Original Article

Autoantibodies to neuronal surface antigens in thyroid antibody-positive and -negative limbic encephalitis

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Abstract

Background: Thyroid antibodies (Thy-Abs) are frequently detected in various autoimmune disorders in coexistence with other systemic autoantibodies. In association with an encephalopathy, they are often taken as evidence of Hashimoto's encephalitis (HE). However, the presence of Thy-Abs in a cohort of limbic encephalitis (LE) patients and their association with anti-neuronal autoimmunity has not been explored. **Patients and Methods:** We investigated thyroid and anti-neuronal antibodies in the sera of 24 LE patients without identified tumors by cell-based assay and radioimmunoassay and evaluated their clinical features. **Results:** There was a female predominance in Thy-Ab-positive LE patients. Five of the eight Thy-Ab-positive patients and six of the 16 Thy-Ab-negative patients had antibodies to voltage-gated potassium channel (VGKC), N-methyl-D-aspartate receptor (NMDAR) or undefined surface antigens on cultured hippocampal neurons. There were trends towards fewer VGKC antibodies (1/8 vs. 5/16, $P = 0.159$) and more NMDAR antibodies (2/8 vs. 1/16, $P = 0.095$) among the Thy-Ab-positive LE patients; antibodies to undefined surface antigens were only identified in Thy-Ab-positive patients (2/8 vs. 0/16, $P = 0.018$). There were no distinguishing clinical features between Thy-Ab-positive patients with and without neuronal antibodies. However, patients with anti-neuronal antibodies showed a better treatment response. **Conclusion:** Thy-Abs can be found in a high proportion of patients with non-paraneoplastic LE, often in association with antibodies to specific or as yet undefined neuronal surface antigens. These results suggest that acute idiopathic encephalitis patients with Thy-Abs should be closely monitored for ion-channel antibodies and it should not be assumed that they have HE.

Key words: *Antibody, Hashimoto's encephalopathy, limbic encephalitis, N-methyl-D-aspartate receptor, voltage-gated potassium channel*

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Introduction

Patients with several autoimmune disorders including myasthenia gravis, Type 1 diabetes mellitus and rheumatoid arthritis show a higher thyroid antibody (Thy-Ab) frequency than healthy individuals,[1] suggesting a pathogenic relationship between autoimmune thyroid diseases and other immune-mediated diseases. Thy-

Abs have also been detected in a few autoimmune encephalitis patients' sera in association with ion-channel antibodies.[2,3] However, increased Thy-Ab levels are found in approximately 10-15% of the general population,[4,5] raising the possibility that the association between anti-thyroid and anti-neuronal immune responses might be purely coincidental. Nevertheless, in association with an encephalopathy, they are often taken as evidence of Hashimoto's encephalitis (HE).

To assess the incidence of Thy-Abs in limbic encephalitis (LE) and analyze the association between anti-thyroid and anti-neuronal autoimmunity, we investigated the presence of antibodies directed against specific and undefined neuronal surface antigens in the sera of acute Thy-Ab-positive or -negative LE patients with no identifiable etiology.

Patients and Methods

Patients

This study included 24 consecutive patients (15 women/9 men; age range 28-58 years) presenting with recent onset (<eight weeks' duration) symptoms of limbic dysfunction of unknown cause. The inclusion criteria were presence of psychiatric symptoms, short-term memory loss, confusion or seizures in association with at least one of the findings of medial temporal lobe (MTL) involvement [detected by magnetic resonance imaging (MRI), positron emission tomography (PET) or single photon emission computed tomography (SPECT)] and inflammatory findings in the cerebrospinal fluid (CSF) analysis. A comprehensive screening for metabolic (including thyroid function tests), infectious, systemic and paraneoplastic autoimmune disorders and whole-body computed tomography (CT) scan were negative in all the patients. The antibody panel included anti-nuclear antibody, anti-double-stranded DNA, Rnp/Smith-antibody, SS-A/SS-B antibodies, anti-neutrophilic cytoplasmic antibodies, anti-cardiolipin antibody and paraneoplastic antibodies (anti-Hu, Yo, Ri, Ma2, CV2 and amphiphysin). Patients with other central nervous system disorders (metastases, infections, stroke etc.) were excluded. The median length of the follow-up period was 26 months with a range of 10 to 38 months. None of the enrolled patients in the study had malignancy despite periodic imaging surveillance. Controls included healthy individuals with (13 women/3 men; age range 34-51 years) and without (16 women/4 men; age range 24-52 years) Thy-Abs. The study was approved by the Instutional Review Board of Istanbul University.

