Variability of β-Amyloid Protein Deposited Lesions in Down's Syndrome Brains

SHU-ICHI IKEDA, TAKAHIKO TOKUDA, NOBUO YANAGISAWA, FUYUKI KAMETANI*, TOSHIÖ OHSHIMA† and DAVID ALLSOP‡

Department of Medicine (Neurology), Shinshu University School of Medicine, Matsumoto 390, *Department of Molecular Biology, Tokyo Institute of Psychiatry, Tokyo 156, †The Third Department of Internal Medicine, University of Yamanashi Medical School, Yamanashi 409-38, and ‡Department of Molecular Neuropathology, SmithKline Beecham Pharmaceuticals, Harlow, Essex CM19 5AD, UK

IKEDA, S., TOKUDA, T., YANAGISAWA, N., KAMETANI, F., OHSHIMA, T. and ALLSOP, D. Variability of β-Amyloid Protein Deposited Lesions in Down's Syndrome Brains. Tohoku J. Exp. Med., 1994, 174 (3), 189-198 — An immunohistochemical study was carried out on the brains of 7 adult Down's syndrome cases (ages 31 to 62) using antibodies to β-protein, β-amyloid protein precursor and tau-protein. Variable forms of β-protein deposited lesions (including senile plaques and cerebrovascular amyloidosis) were observed in extensive areas of the neocortex of all cases and coexistence of both β-protein amyloid fibrils and β-amyloid protein precursors was also seen in some of these lesions. Moreover, 3 cases at an advanced stage showed a few plaque-like lesions with β-protein immunoreactivity in the white matter. The following temporal morphological change is suggested for the pathogenesis of Alzheimer's disease: senile plaque undergo sequential structural changes and β-protein amyloid deposits in the form of "early plaque" precede the development of tau-immunoreactive neurofibrillary degeneration. ——— Down's syndrome; Alzheimer's disease; dementia; amyloid; β-protein

The presence of cerebrovascular amyloidosis, senile plaques, and neurofibrillary tangles (NFTs) is a characteristic pathological finding in the brains of individuals with Alzheimer's disease (AD), and Down's syndrome (DS) is a genetically determined disorder in which the typical neuropathological features of AD invariably develop in middle age (Burger and Vogel 1973). It has been shown that amyloid fibrils isolated from cerebrovascular and senile plaque

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Address for reprints: Shu-ichi Ikeda, M.D., Department of Medicine (Neurology), Shinshu University School of Medicine, Matsumoto 390, Japan.
Amyloid in both AD and DS are composed of a unique 4.2 kDa polypeptide termed “β-protein” (Glenner and Wong 1984; Masters et al. 1985), and this amyloid protein is thought to be derived from a much larger glycoprotein (β-amyloid protein precursor, β-APP), the gene for which is located on chromosome 21 (Kang et al. 1987).

Recent histochemical and immunocytochemical studies have suggested that senile plaques may undergo sequential structural changes in their development, showing variable forms of plaque lesions (Ikeda et al. 1989, 1990). However, the chronological correlation between senile plaques and neurofibrillary degeneration (including NFTs, dystrophic neurites and neuropil threads) is still incompletely understood. In the present study we examined a number of adult DS brains using immunohistochemistry with antibodies to β-protein, β-APP and tau, with special attention to the morphological variability of β-amyloid protein deposited lesions, and report previously unknown findings on the cerebral white matter pathology in this disorder.

**Materials and Methods**

The brain tissues of 7 cases with DS (ages 31 to 62) were examined (Table 1). Sections from formalin-fixed and paraffin-embedded brain blocks were stained with hematoxylin-eosine, alkaline Congo red, and by immunocytochemical methods using the avidin-biotin peroxidase technique. The primary reagents were a monoclonal antibody (4D12/2/6) for β-protein (Allsop et al. 1986) and anti-tau antiserum (Ihara 1988), and in the staining with anti-β-protein antibody sections were pretreated with 98% formic acid. Fresh frozen 6 μm sections were

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Sex</th>
<th>β-Protein</th>
<th>tau-Protein</th>
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<tr>
<td></td>
<td></td>
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<td>Early plaques</td>
<td>Mature plaques</td>
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<tr>
<td>DS1</td>
<td>31</td>
<td>M</td>
<td>+</td>
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<td>DS2</td>
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<td>DS7</td>
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DS, Down's syndrome; CAA, cerebral amyloid angiopathy.

