

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

Stéphanie Bombois, MD; Claude-Alain Maurage, MD, PhD; Marie Gompel, PhD; Vincent Deramecourt, MD; Marie-Anne Mackowiak-Cordoliani, MD; Ronald S. Black, MD; Rodolphe Lavielle, MD; André Delacourte, PhD; Florence Pasquier, MD, PhD

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 μ g of AN-1792 (β -amyloid [$A\beta$]) plus 50 μ g 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- $A\beta$ 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, $A\beta$ 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 $A\beta$ -immunization trials.

Arch Neurol. 2007;64:583-587

Author Affiliations: Resource and Research Memory Center, University Hospital, EA2691 (Drs Bombois, Deramecourt, Mackowiak-Cordoliani, and Pasquier), Service d'Anatomie Pathologique, Centre Hospitalier Régional Universitaire de Lille, EA2691 (Dr Maurage), and Unité Inserm 815 (Drs Maurage, Gompel, and Delacourte), Lille, France; Wyeth Research, Collegeville, Pa (Dr Black); and Wyeth Pharmaceuticals, Paris, France (Dr Lavielle).

ALZHEIMER DISEASE (AD) IS characterized by amyloid deposits and neurofibrillary degenerations. Almost 35% of AD cases have Lewy bodies and Lewy neurites,¹ resulting from the aggregation of α -synuclein, and most cases of dementia with Lewy bodies (DLB) have some AD changes.² Studies in transgenic mouse models for AD have shown that immunization with synthetic, preaggregated β -amyloid ($A\beta$) has reduced the extent and progression of AD pathological features and has prevented the cognitive decline in these animals.^{3,4} An international multicenter, double-blind phase 2 study of active immunization with $A\beta_{42}$ (AN-1792) combined with the adjuvant QS-21 in mild to moderate AD was initiated in 2001 and discontinued after

meningoencephalitis was reported.⁵ Neuropathological examination of 3 immunized patients showed evidence of partial clearance of amyloid plaques and inflammatory changes.⁶⁻⁸ We report the clinicopathological 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

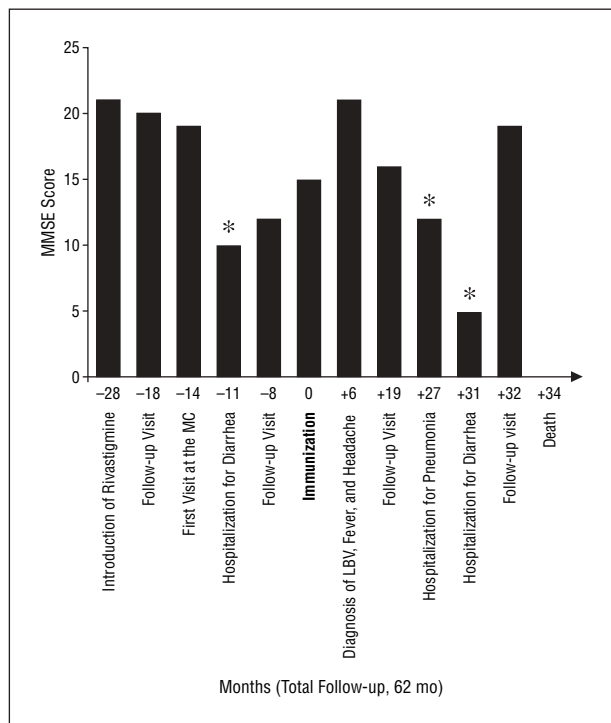


Figure 1. Subsequent Mini-Mental State Examination (MMSE) scores during the clinical follow-up of the patient. After immunization, cognitive evaluations remained globally stable despite fluctuations and confusional episodes due to somatic symptoms. Rivastigmine was given as rivastigmine tartrate. MC indicates memory center; LBV, Lewy body variant of Alzheimer disease. * Hospitalization for concomitant somatic episode.

