Anti-RAGE and Aβ Immunoglobulin Levels Are Related to Dementia Level and Cognitive Performance

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Background. Blood-based immunoglobulins (IgGs) may mark the presence of amyloid plaques characterizing the progression of Alzheimer’s disease (AD). Previous studies suggest that anti-RAGE and anti-Aβ IgGs increase proportionately with accumulation of amyloid-beta (Aβ) peptides at receptor sites for advanced glycation end products (RAGE), within cortical areas of brain tissue. We assessed the relationship between these potential markers and an AD-type cognitive profile. We hypothesized that these specific IgG levels would be positively correlated with Clinical Dementia Rating (CDR) scores as well as index scores on the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) in domains associated with cortical function.

Methods. Participants were 118 older adults (mean age = 74, standard deviation = 10.5) drawn from the community and local physician referrals. Participants were reassigned into five groups based on CDR. Blood IgG levels were determined through an affinity purification process.

Results. Analysis of covariance analyses revealed that CDR scores were significantly related to anti-RAGE, F(4,106) = 12.93, p < .001, and anti-Aβ, F(4,106) = 17.08, p < .001, after controlling for age and total IgG levels. Regression analyses indicated significant variance accounted for by anti-RAGE and anti-Aβ above and beyond total IgG effects. Additional regression identified specific RBANS domains accounting for significant variance in anti-RAGE levels including language (t = −3.74, p < .001) and delayed memory (t = −2.31, p < .05), whereas language accounted for a significant amount of variance in anti-Aβ levels (t = −3.96, p < .001).

Conclusions. Anti-RAGE and anti-Aβ IgGs correlate strongly with global scores of dementia. Furthermore, they are associated with a profile of deficiency in domains associated with specific cortical function. Results suggest potential for anti-Aβ and anti-RAGE IgGs as blood biomarkers for AD.

Key Words: Dementia—Alzheimer’s disease—Biomarker—RAGE—Aβ—Immunoglobulin—Cognition.

AGE distributions have shifted toward a more aged population (1,2), and planning for resultant changes in health-related needs requires significant attention. The leading causes of death in industrialized nations have changed over the past century from infectious to chronic diseases (3). Among major age-related diseases, such as atherosclerosis, diabetes, cancer, and Alzheimer’s disease (AD), the main factor contributing to disease pathology is immune chronic inflammation (4). This study assesses the role of the immune system in dementing illness with particular attention to AD.

AD is a neurodegenerative disease marked by increasing deterioration of brain tissue, concentrated in the neocortex and hippocampus. Core symptoms include deficits in memory and cognition. The pathology of AD is complicated, but several abnormalities of structure and function in the brain are hallmarks of the disease. The acetylcholine (ACh) neurotransmitter system—in involved in cognition and memory—is underproductive in the AD brain. Primary treatments for the disease, acetylcholinesterase (AChE) inhibitors, work to revitalize cholinergic functioning by blocking the activity of AChE, which normally breaks down ACh (5). Lack of function in this system is probably due to physical degeneration of neuronal cell tissue, a process resulting in ventricular enlargement, development of neurofibrillary tangles, and accumulation of extracellular amyloid plaques.

Research supports a central role for amyloid-beta (Aβ) proteins in these plaques. Aβ protein is composed of 40–42 amino acid peptides in the amyloid protein family; is known for having a high beta-sheet secondary structure making it resistant to degradation; and is associated with a tendency to aggregate (for review see 6). Although Aβ formation was once viewed as the major contributor to neuronal death in AD, this theory has been challenged (7). Recent studies support a complex etiology of AD, with interactions among many types of molecules in the AD brain and periphery (8,9,10).
One pathway begins with the neuronal membrane receptor for advanced glycation end products (RAGE) that normally binds advanced glycation end products (AGE) and mediates normal aging processes in tissues. Evidence suggests that the gene that codes for RAGE is overexpressed in areas that degenerate in AD—the hippocampus and frontal lobe—and that this receptor binds Aβ strands (11). This not only allows for amyloid aggregation but also stimulates an immune inflammatory response. RAGE-Aβ interaction causes macrophage colony-stimulating factor to be released, which interacts with its complement receptor, c-fms, on microglial cells. This immune activation triggers many characteristics of AD pathology and leads to cytotoxicity of neurons (8).

