Brain Infiltration of Immune Cells in CASPR2–Antibody Associated Mesial Temporal Lobe Epilepsy with Hippocampal Sclerosis

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ABSTRACT

Introduction: Antibodies directed against neuronal surface antigens have recently been identified in patients with focal temporal lobe epilepsy (TLE) of unknown cause and mesial TLE with hippocampal sclerosis (MTLE-HS), thereby emphasizing the role of autoimmunity in TLE. Antibodies to contactin-associated protein-like 2 (CASPR2) are prevalent in MTLE-HS patients. We aimed to find out whether anti-neuronal autoimmunity might be involved in CASPR2 antibody-related MTLE-HS.

Methods: Surgically resected medial temporal lobe specimens of seropositive and seronegative MTLE-HS patients were examined with hematoxylin and eosin and immunohistochemical staining using specific immune cell markers.

Results: Two of 5 CASPR2 antibody-positive MTLE-HS patients showed polymorphonuclear and mononuclear cells infiltrating

the subarachnoidal region. One of these patients also showed mononuclear cell infiltration in the parenchyma of the temporal lobe cortex. Subarachnoidal and parenchymal infiltrates contained CD3+, CD8+, and CD68+ cells. None of the 13 seronegative MTLE-HS patients displayed cellular infiltrates in their brain samples, and all MTLE-HS patients showed marked neuronal cell loss but no immune cell infiltration in their hippocampi.

Conclusion: Our results show that CASPR2 antibody-associated MTLE-HS can present with central nervous system inflammation; thus, this subtype of MTLE-HS might have an autoimmune origin.

Keywords: Antibody, CASPR2, hippocampal sclerosis, temporal lobe epilepsy, autoimmunity

INTRODUCTION

Neuronal cell surface antibodies directed against ion channels or synaptic membrane proteins have recently been shown in several epilepsy cohorts (1,2). Among diverse epilepsy syndromes, focal temporal lobe epilepsy (TLE) of unknown cause and mesial TLE with hippocampal sclerosis (MTLE-HS) particularly stand out with their high anti-neuronal antibody positivity rates and favorable response to immunosuppressive treatment (2). These findings corroborate the previously established significance of autoimmunity in the pathogenesis of TLE (3,4,5,6). Although MTLE-HS and TLE patients might present with a wide variety of neuronal cell surface antibodies, contactin-associated protein-like 2 (CASPR2) antibody is by far the most prevalently found antibody in this epilepsy subgroup (2). To find further evidence for the potential autoimmune nature of CASPR2-related MTLE-HS, we examined surgically removed temporal lobe specimens of treatment-resistant CASPR2 antibody-positive and -negative MTLE-HS patients using immunohistochemical methods.

METHODS

Patients

Eighteen MTLE patients (13 women, 5 men; 38.9±11.9 years old) fulfilling the magnetic resonance imaging (MRI) criteria for HS and with surgically resected temporal lobe specimens available for immunohistochemical studies were included. Average age at onset of seizures was 8.5±6.9 years, and average age at the time of epilepsy surgery was 21.2±9.5 years. None of the patients had a concomitant autoimmune disease, another neurological disorder, or history of autoimmune or viral encephalitis. İstanbul University Ethics Committee has approved the study, and written informed consent was obtained from all patients.

For the verification of HS diagnosis, MRIs were investigated. The presence of atrophy on T1-weighted images and high signal changes on T2-weighted images and FLAIR series in any one or more parts of the hippocampus were considered as the major criteria necessary to establish the neuroradiological diagnosis of HS. Magnetic resonance imaging studies were performed with a 1.5-T scanner (Magnetom Siemens Symphony, Erlangen, Germany) using previously reported parameters (7). Patients with HS and dual pathologies were excluded.

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Detailed clinical, demographic, and electrophysiological data were obtained from all patients (Table 1). All available EEGs (routine, video-EEG, and invasive monitoring) were evaluated independently. Impaired background activity, interictal slow waves, extratemporal and temporal epileptic foci, fast activity, activation of foci during hyperventilation, intermittent photic stimulation, and sleep were examined with a standardized form by two investigators systematically.

Autoantibody Testing

The sera of patients and controls were kept at -80°C until assayed and tested for antibodies against CASPR2, leucine-rich glioma inactivated 1 (LGI1), N-methyl-D-aspartate receptor (NMDAR), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA), and type B gamma aminobutyric acid receptor (GABA_B) using an immunofluorescence-based commercial kit utilizing HEK-293 cells transfected with plasmids encoding relevant ion channel complex subunits (Euroimmun, Luebeck, Germany). The binding was scored visually on a range from 0 (negative) to 4 (very strong), as described previously (8).

