Distinct Patterns of Antiamyloid-β Antibodies in Typical and Atypical Alzheimer Disease

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Objective: To compare serum antiamyloid-β (Aβ) antibodies in typical and atypical Alzheimer disease (AD).

Design: Preliminary observations.

Subjects: Thirteen patients with AD, 8 patients with posterior cortical atrophy with evidence of AD (PCA-AD) pathophysiological process by both cerebrospinal fluid (CSF) biomarkers and amyloid imaging, and 12 age-matched control individuals.

Interventions: The class and subclass levels of serum anti-Aβ antibodies were measured using an oligomer-based enzyme-linked immunosorbent assay. This method allowed measuring both free antibodies and, after acidic treatment, the total fraction that includes all antibodies complexed with circulating Aβ42 and any cross-reacting antigen.

Results: Anti-Aβ IgG were restricted to the IgG1 and IgG3 subclasses. Their total levels were strikingly lower and more homogeneous in patients with PCA compared with both typical AD and controls, while biomarkers of amyloid deposition (CSF Aβ42 and positron emission tomography amyloid imaging) were similar in patients with AD and patients with PCA.

Conclusions: Serum anti-Aβ IgG1 and IgG3 antibodies differ between distinct forms of AD. Its significance is discussed for possible implications as immune effectors in the specific pathophysiology of AD variants.


Brain amyloid-b peptide (Aβ) deposition is a major feature of Alzheimer disease (AD). IgG antibodies directed to Aβ naturally occur in elderly persons, and their serum and cerebrospinal fluid (CSF) levels may be significantly altered in the course of AD. While anti-Aβ antibodies may help to control the development of amyloid plaques, the clinical significance of their serum levels remains unclear.

We, and others, recently showed that posterior cortical atrophy (PCA) may be defined as an atypical focal form of AD (PCA-AD). While AD and PCA-AD display clearly distinct clinical presentations, they are similar for biomarkers of amyloid deposition, such as CSF Aβ42 levels and Pittsburgh Compound B (PiB) binding patterns. In the present study, we compared the pattern of serum anti-Aβ antibodies in typical AD and PCA-AD.

Methods

Patients

Eight patients with PCA-AD were enrolled according to the following diagnostic criteria: (1) gradual progression of cognitive impairment beginning with visual complaints; (2) presentation with visuospatial deficits with intact primary visual function; (3) features suggestive of Balint syndrome associated or not with Gerstmann syndrome; (4) proportionally less episodic memory impairment; (5) relatively preserved insight; (6) no parkinsonian signs; (7) glucose hypometabolism on fluorodeoxyglucose F18–positron emission tomographic (PET) examination; (8) cortical atrophy on magnetic resonance imaging with a predominance in the posterior cortical region; (9) an AD profile of CSF biomarkers (see the “CSF Biomarker Analysis” subsection); and (10) a global cortical index of radiolabeled carbon 11 (11C)–PiB uptake on PiB-PET > 1.6 (see Table note b for details).

