kitamoto, T., Ogomori, K., Tateishi, J. & Prusiner, S. B. (1987) *Lab. Invest.* **57**, 230–236.
41. Vandesande, K. (1983) in *Immunohistochemistry*, ed. Cuello, A. C. (Wiley, Chichester), pp. 101–119.
42. Dyrks, T., Weidemann, A., Multhaup, G., Salbaum, J. M., Lemaire, H. G., Kang, J., Müller-Hill, B., Masters, C. L. & Beyreuther, K. (1988) *EMBO J.* **7**, 949–957.
43. Perry, G., Lipphardt, S., Mulvihill, P., Kancherla, M., Mijares, M., Gambetti, P., Sharma, S., Maggiora, L., Cornette, J., Lobl, T. & Greenberg, B. (1988) *Lancet* **ii**, 746.
44. Selkoe, D. J., Podlisny, M. B., Joachim, C. L., Vickers, E. A., Lee, G., Fritz, L. C. & Oltersdorf, T. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7341–7345.
45. Palmert, M. R., Podlisny, M. B., Witker, D. S., Oltersdorf, T., Younkin, L. H., Selkoe, D. J. & Younkin, S. G. (1988) *Biochem. Biophys. Res. Commun.* **156**, 432–437.
46. Ishii, T., Kametani, F., Haga, S. & Sato, M. (1989) *Neuropathol. Appl. Neurobiol.* **15**, 135–147.
47. Kirschner, D. A., Inouye, H., Duffy, L. K., Sinclair, A., Lind, M. & Selkoe, D. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6953–6957.
48. Gorevic, P. D., Castano, E. M., Sarma, R. & Frangione, B. (1987) *Biochem. Biophys. Res. Commun.* **147**, 854–862.
49. Masters, C. L., Multhaup, G., Simms, G., Pottgiesser, J., Martins, R. N. & Beyreuther, K. (1985) *EMBO J.* **4**, 2757–2763.
50. Behrouz, N., Defossez, A., Delacourte, A., Hublau, P. & Mazzucca, M. (1989) *C.R. Acad. Sci. Paris Ser. 3* **308**, 437–444.
51. Snow, A. D., Mar, H., Nochlin, D., Kimata, K., Sato, M., Suzuki, S., Hassell, J. & Wight, T. N. (1988) *Am. J. Pathol.* **133**, 456–463.