Although nonspecific immune inflammatory responses have been investigated, specific immune processes have only recently been considered in dementia. Schenk and colleagues (12) showed near-complete prevention of Aβ deposition in a transgenic platelet-derived growth factor-driven human amyloid precursor protein mouse immunized with synthetic human Aβ. Subsequent studies have shown anti-Aβ autoantibodies present at low levels in blood plasma samples of an elderly population (13). Other studies have shown circulating anti-Aβ immunoglobulins (IgGs) in periphery, although levels were not always specific to AD (14,15).

Such results suggest potential immunotherapies for AD—if low levels of autoantibodies are present naturally, then passive immunization with similar antibodies directed at autoantigens characteristic of the disease probably will not be toxic. In support, Dodel and colleagues (16) showed a reduction in levels of Aβ in blood plasma and cerebrospinal fluid samples in human AD patients after immunization with anti-Aβ IgG and associated improvements in cognitive abilities for a 6-month period. Active immunization using prime and booster aggregates of Aβ-42 also have shown slowing of cognitive decline as a function of levels of anti-Aβ antibodies (17). The mechanism for interaction between degenerative processes in the brain and circulating IgGs could result from compromises in the integrity of the blood brain barrier (BBB) (18) via microtrauma, microvascular pathology, or local inflammation (19).

These studies indicate that Aβ may play a part in cognitive decline seen in AD and interacts with general and specific immune systems. Understanding specific immunity may be key to finding better therapies and diagnostic methods.

Existing diagnostic methods are limited and restricted to ex vivo techniques to diagnose AD on a biologic level. Current clinical diagnostic methods include behavioral and neuropsychological tests, neuroimaging, blood chemistry markers, and other disease-status rule-outs. However, a biologic substrate specific to the disease would be an ideal identifier of AD brain pathology. Past potential biomarkers have been too expensive, invasive, and inconclusive (20). However, recent studies by Zhang and Pardridge (18) and Bouras and colleagues (19) showing evidence for BBB permeability support the hypothesis that an immune reaction to AD pathology may be seen in circulating blood. Thus, a blood measure would be a good option for diagnosis.

This idea was supported in two studies. Mice immunized with a human neurofilament-derived AGE unexpectedly developed antibodies directed against both Aβ and a RAGE peptide fragment (21). Results suggested that RAGE-Aβ interaction was a catalyst for autoimmune responses and that immune activation may not just be general inflammation observed by Yan and colleagues (8), but may include the generation of specific antibodies. In a study comparing plasma levels of Aβ and RAGE IgGs with cognition in demented and healthy control participants, Mruthini and colleagues (22) found that when an affinity purification method was used to determine antibody (IgG) titer values specific to RAGE or Aβ antigen molecules in blood, AD patients had significantly higher endogenous levels of both IgGs than healthy controls of the same age. This indicates that IgGs specific to RAGE and Aβ may be of use as a diagnostic tool for AD. Low—molecular weight (LMW) 1-42 amino acid Aβ peptide fragments may be more damaging to cells than other high—molecular weight proteins. It has been shown that protein hydrolyzed into peptides resulted in a greater increase in cellular injury compared with an equivalent amount by weight of unhydrolyzed protein (23). AGEs can also lead to enhanced formation of free radicals both directly through catalytic sites in their molecular structure and via stimulation of membrane-bound NAD(P) H oxidase through the RAGE receptor and depletion of cellular antioxidant systems (24). LMW-AGEs are able to bind in a stable fashion with AGE receptors, and the predominant effector of pathogenic receptors (such as RAGE) may be LMW-AGEs rather than AGE proteins (25). The serum concentration of LMW-AGEs has been correlated with the severity of renal disease and with aging (23,26).

This study examined the relationships between IgGs directed at both RAGE and Aβ and specific areas of cognition. Participants across levels of cognitive functioning were given a Clinical Dementia Rating (CDR) (27), the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) (28), and provided a blood sample. CDR and RBANS scores were used as standards of functional dementia with which concentrations of anti-RAGE and anti-Aβ IgGs in blood plasma were compared.