Immunohistochemistry Studies

Surgically resected medial temporal lobe specimens of all MTLE-HS patients were fixed in formalin and embedded in paraffin. Paraffin-embedded spleen samples obtained from neurologically normal individuals, and three adenocarcinoma samples with abundant inflammatory infiltrates (2 lung and 1 breast cancer) were used as positive controls. After a standard hematoxylin and eosin staining, immunohistochemical analysis of inflammatory infiltrates was performed as previously reported (9). Paraffin-embedded tissues were deparaffinized, and the antigens were retrieved. Tissue sections were serially incubated with 0.3% H_2O_2 for 20 min, 10% goat serum for 1 h, and primary antibodies against CD3 (1:100, rabbit anti-human, Abcam, Cambridge, MA, USA), CD8 (1:200, mouse anti-human, Abcam), CD68 (1:200, mouse anti-human, Santa Cruz, Dallas, TX, USA), CD20 (1:100, mouse anti-human, Novus Biologicals, Littleton, CO, USA), and CD79a (1:400, rabbit anti-human, Novus Biologicals) diluted in 10% goat serum in phosphate-buffered saline (PBS) overnight at 4°C .

After using the appropriate secondary antibodies (all diluted 1:2000), the reactivity was developed with the avidin-biotin-peroxidase method. Hematoxylin was used for counterstaining. The cells were identified based on the marker positivity and morphological features.

RESULTS

Antibody screening showed CASPR2 antibody in sera of 5 of 18 MTLE-HS patients (Table 1). None of the patients showed antibodies against LGI1, NMDAR, AMPAR, and GABA_B .

All patients showed significant neuronal loss and gliosis in the hippocampal region in compliance with the diagnosis of HS. Hematoxylin and eosin staining showed an abundance of cellular infiltrates (Figure 1 a-f) in 2 of 5 CASPR2 antibody-positive (Cases 1 and 2) and in none of the 13 seronegative MTLE-HS patients ($p=0.017$ with Fisher's exact test). Cellular infiltrates were located in the subarachnoid region in both CASPR2 antibody-positive patients (Figure 1a, e). In Case 2, there were additional infiltrating cell foci within the parenchyma of the temporal cortex (Figure 1d). The subarachnoid infiltrates contained both polymorphonuclear and mononuclear cells (Figure 1e), whereas the parenchymal infiltrates contained only mononuclear cells (Figure 1d). Notably, none of the patients showed infiltrating immune cells in the hippocampus and surrounding areas (Figure 1f).

Immunohistochemical analysis of the subarachnoid and parenchymal infiltrates showed CD3+ T cells (Figure 1b), CD8+ T cells (not shown), and CD68+ macrophages (Figure 1c), but no CD20+ or CD79a+ B cells.

DISCUSSION

The involvement of immunological mechanisms in the pathogenesis of TLE with or without HS has been extensively investigated, and TLE patients have been shown to exhibit increased numbers of peripheral blood T cells and proinflammatory cytokine expression (3,6). Moreover, infiltrating CD3+ and CD4+ T cells have been identified in brain samples of TLE patients with HS (4,5). Recent characterization of antibodies directed

Table 1. Clinical and serological features of MTLE-HS patients with anti-neuronal antibodies

Case no/ Age/ Gender	Antibody ^a ; level	Age at onset	History	Seizure types	MRI findings	Major EEG findings	Epilepsy duration during operation	Prognosis and treatment
1/ 41/ F	CASPR2, 3	17	FS, depression	FSwLoC, F- BCS	RHS	R FT spikes and L FT sharp waves	23 years	Sz free for 1 year after R anterior temporal lobectomy
2/ 39/ M	CASPR2, 2	20	None	FSwLoC, F- BCS	LHS	L FT sharp waves	11 years	Sz free for 8 years after L amygdalo- hippocampectomy
3/ 36/ F	CASPR2, 3	7	None	FSwLoC, F- BCS	LHS	L FT sharp waves	23 years	Sz free for 6 years after L amygdalo- hippocampectomy
4/ 35/ M	CASPR2, 3	1	FS, depression	FSwLoC, F- BCS	LHS	L FT sharp waves	33 years	Partial remission for 1 year after L temporal lobectomy
5/ 29/ F	CASPR2, 3	6	None	FSwLoC, F- BCS, SE	BHS	R FT sharp waves	15 years	Partial remission for 8 years after R anterior temporal lobectomy