The immunoreactive lesions in each case were divided into four grades of severity under low power magnification: −, not observed; +, a few lesions were observed, but only after extensive search; #, a substantial number of lesions were seen, but some optic fields were still free of lesions; †, many lesions were visible in every field.

**Table 1. Semiquantitative estimation of severity of immunoreactive lesions in the cases with Down's syndrome**
prepared from some neocortical tissues of case 5 and were processed for the indirect immunofluorescence study using rabbit antisera to β-APP: these were raised against N-terminal T97 (residues 18–38 of β-APP695), R36 (residues 527–540 of β-APP), C-terminal R37 (residues 681–695 of β-APP 695) and Kunitz-type

Fig. 1. Representative photomicrographs of β-protein immunoreactive plaque lesions.
A: Early plaques seen in case 1, showing ill-defined immunoreactive area.
B: Neocortex in case 2. Many weakly immunoreactive lesions (early plaques) and several intensely stained discrete plaques (mature plaques) are seen. Insert shows senile plaque-related degenerating neurites with tau-immunoreactivity. (Bars=100 μm)
inhibitor insert (KPI) R98 (residues 681-695 of β-APP770) (Ishii et al. 1989). The immunospecificity of the staining produced by all these antibodies has been well characterized. An electron microscopy study was performed on the cerebral white matter of case 5: formalin-fixed, plastic embedded tissue blocks were screened for the presence of amyloid deposits with β-protein immunoreactivity (Ikeda et al. 1987). Ultrathin sections were made from those blocks that stained positive for β-protein, and then were observed under a Hitachi HS-9 electron microscope.

Results

The histopathological and immunocytochemical staining results of all 7 cases are summarized in Table 1, and the pattern of β-protein immunoreactive lesions seen in these cases is briefly as follows: cases 1 and 3 showed variable numbers of early plaque lesions consisting of ill-defined β-protein immunoreactive areas with a reticulogranular appearance (Fig. 1-A). In addition to these lesions, typical senile plaques with a discrete core of amyloid were also observed in the sections from cases 2, and 4 to 7, and the number of these plaques increased with age (Fig. 1-B). Moreover, cases 2, and 5 to 7 revealed cerebrovascular involvement: in small or medium-sized vessels the entire vascular walls often showed β-protein immunoreactivity, while these lesions on large vessels were mostly confined to the

Fig. 2. Representative photomicrographs of cerebrovascular amyloid deposits with β-protein immunoreactivity.
A: Vascular wall lesions seen in the leptomeningeal vessels of case 2. Note that the media is mainly involved in large vessels. B: Capillary lesions seen in case 5. Arrowheads indicate plaque-like degeneration. (Bars = 100 μm)
media or adventitia (Fig. 2-A). Additionally, in case 5 strong immunoreactivity to anti β-protein antibody was also seen in many capillaries and arterioles, some producing the “plaque-like degeneration” originally proposed by Scholz (Fig. 2-B). These β-protein immunoreactive lesions seemed much more abundant and showed a wider distribution than those obtained from Congo-red-stained preparations. Immunofluorescence histochemistry using the brain tissues of case 5 disclosed that both senile plaques and cerebrovascular amyloid deposits were positively stained by anti-β-APP antisera against T97 and R36 (Fig. 3-A), whereas they lacked any consistent immunoreactivity to either anti-R98 antibody or anti-R37 antibody. The distribution and morphologic appearance of these

Fig. 3. Representative fluorescent photomicrographs of β-APP-immunoreactive lesions. A: Both vascular wall and senile plaque amyloid deposits are immunoreactive to anti-β-APP (T97) antibody. B: Closely adjacent two sections were stained by immunofluorescence method with anti-β-APP antibody and by immunoperoxidase method with anti-β-protein antibody (insert) respectively. Only amyloid-laden vascular walls with β-protein immunoreactivity (arrowheads) were immunolabeled by anti-β-APP (R36) antibody. (Bars = 100 μm)
β-APP immunoreactive lesions closely resembled that observed with β-protein immunostaining (Fig. 3-B).

Concerning tau-immunohistochemistry, no tau-immunoreactive lesions were observed in cases 1 and 3, and degenerating neurites accompanied by senile plaques were occasionally detected in case 2 (Fig. 1-B insert). However, case 4 disclosed significant numbers of tau-immunoreactive lesions including NFTs and degenerating neurites, and cases 5, 6 and 7 revealed innumerable neuropil threads with tau-immunoreactivity in addition to many NFTs and senile plaque-related degenerating neurites (Fig. 4). Moreover, in cases 6 and 7 showing numerous neuropil threads, some NFT-bearing neurons revealed a tortuous apical dendrite and several basal dendrites, which reacted with anti-tau antibody.

