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Antibodies to Myelin Basic Protein are Associated with Cognitive Decline after Stroke

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Abstract

B lymphocytes cause post-stroke cognitive decline in mice. We therefore evaluated the association between autoantibodies and post-stroke cognitive decline in a prospectively collected human cohort. The mini-mental state exam (MMSE) was administered 30, 90, 180, and 365 days after stroke. Antibody titers to myelin basic protein (MBP), proteolipid protein, and several non-specific proteins were determined. Among 58 subjects with initial MMSE ≥ 20 and at least 2 MMSE examinations in the year after stroke, cognitive decline (MMSE decrease ≥ 2) occurred in 10 (17%) subjects. In multivariate analysis, MBP antibody titers were the only independent predictor of cognitive decline (OR=9.02 [1.18, 68.90]; $P=0.03$).

Keywords

Stroke; Cognitive Decline; Autoimmune; MBP antibodies

Patients who suffer stroke are at increased risk of cognitive decline and dementia. While some of the cognitive decline may be attributable to the stroke itself, the increased risk of cognitive decline is present for at least a decade after the index stroke (Desmond et al. , 2002, Ivan et al. , 2004). Moreover, in a large study with cognition analyzed before and after stroke, the trajectories over time of different cognitive domains, particularly executive function, worsen after stroke (Levine et al. , 2015). The increased risk of cognitive worsening after stroke is thus not solely due to the acute loss of neurons related to the ischemic insult. The reason for this increased risk of cognitive decline in the years after stroke is not known.

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Conflicts of Interest

There are no conflicts of interest to disclose.

In mice initially cognitively normal after stroke, we recently discovered that B lymphocytes infiltrate the brain and cause cognitive decline between 1 and 7 weeks after stroke (Doyle et al. , 2015). In human stroke survivors there are autoantibodies against brain antigens in blood (Bornstein et al. , 2001, Dambinova et al. , 2003, Kalev-Zylinska et al. , 2013, Shibata et al. , 2012) and B cells in the stroke lesions of those with dementia (Doyle, Quach, 2015). In addition to the humoral responses, cellular responses against brain antigens also occur after stroke and both appear to predictive of functional outcome (Becker et al. , 2011, Becker et al. , 2005, Shibata, Cain, 2012). The contribution of these immune responses to cognitive outcomes, however, has not been evaluated.

In this study we followed cognitive function in the year after stroke using the mini-mental state examination (MMSE) and assessed antibodies to two central nervous system antigens, myelin basic protein (MBP) and proteolipid protein (PLP). These responses were compared to acquired autoantibodies to phospholipids and a vaccine-generated antibody against tetanus toxin (TT).

Materials and Methods

Research Subjects

We prospectively enrolled patients with ischemic stroke admitted to Harborview Medical Center from 9/2005 through 5/2009 whom were at least 18 years of age, could be enrolled within 72 hours of symptom onset and were felt not likely to die from their stroke. Patients with ongoing therapy for malignancy, known HIV, Hepatitis B or C, brain tumor, anemia, and those taking immunomodulatory drugs were excluded. Blood was drawn at 3, 7, 30, 90, 180 and 365 days after stroke onset. A group of 40 subjects with no history of stroke was recruited as controls. Serum was stored at -80° until use. The study was approved by the Institutional Review Board and all subjects or their surrogates provided informed consent.

Clinical Data

Demographic and clinical data were collected on all patients. Total infarct volume on initial diffusion weighted MRI was calculated by the ABC/2 method. The MMSE was administered at days 30, 90, 180 and 365. Only individuals with an initial MMSE score ≥ 20 were included.

Laboratory Studies

Serum antibody titers to MBP, PLP, and TT were determined in subjects with stroke as well as in controls as described (Shibata, Cain, 2012). Briefly, antibody titers (immunoglobulin G [IgG]) to TT were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (IBL International). To determine relative antibody titers to MBP and PLP, 96 well plates (NUNC MaxiSorp™) were coated with either human MBP (Sigma; 0.10 μ g/well) or PLP (Biogenesis; 0.10 μ g/well) and incubated overnight at 4°C. Following extensive washing, the plates were incubated overnight at 4°C with serum (diluted 1:10) samples (100 μ l/well). After washing, antigen bound human IgG was detected with peroxidase conjugated goat anti-human IgG antibodies (Pierce) and the plates developed with tetramethyl benzidine (TMB; Pierce). The absorbance was assessed at 450 nm

(BioTek®). All experiments were performed in duplicate. Control wells included (1) those with serum but no secondary antibody, (2) those without serum but with secondary antibody and (3) and those without serum or secondary antibody. Antibody titers >95th percentile of the control group were considered to be elevated. In subjects with stroke, serum samples were additionally screened for the presence of acquired antiphospholipid antibodies (anticardiolipin IgM and IgG and β -2-glycoprotein-1 IgG) by the hospital clinical laboratory using ELISA; titers >15 IU/mL were considered positive.