We hypothesized that participants with higher CDR scores would have higher levels of IgGs in their blood plasma titers. Participants with low RBANS scores in cognitive areas associated with cortical functioning, that is, language and memory, would have high levels of the target plasma IgGs. RBANS has been shown to differentiate between “cortical” and “subcortical” dementias (29), and one hallmark of AD is degeneration in temporal and frontal cortical areas of the brain.

In order for anti-RAGE and anti-Aβ IgGs to be good markers of AD, they must also be unrelated to other possible
confounding factors. As age is the largest predictor of AD (30), this study examined whether participant age might account for any variance in IgG levels. Finally, circulating levels of total IgGs may confound findings of specific IgGs, so we additionally examined this role.

METHODS

Neurological Disorders Database Repository Database

The Neurological Disorders Database Repository (NDDR, Alzheimer’s Research Center, Medical College of Georgia, Augusta, GA.) is an ongoing collaborative effort hosted by the Medical College of Georgia (MCG) that collects information useful for research of neurological disorders, focusing on AD. For each participant, the NDDR contains scores of cognitive functioning, functional measures, and biologic indices. Based on a clinical diagnosis, local physicians refer two groups of participants—patients with AD, and those with other neurological disorders. Healthy control participants are recruited for the database primarily from caretakers accompanying participants and through advertisement. All data in this study are drawn from this repository.

Participants and Procedures

This study drew from NDDR data for 118 participants between October 29, 2002, and February 28, 2006. Because original diagnoses were based on physician report, participants’ data was broken down into five groups of staging level of dementia based on the CDR Scale (27). Participants were generally interviewed, assessed, and had blood draws on a single day. Participants underwent an in-depth clinical interview by a trained interviewer (R.F.S.), followed by cognitive testing. CDR scores were based on all available data including collateral report and physician and chart notes if available. The same interviewer conducted the clinical interview and cognitive testing for all participants in the database and reliably followed the same semistructured format specified for each behavioral measure (31,32).

Behavioral Measures

Clinical Dementia Rating Scale.—The CDR is an observation and data-driven procedure used in staging severity of dementia commonly used in studies of AD (27). Patients are assessed based on six cognitive and functional domains—memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care. Each category is rated on a 5-point scale of impairment: 0 = none; 0.5 = questionable; 1 = mild impairment; 2 = moderate impairment; and 3 = severe impairment. Impairment scores determine the CDR global score of overall dementia, memory being the primary category considered.

RAGE and Aβ IgG Purification From Plasma

Plasma (0.5 mL) IgG was purified via a two-step purification process involving initial ammonium sulfate precipitation, followed by protein-G isolation as described elsewhere (22). Nonspecific total IgG was further purified to isolate RAGE and Aβ-specific IgG fractions by passing through aminohexyl sepharose 4B beads (1 mg/mL) conjugated with either RAGE or Aβ-42 peptides. After eluting unbound IgGs with three washes of phosphate-buffered saline (PBS), bound antigen-specific IgG was eluted with Tris-glycine-HCl (pH 2.8). The pH of the eluate was adjusted to 7.0 with Tris-HCl, dialyzed in PBS, concentrated, and assessed for final protein content.

Quantifying Specific IgGs

RAGE peptide or Aβ-42 (1 μg/100 μL per well) was coated to electroimmunodiffusion/radioimmunoassay strip plates and incubated overnight at 4°C. Antigen was discarded, and wells were blocked with 200 μL of Tris-buffered saline + Tween 20 (TBST) in 0.2% gelatin for 1 hour. Affinity-purified anti-Aβ-42 or anti-RAGE IgG (10 μg) derived from AD or control participants was added to each well and incubated overnight at 4°C. After washing three times with TBST, 200 μL of donkey anti-human IgGF(ab’2)-horseradish peroxidase–conjugated secondary antibody (1:1000) was added and incubated for 2 hours at 37°C. Plates were washed three times with TBST followed by adding 200 μL of ready made tetramethylbenzidine substrate (Sigma-Aldrich, St. Louis, MO) for blue color development, which was stopped after 15 minutes by adding 0.2 M H2SO4. Absorbance was read at 450 nm providing the titer values used in this study. Each sample was run in triplicate.