Thirteen patients with typical AD (Clinical Dementia Rating [CDR] ≥0.5) matched to patients with PCA-AD for age, disease duration, and Mini-Mental State Examination (MMSE) score were selected according to the New Research Criteria. Twelve healthy elderly controls were recruited according to the following criteria: (1) MMSE score ≥27/30 and CDR = 0; (2) no history of neurological or psychiatric disorders; and (3) no memory complaint or cognitive deficit.
Table. Demographic and Clinical Data of Studied Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients With PCA-AD (n = 8)</th>
<th>Patients With AD (n = 13)</th>
<th>Control Individuals (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/male</td>
<td>6/2</td>
<td>4/9</td>
<td>4/8</td>
</tr>
<tr>
<td>Age, (SD) [minimum-maximum], y</td>
<td>63.3 (3.6) [59.5-69.0]</td>
<td>63.7 (9.8) [54.5-89.1]</td>
<td>67.3 (8.5) [52.9-81.3]</td>
</tr>
<tr>
<td>Disease duration, (SD) [minimum-maximum], y</td>
<td>3.1 (1.5) [1-5]</td>
<td>3.1 (1.1) [1-5]</td>
<td>NA</td>
</tr>
<tr>
<td>MMSE</td>
<td>17.5 (6.2) [9-27]a</td>
<td>19.1 (5.1) [10-27]a</td>
<td>29.5 (1.0) [27-30]</td>
</tr>
<tr>
<td>CDR</td>
<td>CDR = 0.5 (n = 3)</td>
<td>CDR = 0.3 (n = 4)</td>
<td>CDR = 0 for all controls</td>
</tr>
<tr>
<td>CDR</td>
<td>CDR = 1 (n = 3)</td>
<td>CDR = 1 (n = 7)</td>
<td>NA</td>
</tr>
<tr>
<td>CDR</td>
<td>CDR = 2 (n = 2)</td>
<td>CDR = 2 (n = 2)</td>
<td>NA</td>
</tr>
<tr>
<td>CSF biomarkers: ratio Aβ42/Tau, (SD) [minimum-maximum]</td>
<td>0.39 (0.15) [0.19-0.64]</td>
<td>0.30 (0.11) [0.16-0.43]</td>
<td>NA</td>
</tr>
<tr>
<td>[11C]-PiB SUVR global index, (SD) [minimum-maximum]</td>
<td>2.57 (0.44) [1.72-3.17]a</td>
<td>2.53 (0.47) [1.65-3.27]a</td>
<td>1.23 (0.17) [0.86-1.39]</td>
</tr>
</tbody>
</table>

Abbreviations: Aβ, amyloid β; AD, Alzheimer disease; CDR, Clinical Dementia Rating; CSF, cerebrospinal fluid; MMSE, Mini-Mental State Examination; NA, not applicable; PCA-AD, posterior cortical atrophy AD; PiB SUVR, Pittsburgh Compound B standard uptake value ratio.

a Calculated with the formula Aβ42/[240 + (1.18 × T-Tau)].

b Positron emission tomographic examinations were performed with an HRRT tomograph (Siemens). Images were analyzed using BrainVISA software as previously described.

c Positron emission tomographic examinations were performed with an HRRT tomograph (Siemens). Images were analyzed using BrainVISA software as previously described. Briefly, parametric images of the SUVR constructed on late images (after 50-70 min acquisition) with the cerebellum as the reference region were coregistered individually with the 3-dimensional magnetic resonance (MR) T1-weighted images. The volumes of interest (VOIs) were delineated on the individual MR images for each subject after segmentations in 78 anatomical regions using an automated anatomical labeling atlas (cortical and subcortical structures) and SACHA software (amygdala and hippocampi). Then, anatomical regions were pooled from regions provided by automated anatomical labeling atlas segmentation and were defined as the following: (1) frontal cortex by grouping orbitofrontal, polar prefrontal and dorsolateral cortex; (2) anterior cingulate; (3) medium cingulate; (4) posterior cingulate; (5) precuneus; (6) occipital cortex; by grouping calcicortex, occipital cortex and cuneus; (7) temporal cortex by grouping anterior and lateral temporal cortex; (8) hippocampus; and (9) parietal cortex by grouping inferior and superior parietal cortex and the parieto-temporal junction. The radiolabeled carbon 11-PiB global index represents the subject’s mean SUVR in all the defined regions.

**CSF BIOMARKER ANALYSIS**

Levels of total Tau (T-Tau), T181-phosphorylated Tau (P-Tau), and Aβ42 were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s (Innogenetics) instructions in CSF samples from all the patients except 3 patients with AD. We calculated derived ratios from single biomarkers, including T-Tau/Aβ42 and P-Tau/Aβ42. We also calculated the Aβ:Tau ratio, defined by the formula Aβ42/[240 + (1.18 × T-Tau)], where a score below 0.8 is suggestive of AD.

**SERUM ANTI-Aβ ANTIBODY MEASUREMENT**

Serum samples were diluted at 1:50, and levels of anti-Aβ were analyzed by indirect ELISA using oligomeric Aβ-coated plates, with and without prior dissociation of immune complexes at pH 3.5, as described by Britschgi et al. IgG, IgM, and IgG subclasses were revealed using specific antibodies (Jackson ImmunoResearch) and monoclonal antibodies N1L6, GOM2, ZG4, and Rj4 (University of Birmingham). Anti-Aβ monoclonal antibody BAM-10 diluted to 0.5 µg/mL was used as an internal reference in each ELISA plate, and results were expressed as ratios of the samples’ optical densities to that yielded by BAM-10.