Anti-thyroid and neuronal antibody measurements

All enrolled patients and controls were investigated for thyroid and neuronal antibodies. Sera were obtained from all the patients during diagnostic work-up and kept frozen at -80°C until assayed. Thyroid peroxidase (TPO)- and thyroglobulin (TG)-antibody concentrations were measured by a chemiluminescent analyzer (nv < 50 IU/ml) (Siemens Healthcare Diagnostics Products, Deerfield, IL). Serum voltage-gated potassium channel (VGKC) and voltage-gated calcium channel (VGCC)-Abs were measured by radioimmunoassay using whole rat brain homogenate, as described previously (nv < 100 pM).[6] For N-methyl-D-aspartate receptor (NMDAR)-Ab detection, HEK293 cells were transfected with plasmids containing NR1 and NR2 subunits of the NMDAR. All cells were grown in the presence of 500 μM ketamine after transfection. Transfected cells were then incubated with the sera (1:20) and the appropriate Alexa Fluor-secondary antibody, as described earlier.[7] For detection of antibodies to undefined neuronal surface antigens, sera (1:200) were incubated for 1 h at room temperature with the live rat hippocampal neurons (LHN) grown on cover slips. Neurons were then fixed with 4% paraformaldehyde and immunolabeled with the appropriate Alexa Fluor-secondary antibody.[7] Incubation of the neurons with the sera before fixation prevents the binding of serum immunoglobulin G (IgG) to intracellular antigens and provides the detection of antibodies reactive with the neuronal cell surface only.

Results

Eight of 24 LE patients displayed both TPO- and TG-Abs (all > 200 IU/ml). Antibodies against VGKC (n=6), NMDAR (n=3) or undefined surface antigens of LHN (LHN-Ab) (n=2) were detected in 11 LE patients. No patients showed VGCC-Abs and none of the healthy control sera was positive for any antibodies [Tables 1 and 2].

There was a trend towards a higher fraction of Thy-Ab-positive LE patients with serum antibodies against specific ion-channels or undefined neuronal surface antigens (5/8 vs. 6/16, P = 0.123). There were trends towards a higher NMDAR-Ab incidence in the Thy-Ab-positive group (2/8 vs. 1/16, P = 0.095) and a higher VGKC-Ab incidence in the Thy-Ab-negative group (1/8 vs. 5/16, P = 0.159) [Table 1]. Although only two patients had evidence of antibodies binding to undefined neuronal surface antigens, these were both in the Thy-Ab-positive group (2/8 vs. 0/16, P = 0.018).

Patients with Thy-Abs had an apparent female predominance. Otherwise, patients with and without Thy-Abs were essentially identical in terms of age, clinical features and laboratory findings. Notably, six of eight Thy-Ab-positive patients with or without neuronal antibodies showed MTL involvement by MRI, PET or SPECT. MTL involvement was detected by PET or SPECT but not MRI in two patients (Cases 2 and 4, Table 2).

All 24 patients received steroids, nine intravenous immunoglobulin, three plasma exchange and one cyclophosphamide. Previously reported criteria were used for evaluation of the treatment response.[8] Complete improvement was considered for patients who could return to work and normal daily activities. Patients who could not return to work but could retain some of their daily activities were considered as partial responders. Overall, 21 patients showed neurological improvement (11 complete, 10 partial). Three patients with no clinical improvement were negative for thyroid and neuronal antibodies. Five of the complete responders were Thy-Ab-positive patients and the remaining six patients were in the Thy-Ab-negative group (P = 0.123 by chi-square test). Notably, nine of 11 patients with neuronal surface antibodies showed complete neurological improvement as opposed to two of 13 patients with no neuronal antibodies (P = 0.001 by chi-square test).

Table 1: Comparison of demographic features and antibody responses to neuronal surface antigens in limbic encephalitis patients and healthy controls with and without thyroid antibodies

Patients	LE patients without Thy-Abs (n=16)	LE patients with Thy-Abs (n=8)	*P* value
Age (mean±SD, range)	42.75±8.1 (28-55)	47.75±8.4 (37-58)	0.187
Gender	8 women, 8 men	7 women, 1 man	0.037*
VGKC-Abs	5	1	0.159
NMDAR-Abs	1	2	0.095
Undefined neuronal surface-Abs	0	2	0.018*
VGCC-Abs	0	0	NA
Total	6	5	0.123
	HC without Thy-Abs (n=20)	**HC with Thy-Abs (n=16)**	
VGKC-Abs, NMDAR-Abs, VGCC-Abs, undefined neuronal surface-Abs	0	0	NA

LE - Limbic encephalitis; HC - Healthy controls; Thy-Abs - Thyroid antibodies; SD - Standard deviation; NMDAR-Abs - N-methyl-D-aspartate receptor antibodies; VGKC-Abs - Voltage-gated potassium channel antibodies; VGCC-Abs - Voltage-gated calcium channel antibodies; NA - not applicable. Statistical analyses were performed by Student's t-test or chi-square test as appropriate.* P<0.05