^aNumbers indicate the antibody binding intensity scored visually on a range from 0 (negative) to 4 (very strong). M: male; F: female; CASPR-2: contactin-associated protein-like 2; SE: status epilepticus; FSwLoC: focal seizure with impairment of consciousness; FS: febrile seizure; F- BCS: focal seizure evolving to bilateral convulsive seizure; sz: seizure; L: left; R: right; B: bilateral; HS: hippocampal sclerosis; SLE: systemic lupus erythematosus; FT: frontotemporal; T: temporal

Figure 1. a-f. Histopathologic and immunohistochemical findings in CASPR2 antibody-positive MTLE-HS patients. Case 1 had mononuclear cells infiltrating the subarachnoid region (a), some of which were immunohistochemically positive for CD3 (brown) (b) and CD68 (brown) (c). Another CASPR2 antibody-positive patient (Case 2) displayed mononuclear inflammatory infiltrates in the cortical brain parenchyma (d). Polymorphonuclear and mononuclear cells were detected in the subarachnoid region of Case 2 (e). There were no inflammatory cells in the sclerotic hippocampus of the same patient (f). Hematoxylin and eosin staining (a, d, e, f) and immunohistochemical staining (b, c); original magnification 40× in D and 400× in other panels. P denotes normal appearing brain parenchyma with no signs of inflammation.

against neuronal membrane antigens in MTLE-HS patients has further corroborated the significance of antigen-specific autoimmunity in this specific epilepsy syndrome (2). In the current study, we have shown that infiltrating immune cells are particularly found in CASPR2 antibody-positive MTLE-HS patients, suggesting an antibody-mediated brain inflammation at least in this subcategory of MTLE-HS. Whether these inflammatory infiltrates exist in the brain samples of MTLE-HS patients with other neuronal membrane antibodies requires to be further studied.

Similar subarachnoidal and/or parenchymal cellular infiltrates have been shown in brain samples of NMDAR, CASPR2, and LGII antibody-positive autoimmune encephalitis patients (9,10,11,12,13). It has also been well documented that autoimmunity directed against the voltage-gated potassium channel complex might cause MTLE-HS within less than a year as a sequela of autoimmune encephalitis (14). Moreover, we have previously shown that CASPR2 antibody-positive epilepsy patients present with psychiatric manifestations (mostly depression or psychosis) more often than seronegative epilepsy patients (2), and these psychiatric presentations are typically found in patients with autoimmune encephalitis (15). It is thus tempting to speculate that CASPR2 autoimmunity might be causing hippocampal cell loss and subsequently a chronic disease characterized with seizures, psychiatric symptoms, and behavioral alterations, as proposed previously (15).

Major drawbacks of this hypothesis are absence of antibody-producing plasma cells within the inflammatory infiltrates observed in our study and absence of immune cell infiltration in the sclerotic hippocampus as these findings are frequently observed in brain samples of autoimmune encephalitis cases (9,10,11,12,13). However, in our cohort, surgical operations had been conducted long after the onset of seizures. Thus, observed infiltrating immune cells might presumably be the residual findings of a long-gone inflammation. Prolonged duration between on-

set of seizures and surgery might also possibly explain the absence of inflammation in 3 CASPR2 antibody-positive MTLE-HS patients (Cases 3–5). If brain samples of these patients could have been investigated in earlier periods of the clinical course, the number of brain samples with infiltrates could have been higher; infiltrating cells could possibly have been found in the sclerotic hippocampal region, and antibody-producing plasma cells could also have been observed. Also, none of our antibody-positive patients with or without brain infiltrates described an acute-onset encephalopathy suggestive of autoimmune encephalitis. Autoimmune encephalitis may present without the typical syndromic manifestations but with seizures as the sole or predominant manifestation (16) and thus might be overlooked. Moreover, autoimmune encephalitis might also present with a chronic and insidious clinical course, as observed in the case of IgLON5 autoimmunity (17).

Alternatively, CASPR2 autoimmunity might not be the direct cause of HS and simply be an epiphenomenon of chronic seizures and neuronal loss. Even in this case, since CASPR2 is a membrane-bound protein (18) exposed to the effects of circulating antibodies, CASPR2 antibodies might still display a pathogenic action by activating the complement cascade, releasing chemotactic complement breakdown products and thus attracting infiltrating immune cells to the hippocampal region.

Mesial TLE with hippocampal sclerosis is a severe and irreversible disorder often presenting with treatment refractory seizures (19). We had previously reported a TLE patient with glycine receptor antibodies and treatment-resistant seizures, who gave remarkable response to pulse steroid and intravenous immunoglobulin treatment; thus, epilepsy surgery was avoided (2). Therefore, investigation of the potentially immunosuppressant-responsive subtypes of MTLE-HS through further examination of anti-neuronal antibodies and surgically removed brain specimens is highly recommended.

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Informed Consent: Written informed consent was obtained from patients who participated in this study.

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