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Immuno-electron-microscopic Localization of Complements in Amyloid Fibrils of Senile Plaques*

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Summary. The presence of components of immunoglobulins (Ishii et al. 1975) and complements (Eikelenboom and Stam 1982) in senile plaques suggests that the immunologic mechanisms are involved in the causation of pathologic processes in the brain of patients with Alzheimer's disease. Senile plaques consists of amyloid degenerated neuritis and glia,and exact localization of complements among these tissue elements will provide an important clue to the pathogenesis of the Alzheimer brain. This report deals with light- and electron-microscopic localization of complements in amyloid fibrils of senile plaques by immunoperoxidase histochemistry. The presence of C_{1q}, C_4, and C_3 is confirmed light-microscopically. At the ultrastructural level, anti-complement C_{1q}, C_4, and C_3 peroxidase reaction products are exclusively localized on the amyloid fibrils, but no other tissue elements, such as normal or degenerated neurites, neurofibrillary tangles, or glia. The results indicate the presence of immune complex in amyloid fibrils of senile plaques, and little association of complements in senile plaques with neurofilament protein.

Key words: Alzheimer's disease – Senile plaque – Amyloid – Complement – Immuno-electron microscopy

Introduction

Senile plaques are one of the most important neuropathologic findings in patients with senile and presenile Alzheimer's disease (Alzheimer 1907; Simchowicz 1911), and the degree of their presence in the cerebral cortex has been found to correlate with the degree of dementia (Blessed et al. 1968). Amyloid fibrils are regarded as the most characteristic constituent of senile plaques (Divry 1952; Terry et al. 1964), together with degenerated neurites and glia. Thus, elucidation of both chemical nature and genesis of amyloid fibrils in senile plaques will likely provide an important clue to the pathogenesis of the Alzheimer brain.

Ishii et al. (1975) and Ishii and Haga (1976) reported the presence of immunoglobulins in amyloid fibrils of senile plaques, using immunofluorescent and ultrastructural immunoperoxidase methods. Ihara et al. (1981a) confirmed immunoglobulin light chain L protein in the cores of senile plaques. Powers et al. (1981) claimed that, besides immunoglobulins, albumin, GFA, and neurofilament proteins are present in senile plaques. Eikelenboom and Stam (1982) demonstrated the presence of complements in senile plaques with light-microscopic immunohistochemistry. They also observed immunoglobulins only in the peripheral parts, but not in the cores, of senile plaques.

The present report deals with the light- and electron-microscopic localization of complement factors in amyloid fibrils of senile plaques by immunoperoxidase histochemistry. The presence of C_{1q}, C_4, and C_3 complements is confirmed light-microscopically. At the ultrastructural level, anti-complement C_{1q}, C_4, and C_3 peroxidase reaction products are exclusively localized on the amyloid fibrils, but in no other tissue elements, such as normal or degenerated neurites, neurofibrillary tangles, or glia. The results indicate the presence of an immune complex in amyloid fibrils of senile plaques.

Materials and Methods

Three cases of Alzheimer brain were investigated. The main clinical and pathologic findings are summarized in Table 1. We used the indirect immunoperoxidase technique by first embedding 2-mm thick

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Table 1. Clinico-pathologic findings of three cases examined

Case	Age (yr)	Disease	Duration (yr)	Brain weight (g)	Neuro-pathology
HK	74	SD Korsakoff	10	1,115	SP, ANT
ET	86	SD	2	1,110	SP, ANT, AA
KT	61	A	15	755	SP, ANT, AA

Abbreviations: SD, senile dementia; A, Alzheimer's disease; SP, senile plaque; ANT, Alzheimer's neurofibrillary tangle; AA, amyloid angiopathy

fresh brain tissue slices from Ammon's horn in Tissue-Tek O.C.T. Compound (Lab-Tek Products) and then freezing them immediately in liquid nitrogen. Fresh 10-μm thick cryostat sections were cut, mounted on slides, air-dried, and fixed in acetone for 10 min. All through the following procedures, the sections were kept on slides. After being washed twice in Tris buffer saline (TBS) for 10 min, the sections were treated with normal horse serum (dilution 1:6) for 20 min. Commercially obtained goat anti-human C_{1q} (Cappel, 1:400), C_3 (Miles, 1:200), C_4 (Miles, 1:400), C_5, C_6, C_7, C_8, and properdin (Miles, 1:100) antibodies were applied on the sections for 1 h, followed by washing twice in TBS for 10 min. Subsequently, the sections were treated with rabbit anti-goat IgG-HRP complex (dilution 1:400) for 30 min, followed by washing twice in TBS for 10 min each time, incuabation in DAB solution (0.05% DAB in 0.05 M Tris buffer, pH 7.6, and 0.01% H_2O_2) for 5 min at room temperature and washing in distilled water.