All lesions described above were seen in extensive areas of the neocortex of cases examined. However, cases 5, 6 and 7 also showed a small number of β-protein immunoreactive plaque-like lesions in the white matter; they lacked a well-formed core and consisted of oval areas with sparse aggregations of irregularly shaped rods or coarse nodules (Fig. 5-A). Some of these lesions were detected by conventional Congo red staining and were also immunoreactive to anti-β-APP-antisera against T97 and R36. Electron microscopy examinations revealed that there were loose bundles of amyloid fibrils in the lesions (Fig. 5-B).

![Fig. 4. Representative photomicrograph of tau-immunoreactive neurofibrillary degeneration seen in case 6. In addition to many NFTs and a senile plaque with degenerating neurites (an arrow), large number of neuropil threads are visible. Insert shows NFT-bearing neurons with tau-immunoreactive dendrites. (Bars=100 μm)
DISCUSSION

The present histopathological and immunocytochemical study of DS brains using antibodies to β-protein and PHF-related tau-protein suggests a temporal sequence for the pathogenesis of AD (Fig. 6): β-protein amyloid deposits in the form of “diffuse” (Yamaguchi et al. 1988) or “early” (Ikeda et al. 1989) senile plaques precede the development of tau-immunoreactive neurofibrillary changes,
including NFTs, degenerating neurites and neuropil threads. Subsequently, there appear both classical plaques with degenerating neurites and NFTs, and neuropil threads develop at around the same time, or in the more advanced stage of the disease. These observations strongly support the concept previously proposed by ourselves (Ikeda et al. 1989, 1990) and others (Giaccone et al. 1989; Mann 1989) that an extensive appearance of extracellular β-protein amyloid deposits in the brain is an early event in the pathophysiology of AD. However, the causative relationship between β-protein amyloid deposition and neurofibrillary degeneration with tau-immunoreactivity in developing AD pathology remains unclear. Neurofibrillary degeneration is composed mainly of bundles of the paired helical filaments (PHFs) containing tau-protein as a major antigenic component (Nukina and Ihara 1986). The abnormally or aberrantly hyperphosphorylated tau-protein is thought to play a crucial role in the formation of PHFs (Trojanowski and Lee 1994), and the foregoing deposition of β-protein amyloid fibrils might induce pathological phosphorylation of tau, possibly by altering the activity of appropriate kinase enzymes.

Taken together with these hypotheses, in considering the pathogenesis of AD, it is very important to clarify the cellular source of β-APP and the processing of this precursor protein into insoluble amyloid fibrils. Three main isoforms of β-APP (β-APP695, 751 and 770) produced by alternatively spliced mRNAs from a single gene have been identified, and CNS neurons have particularly high levels of β-APP695 expression, whereas in peripheral tissues, β-APPs751 and 770 are the predominant forms (Tanaka et al. 1989; Golde et al. 1990). Moreover, soluble secretory derivatives of these β-APP molecules have been detected in serum (Bush et al. 1992), cerebrospinal fluid (Kennedy et al. 1992), brain parenchyma (Kametani et al. 1993) and the growth medium of several cell cultures (Andersson et al. 1991). The present study has shown that β-APP and β-protein amyloid fibrils coexist in both senile plaque and cerebrovascular amyloid deposits. Similar observations have been reported by other laboratories (Tagliavini et al. 1990; Ko et al. 1991) and, particularly in cerebrovascular amyloidosis, it is suggested that the vascular system itself is a source of β-APP, the conversion of soluble β-protein into fibrillar amyloid taking place in situ (Wisniewski and Wegiel 1994).

Finally, we need to discuss the white matter lesions seen in some cases with DS. It is well known that AD pathology exclusively occurs in cerebral gray matter, and therefore, any concurrent white matter disorder has hitherto disregarded. In this investigation of a series of DS cases, some cases with severe cortical involvement also revealed substantial deposits of β-protein amyloid in white matter. Although morphologically, the appearance of these lesions was somewhat different from that of cortical senile plaques, the lesions were demonstrated to consist of genuine amyloid fibrils. It has been revealed that white matter of human brain show appreciable expression of β-APP (Golde et al. 1990; Tokuda
et al. 1994), suggesting the possible role of neuronal or non-neuronal cells in producing β-protein. The origin of this amyloidogenic protein in the brain, however, has not been determined, and further studies are required.

Acknowledgments

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References