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.⁹ 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 approval from Comité Consultatifs de Protection des Personnes se prêtant à des Recherches Biomédicales, Paris-Cochin, France, July 23, 2001). The patient was considered an antibody responder to immunization with an IgG anti-AN-1792 antibody titer exceeding 10 000 (threshold, 1/2200) and an IgM titer exceeding 3500. Maximum serum anti-Aβ titers were reached in 4.7 months. Six months after the first injection, she presented with a fever of 38°C and headache. Although she always refused the lumbar puncture, encephalitis was rejected because of the absence of neurological modification and white matter changes on MRI. In the next months, she developed marked visual hallucinations, somniloquy, dreams, and disorientation on waking. The neurological examination revealed gait instability, right-hand resting tremor, and hypertonia. During the 32 following months after immunization, the Mini-Mental

State Examination score remained globally stable, despite fluctuations and several confusional episodes with pneumonia or diarrhea (**Figure 1**), whereas the Lewy body features became more obvious. She lived at home with a professional caregiver after her husband's death, which she assumed adequately. She remained stable for cognitive functioning and instrumental activities of daily living. The patient died 34 months postimmunization, after an acute abdominal syndrome not related to the brain disease. The abdominal examination revealed numerous liver metastases of an adenocarcinoma.

Autopsy was performed 9 hours post mortem. The brain weighed 920 g (including the cerebellum). The hippocampus was more severely atrophied than the hemispheres. The right hemisphere was frozen and the left hemisphere was fixed in formalin for 6 weeks. The neuropathological examination was established according to consensus recommendations.^{1,10} The amyloid and tau pathological features were semiquantified by immunohistochemical and Western blot analyses as already described.¹¹ Synucleopathy was semiquantified by immunohistochemical analysis using the antibody 3B5 (Innogenetics, Gent, Belgium) and Western blot analysis.² Age-matched controls were 1 patient with AD (apolipoprotein genotype, E3/E4) and 1 woman devoid of any neurological disease (apolipoprotein genotype, E3/E4).

RESULTS

Neither focal amyloid deposit nor amyloid angiopathy was shown on Congo red and thioflavine stains. β-Amyloid immunolabeling did not disclose focal or diffuse deposit 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 stage¹⁰ and 9c/10 according to the Western blot analysis.¹² 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**).¹ There was neither inflammation nor metastatic dissemination. No microglial activation was detected by anti-CD68 (macrophage labeling), anti-CR3/43 (HLA-DP, HLA-DQ, HLA-DR), and tomatolectin 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**).