For light-microscopic observation, the sections were counter-stained with dilute hematoxylin, dehydrated, and mounted on glass slides. For electron microscopy (EM) the sections on slides were fixed with 2% glutaraldehyde after the DAB reaction, postfixed with 1% OsO_4, dehydrated, and embedded in Epon by putting the sections on slides downward on top of the resin in the capsules. After hardening of resin, the slides were removed, and the remaining tissue on the surface of the resin was cut with an LKB ultratome using a diamond knife. The ultrathin sections, either unstained or stained with lead and uranium, were observed under a JEM 200 CX electron microscope at 80 kV. Control sections were treatet in the first step with nonimmunized goat serum (1:100).

Results

Light-microscopic Findings

Senile plaques showed positive reactions, with anti-complements C_{1q} (Fig. 1), C_4, and C_3 antibodies, but negative ones with anti-complements C_5, C_6, C_7, C_8 antibodies or antiproperdin. The pattern of positive reaction products indicated amyloid-like distribution, but sometimes it looked similar to that of degenerated neurites. A few Alzheimer's tangles and astroglial cells were also stained. Staining of astroglia disappeared when the antiserum was diluted, but Alzheimer's tangles remained positive, though weak. From this, we conclude that the reaction on glia was nonspecific. Thus, the exact localilzation of the reaction product was not conclusively clear at the light-microscopic level.

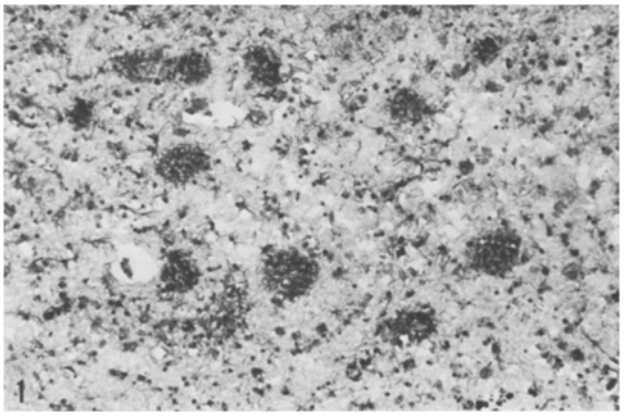


Fig. 1. Senile plaques with positive DAB reaction product of anti-C_{1q} antibody. Counterstained with hematoxylin. Case KT. × 80

Ultrastructural Findings

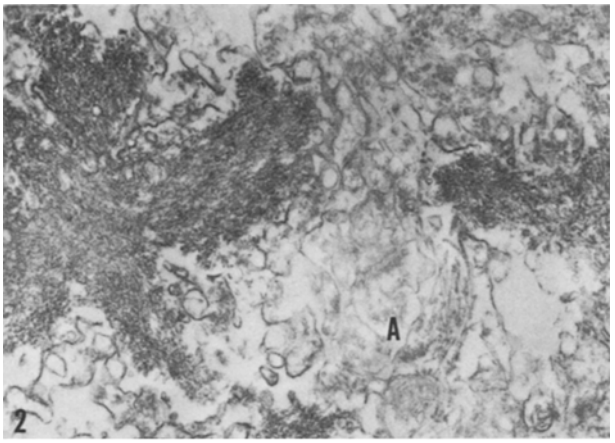
Observation of the stained and unstained sections clearly showed that positive reaction products of C_{1q}, C_4, and C_3 were exclusively on the amyloid fibrils of the senile plaques (Figs. 2–6). No other tissue elements in the senile plaques, such as normal or degenerated neurites (Figs. 3, 5) or Alzheimer's tangles (Fig. 2), glia fibers (Fig. 6), or vascular walls near the amyloid fibrils, showed positive reaction. Sometimes, however, cell membrane near the amyloid fibrils with reaction products emerged dark among the unstained tissue elements (Figs. 2, 5).

Amyloid fibrils with positive reaction products were very electron-dense and appeared thicker than the normal amyloid (Figs. 2–6). When deposits were less in amount, we discerned the fine structure of amyloid fibrils (Fig. 2). However, if deposits were heavy, fibrous profiles of the amyloid became obscured and sometimes appeared as a mass of granules or beads along the amyloid fibrils (Fig. 4). In general, deposits on amyloid fibrils were heavier in the periphery than in the central parts of the amyloid bundles (Fig. 2).