**MEASUREMENT OF SERUM TOTAL IgG AND IgM LEVELS**

Serum IgG and IgM levels were measured by the immunoturbidimetric method (Abbott Labs). Serum IgG subclass levels were measured using a competitive ELISA with specific monoclonal antibodies.

**STATISTICAL ANALYSES**

All the comparisons of antibody levels were performed using the Mann-Whitney nonparametric test for comparison of means between 2 groups and the Wilcoxon test (GraphPad Prism Software, Inc) for paired AD and PCA-AD cases.

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**STANDARD PROTOCOL APPROVALS, REGISTRATIONS, AND PATIENT CONSENTS**

The study was conducted by the Institut National de la Santé Et la Recherche Médicale (INSERM; grant ANR-07-LVIE-002-01) and was approved by the Ethics Committee of Pitie-Salpêtrière Hospital. All the subjects provided written informed consent before participating. The controls underwent the same procedure as did the patients with AD and PCA-AD except for lumbar puncture, which was not proposed for ethical reasons.

**[11C]-PiB PET IMAGING PROCEDURES**

PET imaging with [11C]-labeled PiB was performed in all the subjects except 4 controls. The method was the same as previously described and summarized in table legend c.
RESULTS

CSF BIOMARKERS AND 11C-PiB PET IMAGING

No statistical difference was observed between the AD and PCA-AD groups concerning Aβ42, T-Tau, P-Tau levels, and Aβ:Tau ratios. The mean PiB indices were identical in the PCA-AD and AD groups concerning all the analyzed regions of interest (Figure 1 and Table).

SERUM ANTI-Aβ ANTIBODY LEVELS

Acidic pretreatment of serum samples allowed the evaluation of the total fractions of anti-Aβ antibodies after dissociation of immune complexes, while analyses of untreated sera measured the free antibody fractions. As shown in Figure 2, total serum anti-Aβ antibodies essentially belonged to the IgM class and IgG1 and IgG3 subclasses. This total fraction may include all antibodies that are initially complexed with circulating Aβ40/42 as well as any other cross-reacting antigen. While IgM
antibody levels were quite similar in the 3 groups, patients with PCA-AD displayed significantly lower and more homogeneous concentrations of IgG antibodies than patients with AD and healthy controls (Figure 2A), which was most evident for IgG1 (Figure 2B). Comparison of typical AD and PCA-AD cases paired for age, disease duration, and CDR confirmed that those with PCA-AD have lower IgG1 (P = .008) and IgG3 (P = .023) circulating anti-Ab antibodies.

Overall serum IgG, IgM, and IgG subclass levels were similar in the PCA-AD and AD groups (eFigure 1; http://www.archneurol.com), which ruled out possible immunoglobulin deficiencies. Comparisons of ratios between anti-Ab antibodies and corresponding total immunoglobulin levels for each class and subclass confirmed that anti-Ab IgG1 and IgG3 were strongly and specifically lower in patients with PCA-AD than in patients with AD (P < .001 for both IgG1 and IgG3, eFigure 2).

Anti-Ab antibodies in both groups of samples—total and free anti-Ab antibodies—were found only for iso-types IgM, IgG1 and IgG3. No significant difference was found between the free antibody fractions of controls, patients with PCA-AD and patients with AD, except for free anti-Ab IgG1, which were lower in patients with PCA-AD than in patients with AD (P = .03, data not shown).

Concerning all groups, no significant correlation was found between antibody levels and any of the tested clinical data (age, MMSE, disease duration), levels of CSF biomarkers and PiB global index. No correlation was observed between antibody levels and global PiB index nor CSF biomarkers when we pooled patients with AD and patients with PCA-AD.