Peptides

RAGE peptide was synthesized according to the sequence (35) DQNITARIGKPLVLNCKGAPKPPQLEWKLN representing the nucleotide and amino acid sequence of human RAGE. The peptide was synthesized by the Molecular Biology Central Core facility at MCG. Aβ1-42 was purchased from Sigma-Aldrich. Stock solutions of the peptides were prepared in deionized water (100 μg/mL), and aliquots were stored at −80°C.
Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS 13.0 for Windows; SPSS, Chicago, Ill). Initially, partial correlation was performed to determine relationship strengths among RBANS scores of global cognitive ability, RBANS index scores, and concentrations of anti-RAGE and anti-\( \alpha \)\( \beta \) IgG proteins in blood samples while controlling for age and total IgG level. Analyses of covariance (ANCOVAs) evaluated the direction of the relationships between the level of dementia, as defined by CDR stage, RBANS global scores, and the IgG levels. Multiple regression analyses evaluated the variance accounted for by anti-RAGE and anti-\( \alpha \)\( \beta \) IgG proteins in blood plasma, as well as to rule out patient age and total IgG level as covariates. For anti-RAGE IgG, anti-\( \alpha \)\( \beta \) IgG and age did not account for a significant portion of the variance \((F(1,106)=0.38, p > .05)\). However, CDR stage additionally accounted for a large portion of the variance \((F(4,106)=12.93, p < .001)\) beyond that of total IgG level. For anti-\( \alpha \)\( \beta \), the results were similar, age lacking significance \((F(1,106)=0.01, p > .05)\), total IgG significant \((F(1,106)=25.17, p < .001)\), and CDR stage significantly predictive of additional variance after controlling for age and total IgG level \((F(4,106)=17.08, p < .001)\). Figure 1 illustrates levels of anti-RAGE and anti-\( \alpha \)\( \beta \) IgGs across increasing CDR stages of dementia.

Multiple regression analysis was performed to determine the portion of variance in CDR scores accounted for by anti-RAGE or anti-\( \alpha \)\( \beta \) IgGs over and above that of total IgG levels. As the first step in the model, total IgG accounted for nearly 13% of the variance in CDR rating. Anti-RAGE or anti-\( \alpha \)\( \beta \) IgGs explained an additional 39% of the variance \((R^2\) change; Table 3). Concentration levels of anti-\( \alpha \)\( \beta \) and anti-RAGE IgGs were pitted against each other as possible predictors of CDR global scores. When the two IgGs competed for variance, anti-\( \alpha \)\( \beta \) remained a significant predictor \((t=3.17, p < .01)\). Anti-RAGE IgG lost significance as the second variable in the regression \((t=1.70, p = .09)\). The unexpected change in the coefficient for this variable suggests multicollinearity for anti-RAGE and anti-\( \alpha \)\( \beta \) IgGs as predictors of CDR score variance. Similarly, multiple regression analysis was performed to determine the variance in RBANS total scores accounted for by anti-RAGE or anti-\( \alpha \)\( \beta \) IgG over and above that of total IgG levels. Total IgG accounted for nearly 15% of the variance in RBANS total scale score, and IgG markers explained an additional 23% of the variance \((R^2\) change; Table 4). When anti-\( \alpha \)\( \beta \) and anti-RAGE IgGs competed for the variance, anti-\( \alpha \)\( \beta \)
We used index scores from the RBANS to assess the relationship between IgG concentrations and specific domains of cognition to show that anti-RAGE and anti-Aβ IgGs are specific to dementia that matches an Alzheimer-type profile. Taken together, RBANS index scores accounted for nearly 24% and 25% of variance in anti-RAGE IgGs and anti-Aβ IgGs after controlling for total IgG, respectively (see $R^2$ change scores; Tables 5 and 6). Simultaneous regression confirmed the hypothesis that deficits in language and delayed memory, which are associated with temporal and frontal lobe cortical function, would predict high levels of RAGE- and Aβ-directed antibodies more strongly than cognitive skills associated with subcortical brain activity (29).

The Language Index of the RBANS was negatively and significantly predictive of each IgG. The Delayed Memory Index was significantly predictive of anti-RAGE IgG levels. No other index scores approached significance for either IgG.

