Absence of β-Amyloid Deposits After Immunization in Alzheimer Disease With Lewy Body Dementia

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Objective: To describe the neuropathological and biochemical findings of the brain examination of a patient enrolled in the AN-1792(QS-21) trial with an initial clinical diagnosis of Alzheimer disease (AD), in whom Lewy body variant was thereafter clinically diagnosed.

Design: A case report.

Setting: University memory clinic.

Patient: A 74-year-old woman with clinical features of probable AD.

Intervention: The patient received 2 injections of 225 µg of AN-1792 (β-amyloid [Aβ]) plus 50 µg of the adjuvant QS-21 at an interval of 4 weeks. The patient was an antibody responder with an IgG anti–AN-1792 antibody titer exceeding 10 000 and an IgM titer exceeding 3500. Maximum serum anti-Aβ titers were reached in 4.7 months. During the 3 following years, while the Mini-Mental State Examination score remained globally stable despite several confusional episodes, she developed clinical features of dementia with Lewy bodies. The patient died 34 months postimmunization. An autopsy was performed.

Main Outcome Measures: Neuropathological and biochemical examination of the brain using standardized evaluation for tau, β-amyloid, and synuclein deposits.

Results: Neither neuropathological nor biochemical examinations showed amyloid deposit in the brain of this immunized patient. For tau deposition, Braak stage was IV/VI, and the Western blot analysis score was 9c/10. The neuropathological semiquantitative score for α-synuclein aggregation was 4. There was no inflammation. These results were compared with those of an age-matched patient with AD and a control devoid of any neurological disease.

Conclusion: In this Lewy body variant case, with globally stable functional and cognitive features, Aβ immunization resulted in a significant clearance of amyloid deposits, with remaining tau and synuclein pathological features in the brain. Patients with a Lewy body variant of AD should not be excluded from enrollment in Aβ-immunization trials.

Arch Neurol. 2007;64:583-587

Alzheimer disease (AD) is characterized by amyloid deposits and neurofibrillary degenerations. Almost 33% of AD cases have Lewy bodies and Lewy neurites, resulting from the aggregation of α-synuclein, and most cases of dementia with Lewy bodies (DLB) have some AD changes. Studies in transgenic mouse models for AD have shown that immunization with synthetic, preaggregated β-amyloid (Aβ) has reduced the extent and progression of AD pathological features and has prevented the cognitive decline in these animals. An international multicenter, double-blind phase 2 study of active immunization with Aβ1-42 (AN-1792(QS-21)) combined with the adjuvant QS-21 in mild to moderate AD was initiated in 2001 and discontinued after meningoencephalitis was reported. Neuropathological examination of 3 immunized patients showed evidence of partial clearance of amyloid plaques and inflammatory changes. We report the clinico-pathological observation of a patient with AD treated with AN-1792(QS-21), who subsequently developed clinical evidence of DLB.

REPORT OF A CASE

A 74-year-old patient with a history of hypertension complained of progressive episodic memory disturbance for 3 years. The Mini-Mental State Examination score was 19 of 30, the Mattis Dementia Rating Scale score was 102 of 144, and the digit span was 5 forward and 2 in reverse order. The selective reminding test showed a severe...
impairment in all the memory processes. She had impairment in calculation, gestural and constructional praxis, gnosis, and judgment. She presented with word-finding difficulty and semantic paraphasic errors. Parkinsonism, hallucination, sleep disorder, and fluctuations were absent. The clinical features were consistent with the diagnosis of probable AD. Her apolipoprotein genotype was E3/E3. She was treated with rivastigmine tartrate. Fourteen months later, the Mini-Mental State Examination score was 15 of 30. She was enrolled in the AN-1792 trial and received 2 injections of 225 µg of AN-1792 (Wyeth Research, Collegeville, Pa) plus 50 µg of QS-21 (Elan Pharmaceuticals, San Francisco, Calif) at an interval of 4 weeks (institutional review board approved). Rivastigmine was given as rivastigmine tartrate.

Neither focal amyloid deposit nor amyloid angiopathy was shown on Congo red and thioflavine stains. β-Amyloid immunolabeling did not disclose focal or diffuse deposition using 4 different anti-Aβ antibodies (ie, 3D6, 4G8, 6E10, and 6F3D) (Figure 2A and B). The Gallyas technique showed in the hippocampus and temporal cortex clusters of neuritic processes reminiscent of senile plaques but devoid of amyloid core (Figure 2C) that were tau immunoreactive (Figure 2D). Tau-immunoreactive neurofibrillary tangles and neuropil threads were labeled in the entorhinal cortex, hippocampus, limbic cortices, and, to a lesser extent, the prefrontal lobe, reaching the final score of IV/VI according to the Braak stage and 9c/10 according to the Western blot analysis. Hematoxylin-eosin staining and α-synuclein labeling showed many Lewy bodies in the brainstem and limbic and neocortical regions, with a semiquantitative score of 4 (very severe) (Figure 2E and F). There was neither inflammation nor metastatic dissemination. No microglial activation was detected by anti-CD68 (macrophage labeling), anti-CD68 (macrophage labeling), anti-CD34 (HLA-DR, HLA-DQ, HLA-DR), and tomatodectin at light microscopy examination. Moreover, the burden of CR3/43-positive cells was lower in the brain of the patient compared with the control with AD. All neuropathological data were confirmed by Western blot analysis of frozen tissue (Figure 3).