### COMMENT

This preliminary study explored anti-Ab antibodies in carefully identified populations of patients with typical AD and patients with PCA-AD, the latter being defined as PCA syndrome associated with clear biological and imaging evidence of AD pathophysiological process. The total amount of serum anti-Ab IgG antibodies, especially those of the IgG1 subclass, was strikingly lower in patients with PCA-AD compared with patients with typical AD and aged controls.

Serum anti-Ab antibodies occur naturally in elderly individuals, and their role in AD remains unclear. Recent studies suggested that serum anti-Ab antibodies may have beneficial effects on amyloid pathology and neuron toxicity. In typical patients with AD, previous results showed a striking heterogeneity of serum antibody levels, which may be decreased, increased or unchanged compared with healthy controls. Such divergent results may relate to a diversity of methods used for anti-Ab antibody assessment. A 2010 report by Storace et al showed that the total fraction of serum anti-Ab antibodies was higher in patients with mild cognitive impairment who progressed to AD than stable cases, suggesting that this blood marker is associated with AD progression. Using a similar method, we observed a clear difference in serum total anti-Ab IgG antibody levels between patients with AD and patients with PCA-AD, while their amyloid pathological markers were similar.

Thus, differences in serum anti-Ab antibodies may hardly be explained by mere adsorption onto amyloid deposits. However, this difference may relate to differential intracerebral reactivity with molecular species that do not fix PiB, such as Ab monomers or soluble oligomers. Intergrup differences were evident for IgG1 and IgG3 subclasses, which were lower and more homogeneous in the patients with PCA-AD. IgG1 and IgG3 have the unique ability to bind FcγR-I receptors on monocytes/macrophages, which are actively recruited to the brain parenchyma in the course of AD. Thus, lower antibody levels in patients with PCA-AD might relate to more efficient recruitment of anti-Ab-bearing monocytes to the central nervous system. On the other hand, lower antibody levels in patients with PCA-AD may result from weaker activation of anti-Ab immune responses.

IgG1 and IgG3 are the most potent IgG subclasses in activating the complement classical pathway through C1q binding. Because complement fragments and receptors, including C1q and CR1, are involved in neuroprotection, neuroinflammation, or both, different rates of IgG1 and IgG3 antibody transport to the brain might contribute to pathological differences between focal and classical forms of AD.

Because PCA seems to remain a focal posterior disease even at the late stage of evolution, it provides a model for studying in vivo factors that might influence AD progression and the interactions between amyloid and Tau pathology. Our results point to new targets for investigating this crucial field of research by showing different implications of anti-Ab immune effectors between typical AD and PCA-AD.

In conclusion, our observations support the idea that systemic immunological responses, especially the anti-Ab IgG1 and IgG3 antibodies, could contribute to shaping clinical presentations of AD. The above hypotheses require studies on a larger series of patients and further investigations into the specific pathophysiology of typical and atypical forms of AD, with particular focus on immunological features. Such studies would allow better understanding of the pathophysiology of the disease and could lead to the development of innovative immunotherapy approaches in AD.
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Author Contributions: Drs Dorothee, Moukari, de Souza, Maroy, Dubois, Sarazin, and Aucouturier had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs Sarazin and Aucouturier contributed equally to the manuscript. Study concept and design: Dorothee, Bottlaender, Maroy, Dubois, Sarazin, and Aucouturier. Acquisition of data: Bottlaender, Moukari, de Souza, Corlier, and Sarazin. Analysis and interpretation of data: Dorothee, Bottlaender, Moukari, de Souza, Maroy, Chupin, Lamari, Lehéricy, Dubois, Sarazin, and Aucouturier. Drafting of the manuscript: Bottlaender, Moukari, Maroy, Dubois, Sarazin, and Aucouturier. Critical revision of the manuscript for important intellectual content: Dorothee, de Souza, Maroy, Corlier, Colliot, Chupin, Lamari, Lehéricy, Dubois, Sarazin, and Aucouturier. Statistical analysis: Moukari, de Souza, Maroy, Colliot, Lamari, Dubois, and Aucouturier. Obtained funding: Sarazin. Administrative, technical, and material support: Maroy, Corlier, Chupin, Dubois, Sarazin, and Aucouturier. Study supervision: Dorothee, Maroy, Dubois, Sarazin, and Aucouturier.

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REFERENCES