**DISCUSSION**

Results of this study begin to clarify the role of specific immune responses in AD and point to IgGs directed against RAGE and Aβ as potential biomarkers for the disease. RAGE and Aβ IgGs were both significantly correlated with CDR stage as well as with RBANS global score. These findings were consistently shown above and beyond the effects of both age and total IgG level. This shows a strong relationship between the biomarkers and declines in cognition. Furthermore, ANCOVA showed a significant between-groups difference across CDR stages, even when controlling for age and total IgG effects, further supporting the hypothesis that dementia levels are significantly associated with IgG levels across the sample, for both anti-RAGE and anti-Aβ IgG. A point of caution should be noted, however, as it cannot definitively be ruled out that our findings of increased IgG elevations and CDR stage could include a nonspecific state of heightened autoimmunity in patients with AD, as evidence of such autoimmunity remained a significant predictor ($t = 2.16, p < .05$), whereas anti-RAGE IgG lost significance as the second variable in the regression ($t = 0.92, p > .05$).

**Table 3. Multiple Regression Results for IgG Concentrations as Predictors of Clinical Dementia Rating Score**

<table>
<thead>
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<th>Beta</th>
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<th>Sig</th>
<th>Zero Order</th>
<th>Partial</th>
<th>Part</th>
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<tbody>
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<td>Total IgG</td>
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<td>4.07</td>
<td>.000</td>
<td>.371</td>
<td>.371</td>
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<tr>
<td>2</td>
<td>Total IgG</td>
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<td>-0.87</td>
<td>.388</td>
<td>.371</td>
<td>-.086</td>
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<td>Anti-RAGE</td>
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<td>1.70</td>
<td>.092</td>
<td>.694</td>
<td>.166</td>
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<tr>
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<td>Anti-Aβ</td>
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<td>.002</td>
<td>.716</td>
<td>.300</td>
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<th>$SE$ of estimate</th>
<th>$R^2$ change</th>
<th>$F$ change</th>
<th>$df$</th>
<th>Sig $F$ change</th>
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<td>.9659</td>
<td>.137</td>
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</tr>
<tr>
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<td>.727</td>
<td>.7213</td>
<td>.391</td>
<td>42.259</td>
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<td>&lt;.001</td>
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</table>

*Note: Aβ = amyloid-beta; IgG = immunoglobulin; RAGE = receptor for advanced glycation end products; SE = standard error.*
Table 4. Multiple Regression Results for IgG Concentrations as Predictors of RBANS Total Score

<table>
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<tr>
<th>Model</th>
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<th>SE of estimate</th>
<th>R² change</th>
<th>F change</th>
<th>df</th>
<th>Sig F change</th>
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<td>.612</td>
<td>19.881</td>
<td>.228</td>
<td>15.846</td>
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<td>&lt;.001</td>
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</table>

**Note:** Aβ = amyloid-beta; IG = immunoglobulin; RAGE = receptor for advanced glycation end products; RBANS = repeatable battery for the assessment of neuropsychological status; SE = standard error.

Theories of AD development also point to avenues of possible investigation. The amyloid cascade hypothesis conjectures that the AD process begins as Aβ peptides are first deposited in preamyloid lesions (diffuse plaques) (6). However, these have been found extensively in aged persons with no clinical symptoms (38). Although such findings could support the idea that amyloid deposition may not be specific to AD at all, they could alternatively be interpreted to support the idea that AD could be detected...
with a biomarker of amyloid lesions long before development of symptoms. A longitudinal study examining cognitive symptoms of participants over time, especially in the high–AD risk group of MCI patients, will help to clarify this issue. Examining changes in cognitive abilities and levels of IgGs for a period of time will allow observation of the relationship across the progression of the disease.

Finally, the results of this study may point to new treatment venues. One study examined passive immune response to Aβ IgG immunization, finding actual improvements in cognitive functioning (16). Although RAGE would probably not be a good candidate for active immunization treatments, anti-RAGE IgG could feasibly be used in a passive immunization serum. As the current study has pointed to a relationship between RAGE-specific immune processes and immunization serum, it would be interesting to investigate a treatment of this sort.

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