COMMENT

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,

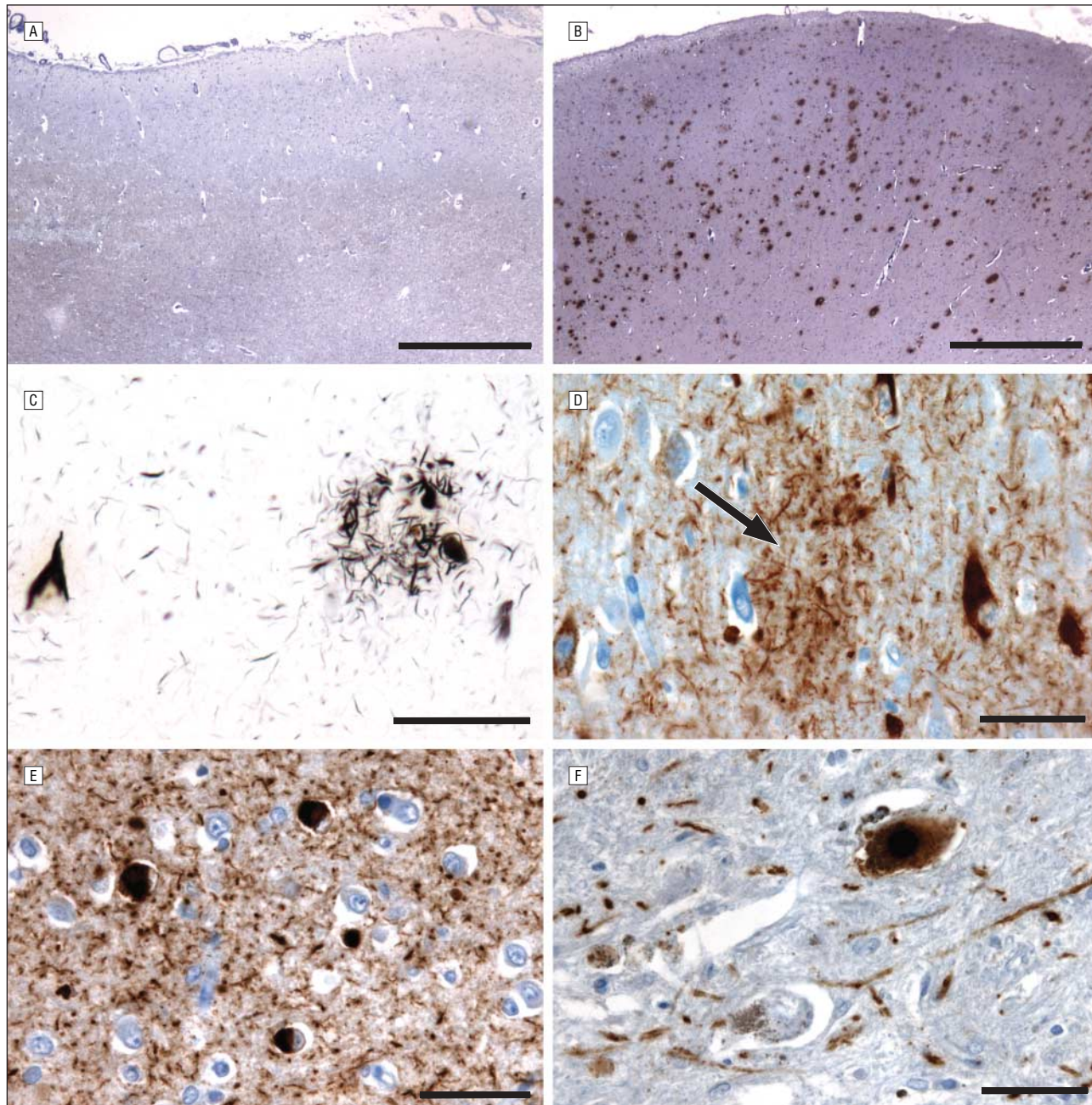


Figure 2. Neuropathological results for detection of amyloid, α -synuclein, and tau aggregation. A and B, β -Amyloid immunolabeling of the frontal cortex of the AN-1792 case (A) and a control with Alzheimer disease (B). Dense deposits shown by the 6F3D antibody (Dako, Glostrup, Denmark) in the control 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 μ m). 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 μ m).

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,^{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 wide-

spread A β burden.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.¹ Lewy bodies were observed in the amygdala of 1 immunized patient,⁷ but this finding is not restricted to DLB and is reported in AD.¹⁴