Discussion

In our study, 33.3% of LE patients had Thy-Abs as opposed to 10-15% of the general population[4] and 62.5% LE patients with Thy-Abs had anti-neuronal antibodies as opposed to 37.5% LE patients without Thy-Abs. Although there are only a few published case reports on LE patients with elevated thyroid and ion-channel antibodies,[2,3] our results suggest that this is not a rare entity or chance association and patients with Thy-Abs are inclined to develop anti-neuronal immune responses and autoimmune encephalitis. These results also support the previously proposed notion that neuronal and thyroid autoimmunities might represent a pathogenic spectrum.[3] Absence of antibodies to neuronal surface antigens in Thy-Ab-positive healthy individuals [Table 1] implies that additional pathogenic factors are required for generation of anti-neuronal immune response.

Thy-Ab-positive patients with anti-neuronal antibodies do not appear to have a distinctive clinical phenotype that would discriminate them from the anti-neuronal antibody or Thy-Ab-negative LE patients. This finding has some relevance regarding the diagnosis of HE. HE is generally defined as steroid-responsive acute-onset encephalitis associated with high titers of Thy-Abs and no other identifiable etiology.[4,5] Tremor, myoclonus and lack of MTL involvement on scans are often associated

Table 2: Clinical features and antibody status of limbic encephalitis patients with thyroid antibodies

Case #	Age	Gender	Clinical findings	Cranial MRI	SPECT/ PET	EEG	Cerebrospinal fluid	Antibody detected
1	38	Female	Delusions, STML, GTCS	FLAIR/T2 HI in both MTLs	Not done	Diffuse slowing	24 WBC, protein 56, OCB+	None
2	42	Female	Delusions, somnolence, STML	Normal	Right temporal hypoperfusion	Normal	Normal, OCB-	None
3	58	Male	STML, GTCS	BL subcortical white matter lesions	Not done	Diffuse slowing	31 WBC, protein 66, OCB-	None
4	58	Female	Confusion, STML, auditory hallucinations	BL subcortical white matter lesions	Right temporal hypometabolism	Diffuse slowing	Normal, OCB+	Undefined
5	51	Female	STML, GTCS	FLAIR/T2 HI in both MTLs	Not done	Diffuse slowing	Normal, OCB-	Undefined
6	45	Female	STML, GTCS	FLAIR/T2 HI in both MTLs	Not done	Normal	Normal, OCB-	VGKC (350 pM)
7	37	Female	STML, GTCS, depression	BL subcortical white matter lesions	Not done	Normal	23 WBC, protein 62, OCB-	NMDAR
8	53	Female	STML, GTCS, somnolence	FLAIR/T2 HI in the left MTL	Not done	Normal	48 WBC, protein 58, OCB-	NMDAR

MRI - Magnetic resonance imaging; PET - Positron emission tomography; SPECT - Single photon emission computed tomography; EEG - Electroencephalography; NMDAR - N-methyl-D-aspartate receptor; VGKC - Voltage-gated potassium channel; GTCS - Generalized tonic clonic seizures; STML - Short-term memory loss; FLAIR - Fluid-attenuated inversion recovery; BL - Bilateral; MTL - Medial temporal lobe; HI - Hyperintensity; OCB - Oligoclonal band; WBC - White blood cells

with HE.[3-5,9] However, there has been a tendency for the diagnosis of HE to be given to any patient who has an acute encephalitis that is Thy-Ab-positive, regardless of the clinical features. Our results indicate that Thy-Abs are frequent in patients with syndromes that are now ascribed to the presence of antibodies to VGKC, NMDAR or undefined neuronal surface antigens. Therefore, a diagnosis of HE should be made only after exclusion of these antibodies – and others that are likely to be defined in the future. This is particularly important since these neuronal antibodies can be associated with tumors.[7]

Our results also imply that a normal MRI does not necessarily rule out MTL involvement. Therefore, patients with clinical suspicion of LE and a normal MRI should undergo PET or SPECT examination, especially if electroencephalography (EEG) and CSF investigations are also unrevealing. However, these examinations may be available to only limited centers and are impractical. Therefore, in the presence of autoantibodies in a clinical setting of LE and normal MRI, clinicians should consider giving a trial of immunosuppressants. Encephalitis patients with Thy-Abs have often been reported to display a good treatment response.[4,5] While our Thy-Ab-positive patients showed trends towards exhibiting better disease outcomes than Thy-Ab-negative ones, this difference did not attain statistical significance. In keeping with the previous observations,[8] LE patients with neuronal surface antibodies were more responsive to treatment in our study. Notably, neuronal surface antibody positivity was more significantly associated with good response to immunosuppressants than Thy-Ab positivity, which constitutes another important reason for monitoring neuronal surface antibodies in LE.

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