Discussion

Amyloid fibrils and degenerated neurites are the two main pathologic alterations in senile plaques (Divry 1952; Terry et al. 1964), and the nature and genesis of amyloid will be of crucial importance in clarifying the pathogenesis of the Alzheimer brain.

Since our first reports (Ishii et al. 1975; Ishii and Haga 1976), the presence of immunoglobulins in amyloid fibrils of senile plaques has been confirmed immunohistochemically by Ihara et al. (1981a), Powers et al. (1981), and Eikelenboom and Stam (1982). The latter authors found immunoglobulins only in the peripheral



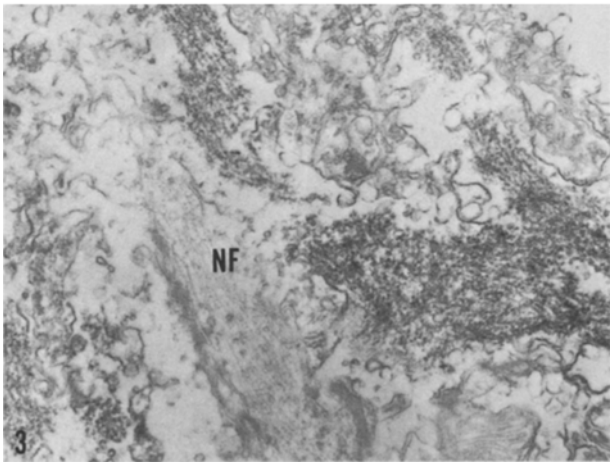
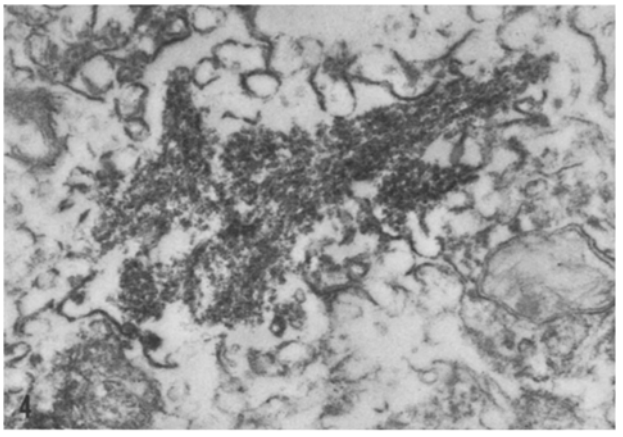


Fig. 2. Amyloid fibrils in a senile plaque are stained very dark with deposits of DAB reaction product of anti-C_{1q}. Reaction product is heavier in the periphery than in the central part of amyloid bundle. Note Alzheimer's tangles (*A*) adjacent to amyloid fibrils are not decorated. Unstained section. Case HK. ×20,000

Fig. 3. Amyloid fibrils with heavy deposits of DAB reaction product are in sharp contrast with adjacent nondecorated neurofilaments (*NF*) in an axon. Unstained section. Case HK. ×21,000



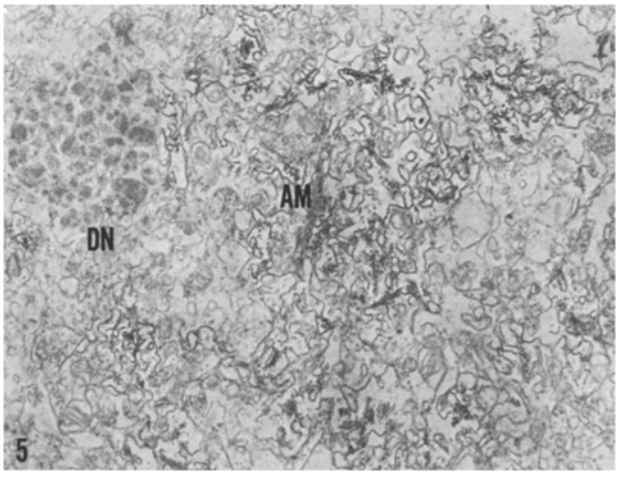


Fig. 4. Higher magnification of amyloid fibrils with heavy deposition of DAB reaction product of anti-C_{1q}. Fibrils look thicker and beads-like. Unstained section. Case HK. ×32,500

Fig. 5. Few amyloid fibrils (*AM*) with intense reaction product of anti-C_{1q}. Note adjacent membranes also are electron-dense. Degenerated neurite (*DN*) is not decorated. Unstained section. Case ET. × 7,500

parts, but not in the cores, of senile plaques. Our recent immunofluorescent study (Ishii and Haga 1983) also revealed that there are many fluorescent-negative plaques with anti-IgG, together with positive ones. Positive fluorescence for IgG was found more often in the peripheral parts of the senile plaques, perivascular plaques, and amyloid deposits in the meninges.