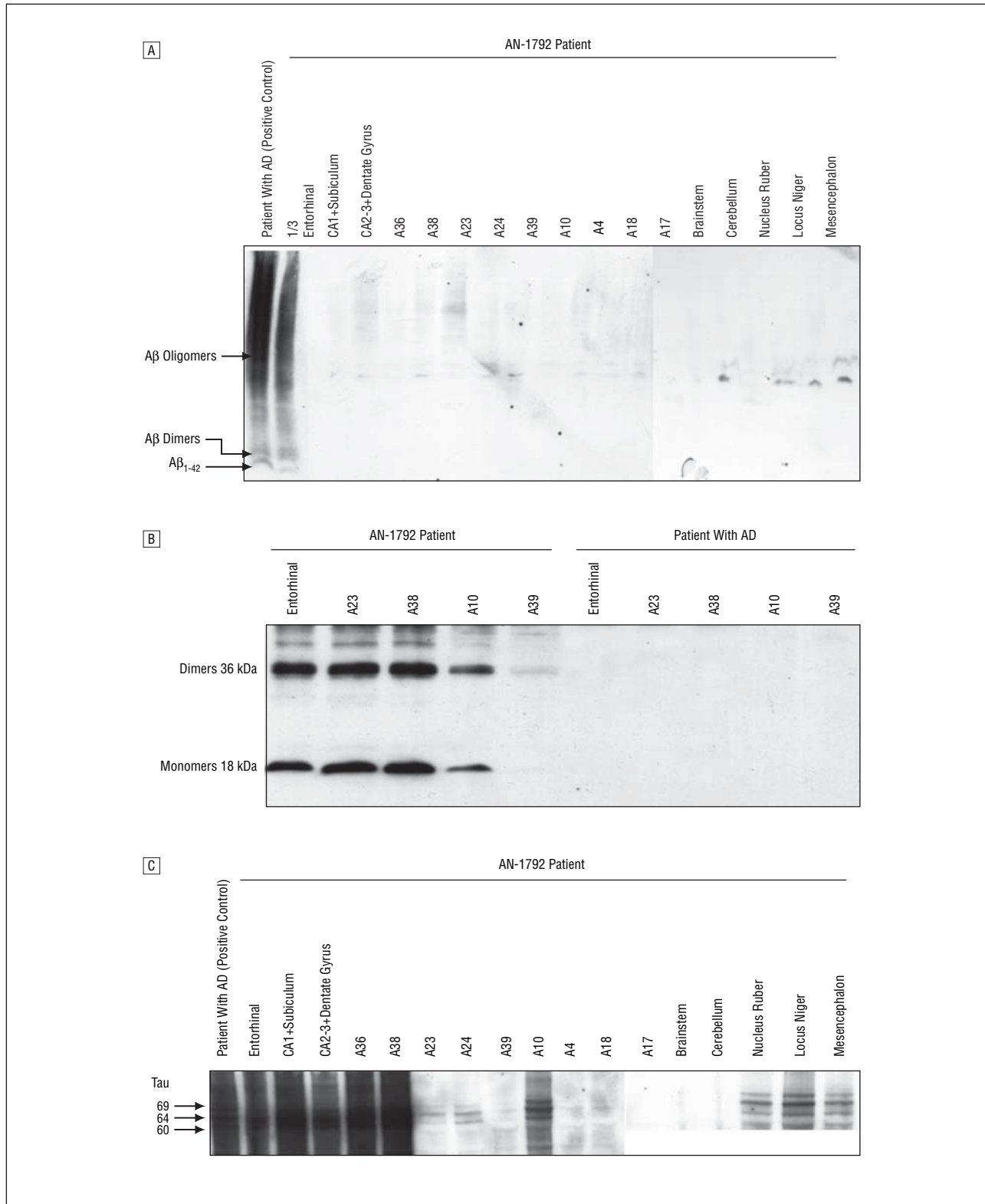


Figure 3. Western blot analysis results for β -amyloid ($A\beta$), α -synuclein, and tau detection. A, Detection of the $A\beta_{42}$ species with the monoclonal antibody 21F12¹¹ showing no trace of aggregated $A\beta_{42}$. The same results were found for the $A\beta_{40}$ species (results not shown). 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 synucleinopathy. C, Analysis of pathological tau proteins with the monoclonal antibody AD2¹² 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 $A\beta$ -immunized amyloid precursor protein transgenic

mice,^{3,15} the 3 previous AN-1792(QS-21)-immunized human cases with AD⁶⁻⁸ showed a focal reduction of amy-

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.⁸ In our case, the use of immunization against A β ₄₂ 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 A β phagocytosis; and (3) amyloid plaques may be more of the diffuse type and less numerous in DLB than in AD¹⁶⁻¹⁸; thus, the clearance may be facilitated. The A β ₄₂ plaque density is more important in patients with DLB than in patients with AD, whereas the A β ₄₀ density is lower in patients with DLB.¹³ However, it may be that antibodies generated after immunization with A β ₄₂ 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,^{6,7} 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 A β 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 A β -immunization trials.