Statistics

Descriptive data are presented as the median and interquartile range (IQR) for continuous variables and percentages for categorical variables. Group comparisons were performed using the Mann-Whitney U test or Fisher's Exact Test. Logistic regression was used to test the association between a decline in MMSE ≥ 2 points and serum antibody titers to MBP as well as other variables found to be associated with such a decline ($P < 0.10$); stroke severity was forced into the equation.

Results

Of the 114 subjects enrolled in the parent study (Becker, Kalil, 2011), the initial MMSE was ≥ 20 and the MMSE could be repeated at least once in 58, who form the study population for these analyses. We excluded patients with an initial MMSE <20 as these were generally the patients with severe aphasia, such that testing could not be reliably performed or repeated. Of the 58 included patients, the MMSE was generally stable or improved over the year after stroke, but the final MMSE was 3 points worse than the best score in 3 (5%), 2 points worse in 7 (12%) and 1 point worse in 6 (10%). If one looks at the 10 patients (17%) who had a worsening in their MMSE of ≥ 2 points, predictors of this decline are shown in **Table 1**.

In comparison to patients without cognitive decline, those with cognitive decline were more likely to have serum antibody titers to MBP that were above the 95th percentile of that seen in controls. They were also older and more likely to have been treated with tPA. Antibodies to PLP, phospholipids, or TT were not associated with cognitive decline. Using those variables associated with a decline in the MMSE of ≥ 2 at $P < 0.10$, a multivariate model was created; NIHSS score was forced into the equation. Only MBP antibody titers $\geq 95\%$ of controls remained significant, with an odds ratio of 9.02 (IQR 1.18, 68.90) for a decrease in the MMSE ≥ 2 (**Table 2**).

Discussion

Antibodies to CNS antigens like MBP, N-methyl-D-aspartate (NMDA) receptors and neurofilaments are detected in stroke survivors (Bornstein, Aronovich, 2001, Dambinova, Khounteev, 2003, Kalev-Zylinska, Symes, 2013, Shibata, Cain, 2012). Whether these antibodies are a mere epiphenomenon of brain injury or contribute to the late sequelae of stroke is unclear. In this study we found that elevated titers of anti-MBP antibodies were independently associated with delayed cognitive decline in the year after stroke, while antibodies to PLP, phospholipids and TT were not, suggesting that anti-MBP antibodies may contribute to the cognitive decline. It is also possible that antibodies to MBP aren't

responsible for worsening cognition after stroke, but correlate with an autoimmune response to other antigens that do mediate the cognitive decline.

Given the small study population, it is possible that our findings represent a false positive. It is also possible that a larger study population would increase the power of the findings, particularly as they are consistent with our mouse data demonstrating that antibody-producing B cells are necessary for post-stroke cognitive decline. The number of possible CNS autoantigens following ischemic brain injury is enormous, and it is likely that many different antibodies emerge after stroke. At least some of these autoantibodies could contribute to delayed cognitive dysfunction and other sequelae of stroke. What represents a clinically relevant increase in antibody titers is unknown, but it is clear that overall serum IgG falls after stroke (Liesz et al. , 2015, Shibata, Cain, 2012). This observation suggests that comparing specific IgG titers of stroke patients to controls may be a flawed approach that underestimates the true effect of the antibodies that arise after stroke. Further studies are needed to better understand how autoantibody repertoires vary among individuals after stroke, to determine how these responses affect cognition, and to validate assays for quantifying these antibodies.

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References

- Becker KJ, Kalil AJ, Tanzi P, Zierath DK, Savos AV, Gee JM, et al. Autoimmune responses to the brain after stroke are associated with worse outcome. *Stroke*. 2011; 42:2763–9. [PubMed: 21799171]
- Becker KJ, Kindrick DL, Lester MP, Shea C, Ye ZC. Sensitization to brain antigens after stroke is augmented by lipopolysaccharide. *J Cereb Blood Flow Metab*. 2005; 25:1634–44. [PubMed: 15931160]
- Bornstein NM, Aronovich B, Korczyn AD, Shavit S, Michaelson DM, Chapman J. Antibodies to brain antigens following stroke. *Neurology*. 2001; 56:529–30. [PubMed: 11222800]
- Dambinova SA, Khounteev GA, Izykenova GA, Zavolokov IG, Ilyukhina AY, Skoromets AA. Blood test detecting autoantibodies to N-methyl-D-aspartate neuroreceptors for evaluation of patients with transient ischemic attack and stroke. *Clin Chem*. 2003; 49:1752–62. [PubMed: 14500616]
- Desmond DW, Moroney JT, Sano M, Stern Y. Incidence of dementia after ischemic stroke: results of a longitudinal study. *Stroke*. 2002; 33:2254–60. [PubMed: 12215596]
- Doyle KP, Quach LN, Sole M, Axtell RC, Nguyen TV, Soler-Llavina GJ, et al. B-lymphocyte-mediated delayed cognitive impairment following stroke. *J Neurosci*. 2015; 35:2133–45. [PubMed: 25653369]
- Ivan CS, Seshadri S, Beiser A, Au R, Kase CS, Kelly-Hayes M, et al. Dementia after stroke: the Framingham Study. *Stroke*. 2004; 35:1264–8. [PubMed: 15118167]
- Kalev-Zylinska ML, Symes W, Little KC, Sun P, Wen D, Qiao L, et al. Stroke patients develop antibodies that react with components of N-methyl-D-aspartate receptor subunit 1 in proportion to lesion size. *Stroke*. 2013; 44:2212–9. [PubMed: 23723305]
- Levine DA, Galecki AT, Langa KM, Unverzagt FW, Kabeto MU, Giordani B, et al. Trajectory of Cognitive Decline After Incident Stroke. *JAMA*. 2015; 314:41–51. [PubMed: 26151265]