Eikelenboom and Stam (1982) denied the presence of immunoglobulins in amyloid fibrils (Ishii and Haga 1976) on the ground that they could not stain cores of senile plaques with the immunoperoxidase method, in spite of a positive reaction in the corona. We attach much importance to the positive reaction in the corona and interpret the nagative reaction in the cores of the plaques as representing the loss of antigenic determinants (burned-out plaques, Wisniewski and Terry 1973).

The presence of a complement (C_3) in senile plaques was first described by Powers et al. (1981). Eikelenboom and Stam's (1982) work with immunoperoxidase histochemistry disclosed the complements C_{1q}, C_3, and C_4 in senile plaques. In the previous immunofluorescent study (Ishii and Haga 1983) we also confirmed C_{1q}, C_4, and C_3 in senile plaques, but no fluorescence was found with antihuman C_5, C_1 esterase inhibitor or other antihuman lysozymes, HLA, B cells, granulocytes, leukocytes, neurons or fibronectin, nor with anti-prealbumin. The present ultrastructural study clearly shows the localization of the bound complements exclusively in the amyloid fibrils of senile plaques. It is noteworthy that the central parts of massive amyloid fibrils tend to show weaker reactions than those in the peripheral, more discrete bundles of amyloid fibrils (Fig. 2). We interpret this as representing a degree of

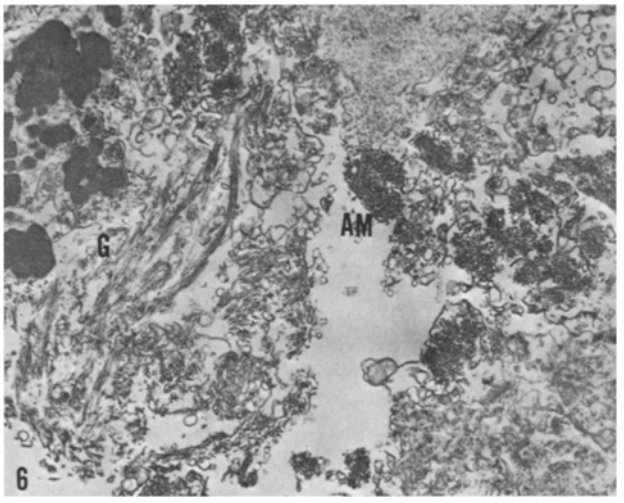


Fig. 6. Glial fibers (*G*) are not decorated, while amyloid fibrils (*AM*) are with heavy deposition of DAB reaction product of anti-C_{1q}. Unstained section. Case HK. ×7,700

accessibility or penetration of the marker protein. The dark profiles of the membrane near the amyloid fibrils with deposits (Figs. 2, 5) are probably due to drifts of the reaction products.

Binding of C_{1q} to amyloid fibrils in senile paques suggests the classical pathway. As mentioned before, the presence of immunoglobulins in amyloid, where complements are bond, is important. The most reasonable inference in this case is the existence of an antigen-antibody reaction or immune complex that activates subsequent complement binding in amyloid of senile plaques, though other interpretations, such as C_1 activation caused by trypsin (fluid phase) or complement binding to intermediate filaments (Linder et al. 1979), are also possible. We do not know yet what kind of antigen or antigens are introduced so massively in the brain of Alzheimer patients. An immunofluorescent study of senile plaques with 20 conventional viral antibodies (Ishii et al. 1982) and evaluation of virus antibodies in sera and cerebrospinal fluid (CSF) of Alzheimer patients with complement fixation and immunofluorescence (Ishii et al. 1983) suggest some associations of papovaviruses. However, these data are still not conclusive. At this time, we can only say that some kind of immunologic processes are present behind the deposition of amyloid fibrils.