Accepted for Publication: September 12, 2006.

Correspondence: Florence Pasquier, MD, PhD, Department of Neurology, University Hospital, 59037 Lille, France (pasquier@chru-lille.fr).

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 ma-*

terial support: Bombois, Maurage, Gompel, Black, Lavielle, Delacourte, and Pasquier. *Study supervision:* Bombois, Lavielle, Delacourte, and Pasquier.

Financial Disclosure: Drs Black and Lavielle are full-time employees of Wyeth Pharmaceuticals and hold stock in the company.

REFERENCES

- McKeith IG, Dickson DW, Lowe J, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. *Neurology*. 2005;65:1863-1872.
- Deramecourt V, Bombois S, Maurage CA, et al. Biochemical staging of synucleinopathy and amyloid deposition in dementia with Lewy bodies. *J Neuropathol Exp Neurol*. 2006;65:278-288.
- Schenk D, Barbour R, Dunn W, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature*. 1999;400:173-177.
- Janus C, Pearson J, McLaurin J, et al. A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature*. 2000;408:979-982.
- Orgogozo J-M, Gilman S, Dartigues JF, et al. Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. *Neurology*. 2003;61:46-54.
- Nicoll JA, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO. Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case-report. *Nat Med*. 2003;9:448-452.
- Ferrer I, Boada Rovira M, Sanchez Guerra ML, Rey MJ, Costa-Jussa F. Neuropathology and pathogenesis of encephalitis following amyloid-beta immunization in Alzheimer's disease. *Brain Pathol*. 2004;14:11-20.
- Masliah E, Hansen L, Adame A, et al. Abeta vaccination effects on plaque pathology in the absence of encephalitis in Alzheimer disease. *Neurology*. 2005;64:129-131.
- McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology*. 1984;34:939-944.
- Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol (Berl)*. 1991;82:239-259.
- Delacourte A, Sergeant N, Champain D, et al. Nonoverlapping but synergetic tau and APP pathologies in sporadic Alzheimer's disease. *Neurology*. 2002;59:398-407.
- Delacourte A, David J-P, Sergeant N, et al. The biochemical pathway of neurofibrillary degeneration in aging and Alzheimer's disease. *Neurology*. 1999;52:1158-1165.
- Lippa CF, Ozawa K, Mann DM, et al. Deposition of beta-amyloid subtypes 40 and 42 differentiates dementia with Lewy bodies from Alzheimer disease. *Arch Neurol*. 1999;56:1111-1118.
- Popescu A, Lippa CF, Lee VM, Trojanowski JQ. Lewy bodies in the amygdala: increase of alpha-synuclein aggregates in neurodegenerative diseases with tau-based inclusions. *Arch Neurol*. 2004;61:1915-1919.
- Bacskaï BJ, Kajdasz ST, Christie RH, et al. Imaging of amyloid-beta deposits in brains of living mice permits direct observation of clearance of plaques with immunotherapy. *Nat Med*. 2001;7:369-372.
- Hansen LA, Masliah E, Galasko D, Terry R. Plaque-only Alzheimer disease is usually the Lewy variant, and vice versa. *J Neuropathol Exp Neurol*. 1993;52:648-654.
- Dickson DW, Crystal H, Mattiace LA, et al. Diffuse Lewy body disease: light and electron microscopic immunocytochemistry of senile plaques. *Acta Neuropathol (Berl)*. 1989;78:572-584.
- Armstrong RA, Cairns NJ, Lantos PL. beta-Amyloid (A beta) deposition in the medial temporal lobe of patients with dementia with Lewy bodies. *Neurosci Lett*. 1997;227:193-196.
- Lee M, Bard F, Johnson-Wood K, et al. Abeta immunization in Alzheimer's disease generates Abeta N-terminal antibodies. *Ann Neurol*. 2005;58:430-435.
- Gilman S, Koller M, Black RS, et al. Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. *Neurology*. 2005;64:1553-1562.