This study showed that Aβ immunization in a patient with a Lewy body variant of AD resulted in the total absence of Aβ plaques or angiopathy despite clear signs of AD,
namely, Braak stage IV neurofibrillary tangle pathological features and apparent “ghost plaques” with neuritic change without amyloid cores in the absence of lymphocyte or macrophage infiltrates. Several studies reported amyloid deposition in most patients with DLB,\textsuperscript{1,2,13} and there are clinical and neuropathological evidences to affirm that there were amyloid plaques in this brain before immunization: the patient had clinical features of AD, and the biochemical stage of tau pathological features was 9c, which correlated with a huge and widespread Aβ burden.\textsuperscript{11} At least, we suggest that clusters of tau-positive neurites may correspond to senile plaques cleared out of the amyloid core ("ghost plaques").

Our patient had indisputable DLB since there was an extensive cortical monoaggregation and dimers aggregation of α-synuclein. A lower Braak stage is consistent with DLB associated with AD pathological features.\textsuperscript{1} Lewy bodies were observed in the amygdala of 1 immunized patient,\textsuperscript{17} but this finding is not restricted to DLB and is reported in AD.\textsuperscript{24}

**Figure 2.** Neuropathological results for detection of amyloid, α-synuclein, and tau aggregation. A and B, Aβ-Amyloid immunolabeling of the frontal cortex of the AN-1792 case (A) and a control with Alzheimer disease (B). Dense deposits shown by the 6F3D antibody (Dako, Glostrup, Denmark) in the control brain tissue were totally absent in the cortex, the meninges, and the vessels of the AN-1792 patient (scale bar = 1 mm). C, The Gallyas silver technique showing neurofibrillary tangles (on the left) and the corona of a plaque (on the right) in the CA2 sector. D, The arrow points to the tau-immunoreactive neurofibrillary tangles and neuropil threads of the corona of a plaque in the same area as C stained by the AD2 antibody (gift from Mourton-Gilles, PhD, Montpellier, France; antibody raised against the phosphorylated serine residues 396-404 of tau) (scale bar = 50 µm). E and F, α-Synuclein immunolabeling of the CA2 sector of the temporal cortex (E) and the substantia nigra (F) from the AN-1792 patient showing extensive α-synuclein aggregation (Lewy bodies and Lewy neurites) (scale bar = 50 µm).
Figure 3. Western blot analysis results for β-amyloid (Aβ), α-synuclein, and tau detection. A, Detection of the Aβ_{42} species with the monoclonal antibody 21F12 showing no trace of aggregated Aβ_{42}. The same results were found for the Aβ_{40} species (results not shown). B, Detection of α-synuclein with the monoclonal antibody 3B5 (Innogenetics, Gent, Belgium) showing signals of 18 and 36 kDa in the main cortical areas, corresponding to a diffuse cortical synucleopathy. C, Analysis of pathological tau proteins with the monoclonal antibody AD2 showing a triplet of pathological tau proteins of the Alzheimer type in most neocortical areas, corresponding to a stage 9c of tau pathological features. AD indicates Alzheimer disease.

Consistent with the neuropathological findings observed in Aβ-immunized amyloid precursor protein transgenic mice, the 3 previous AN-1792(QS-21)–immunized human cases with AD showed a focal reduction of amyloid.
loid plaque burden, which was present to a lesser extent in some other cortical areas. A complete absence of extracellular amyloid deposits was described in 1 patient only in the frontal cortex. In our case, the use of immunization against Aβ42 peptides with AN-1792 (QS-21) resulted in the total absence of amyloid aggregate in the whole brain, possibly because (1) our patient had the highest level of immune response after only 2 injections of AN-1792 (QS-21); (2) autopsy was performed 3 years after immunization (2 years later than the previous cases), leaving more time for the plaques’ clearance. The absence of CD68-positive cells in this case, contrary to the others, might indicate the achievement of an earlier Aβ phagocytosis; and (3) amyloid plaques may be more of the diffuse type and less numerous in DLB than in AD.16-18, thus, the clearance may be facilitated. The Aβ42 plaque density is more important in patients with DBL than in patients with AD, whereas the Aβ40 density is lower in patients with DBL. However, it may be that antibodies generated after immunization with Aβ42 are primarily N-terminal specific.19

Focal inflammatory infiltrates of T lymphocytes in the meninges and the cerebrum, reported in the 2 cases with encephalitis,20 are not necessarily associated with the antibody response to immunization.20 We did not observe such cells in our case. The patient did not have clinical evidence of encephalitis; however, autopsy was performed 3 years after immunization, a delay that could be sufficient to clear inflammatory cells.

This case suggests that Aβ immunization resulted in a significant clearance of amyloid deposits with remaining tau and synuclein pathological features in a brain with Lewy body variant AD. Despite the concomitant digestive disease that caused the patient’s death, the functional and cognitive expression of the underlying degenerative brain disease remained globally stable during the 3 years after immunization. Thus, patients with a Lewy body variant should not be excluded from enrollment in Aβ-immunization trials.

Accepted for Publication: September 12, 2006.
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Author Contributions: Drs Bombois and Pasquier had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Bombois, Maurage, Delacourte, and Pasquier. Acquisition of data: Bombois, Maurage, Gompel, Deramecourt, Mackowiak-Cordoliani, Black, Lavielle, Delacourte, and Pasquier. Analysis and interpretation of data: Bombois, Maurage, Deramecourt, Delacourte, and Pasquier. Drafting of the manuscript: Bombois, Maurage, and Pasquier. Critical revision of the manuscript for important intellectual content: Gompel, Deramecourt, Mackowiak-Cordoliani, Black, Lavielle, and Delacourte. Administrative, technical, and material support: Bombois, Maurage, Gompel, Black, Lavielle, Delacourte, and Pasquier. Study supervision: Bombois, Lavielle, Delacourte, and Pasquier.

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