- Liesz A, Roth S, Zorn M, Sun L, Hofmann K, Veltkamp R. Acquired Immunoglobulin G deficiency in stroke patients and experimental brain ischemia. *Exp Neurol*. 2015; 271:46–52. [PubMed: 25959599]
- Shibata D, Cain K, Tanzi P, Zierath D, Becker K. Myelin basic protein autoantibodies, white matter disease and stroke outcome. *J Neuroimmunol*. 2012; 252:106–12. [PubMed: 22939639]

Highlights

- Cognitive decline occurred in 17% of patients in the year after ischemic stroke.
- PLP and anti-cardiolipin antibodies were not associated with cognitive decline.
- Elevated MBP antibodies were associated with cognitive decline after stroke.

Table 1

Characteristics of the study population with and without cognitive decline during the study (initial MMSE 20).

	Entire Cohort N=58	MMSE decrease by 2		
		No N=48	Yes N=10	P
Patient Characteristics				
Age (years)	52 (42, 63)	49 (41, 62)	64 (54, 70)	0.02
Sex (female)	21/58 (36%)	18/48 (38%)	3/10 (30%)	NS
Past medical history				
Hypertension	25/58 (43%)	18/48 (38%)	7/10 (70%)	0.08
Hyperlipidemia	39/58 (67%)	30/48 (62%)	9/10 (90%)	NS
Coronary heart disease	12/48 (25%)	9/48 (19%)	3/10 (30%)	NS
Atrial fibrillation	8/56 (14%)	7/48 (15%)	1/10 (10%)	NS
Diabetes	13/58 (22%)	10/48 (21%)	3/10 (30%)	NS
Prior stroke	17/58 (29%)	16/48 (33%)	1/10 (10%)	NS
Stroke Characteristics				
NIHSS score	8 (3, 14)	7 (3, 14)	10 (6, 14)	NS
Infarct volume (cc)	4.6 (0.5, 46.9)	4.1 (0.4, 43.5)	11.1 (1.4, 50.1)	NS
Treatment with IV tPA	14/48 (29%)	9/48 (19%)	5/10 (50%)	0.05
Serum Antibodies				
Brain				
MBP>95% control ever	13/58 (22%)	7/48 (15%)	6/10 (60%)	0.005
PLP>95% control ever	10/58 (17%)	8/48 (17%)	2/10 (20%)	NS
Anti-phospholipid				
ACL IgM (+) ever	6/58 (10%)	4/48 (8%)	2/10 (20%)	NS
ACL IgG (+) ever	3/58 (5%)	2/48 (4%)	1/10 (10%)	NS
β2-GP1 IgG (+) ever	4/58 (7%)	4/48 (8%)	0/10	NS
Tetanus Toxoid				
>95% control ever	9/58 (16%)	9/48 (19%)	0/10	0.14

MMSE=mini-mental state exam, NIHSS=NIH stroke scale, IV tPA=intravenous tissue plasminogen activator, MBP=myelin basic protein, PLP=proteolipid protein, ACL=anti-cardiolipin, GPI=glycoprotein I, NS= $P > 0.20$.

Table 2

Multivariate model assessing predictors of MMSE decline 2 points.

Characteristics (controlled for NIHSS)	OR (95% CI)	P
NIHSS (per point)	1.11 (0.97, 1.27)	NS
IV tPA use	3.90 (0.64, 23.82)	NS
Age (per decade)	1.63 (0.59, 4.47)	NS
History of hypertension	4.38 (0.67, 23.82)	NS
Myelin basic protein (MBP) antibody titer >95% controls	9.03 (1.19, 68.68)	0.03

MMSE=mini-mental state exam, OR=odds ratio, CI=confidence interval, IV tPA=intravenous tissue plasminogen activator, NS= $P > 0.20$