Eikelenboom and Stam (1982) speculated that complements are bound to neurofilament protein or its residue in senile plaques, as reported by Linder et al. (1979) in cultured cells. However, many authors, except for Powers et al. (1981), have failed to find the presence of neurofilament protein in amyloid of senile plaques immunohistochemically (Ihara et al. 1981b; Kahn et al. 1980, 1981). Our recent ultrastructural work (Kahn et al. 1983) performed with the monoclonal 210 K RT 97 antineurofilament antibody did not decorate amyloid fibrils, though Alzheimer's tangles were specifically stained. In the present report we have occasionally observed the positive reaction on Alzheimer's tangles at the light-microscopic level. However, at the ultrastructural level, the reaction products of anticomplement C_{1q}, C_4, and C_3 antibodies have been seen only in the amyloid fibrils but not in the normal and degenerated neurites or Alzheimer's tangles, where neurofilament protein is rich. We cannot give any logic explanations, but we are inclined to believe in evidences at the ultrastructural level. Thus, we have observed little association of complements in senile plaques with neurofilament protein, as far as the immunohistochemical evidence is concerned.

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References

Alzheimer A (1907) Über eine eigenartige Erkrankung der Hirnrinde. Allg Z Psychiatr 64:146–156

Blessed G, Tomlinson BE, Roth M (1968) The association between quantititative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. Br J Psychiatry 114: 797–811

Divry P (1952) La pathochimie generale et cellulaire des processes seniles et preseniles. In: Rosenberg, Sellier (eds) Proc First Int Congr Neuropathol, Rome, vol 2. Casa Editrice Libraria, Torino, p 313

Eikelenboom P, Stam FC (1982) Immunoglobulins and complement factors in senile plaques. Acta Neuropathol (Berl) 57:239–242

Ihara Y, Kurizaki H, Nukina N, Sugita H, Toyokura Y, Ebara C (1981a) Presence of immunoglobulin light chain in the cores of senile plaques – An unlabelled antibody peroxidase-anti-peroxidase (PAP) study. Neurol Med (Jpn) 15:292–295

Ihara Y, Nukina N, Sugita H, Toyokura Y (1981b) Staining of Alzheimer's neurofibrillary tangles with antiserum against 200 K component of neurofilament. Proc Jpn Acad 57:152–156

Ishii T, Haga S (1976) Immuno-electron microscopic localization of immunoglobulins in amyloid fibrils of senile plaques. Acta Neuropathol (Berl) 36:243–249

Ishii T, Haga S (1983) Immunoglobulins and complements in senile plaques. Neuropathology (Jpn) 4:31–36

Ishii T, Haga S, Shimizu F (1975) Identification of components of immunoglobulins in senile plaques by means of fluorescent antibody technique. Acta Neuropathol (Berl) 32:157–162

Ishii T, Haga S, Oyanagi S, Tazima M, Kato K, Miyamura T (1982) Virus antibodies in the sera and cerebrospinal fluid (CSF) of patients with Alzheimer's disease and senile dementia (AHD-SD). In: Ohashi H, Nakayama K, Saito M, Saletu B (eds) Proceedings of the World Psychiatric Association Regional Symposium. Kyoto, Japan, pp 261–264

Ishii T, Haga S, Tazima M (1983) Viral antigens in senile plaques. An immunofluorescent study. In: Hirano A, Myoshi K (eds) Neuropsychiatric disorders in the elderly. Igaku-Shoin, Tokyo New York, pp 32–38

Kahn J, Green PG, Thorpe R, Anderton BH (1980) Immunohistochemistry of neurofilaments in Alzheimer's disease. J Cl Exp Gerontol 2:199–210

Kahn T, Anderton BH, Oyanagi S, Ishii T (1983) Ultrastructural localization of 210 K neurofilament protein in Alzheimer's tangles evidenced by monoclonal antibody RT 97. In preparation

Linder E, Lehto VP, Stenman S (1979) Activation of complement by cytoskeletal intermediate filaments. Nature 278:176–178

Powers JM, Schlaepfer WW, Willingham MC, Hall BJ (1981) An immunoperoxidase study of senile cerebral amyloidosis with pathogenetic considerations. J Neuropathol Exp Neurol 40: 592–612

Simchowicz T (1911) Histologische Studien über die senile Demenz. Nissl-Alzheimer'sche Arbeiten 4:267–444

Terry RD, Gonatas NK, Weiss M (1964) Ultrastructural studies in Alzheimer's presenile dementia. Am J Pathol 44:269–279

Wisniewski HM, Terry RD (1973) Reexamination of the pathogenesis of the senile plaque. In: Zimmerman HM (ed) Progress in neuropathology, vol 2. Grune and Stratton, New York, pp 1–26

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