TÜMELAKTİF DEMİYELİNİZAN LEZYONLARA SAHİP MULTİPL SKLEROZ HASTALARINDA NÖRÖNAL YÜZEY ANTİKORLARI

NEURONAL SURFACE ANTIBODIES ARE NOT FOUND IN MULTİPLE SCLEROSIS PATIENTS WITH TUMEFACTIVE DEMYELINATING LESIONS

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ÖZET

Tümefaktif demiyelinizan lezyon (TDL); 2 cm veya daha fazla çapa sahip kütlesel etki, perilezyonal ödem ve halkasal yapı ile karakterize beyin lezyonudur. TDL zaman zaman multipl skleroz (MS) ve nöromiyelitis optika (NMO) hastalarında gözlemlenmektedir. Aquaporin 4 (Aqp-4) ve nöronal yüzey antijenlerine (iyon kanalları ve diğer nöronal membran proteinleri) karşı üretilen antikorlarının TDL patogenezinde rolü olup olmadığını belirlemek için TDL'na sahip yineleyen-düzelen MS (relapsing-remitting MS; RRMS) hastalarında anti-nöronal ve nöronal hücre yüzey antikorlarını taradık.

Bu çalışmada; TDL ile karakterize nörolojik semptomlar gösteren 7 RRMS hastası (6 kadın, 1 erkek; ortalama yaş±standart deviasyon 42.2±11.7) ve kontrol olarak yaş-cinsiyet eşleşmesi gösteren TDL'a sahip olmayan 40 RRMS, 40 otoimmün limbik ensefalit, antikorları bilinen 25 NMO hastası ve 50 sağlıklı kontrol serumu kullanılmıştır.

TDL'a sahip olan veya olmayan MS hastaları ve sağlıklı kontrollerin hiçbirinde, iyi karakterize edilmiş nöronal yüzey antijen veya hücre kültüründeki nöronlarda eksprese olan herhangi bir hücre yüzey antijenine karşı antikor gözlemlenmemiştir. Buna karşın, beklenildiği üzere, otoimmün ensefalit ve NMO hasta serumlarında çeşitli anti-nöronal antikorlar (5 Aqp-4, 3 CASPR2, 3 LGI1, 5 NMDAR, 2 AMPAR, 2 $GABA_BR$, 5 GAD antikor) tespit edilmiştir.

Çalışmamız TDL oluşumu ve nöronal yüzey antikoru arasında ilişki gösterememiştir. Hücre kültürü sonuçlarımızda nöron membran yüzey antijenlerine bağlanan anti-nöronal antikorların olmayışı, TDL hastalarında antikor-ilişkili mekanizmaların rol oynamadığına işaret etmektedir.

SUMMARY

Tumefactive demyelinating lesion (TDL) is a brain lesion with a diameter of 2 cm or more and is often associated with a mass effect, perilesional edema and ring enhancement. TDLs are occasionally encountered in multiple sclerosis (MS) and neuromyelitis optica (NMO) patients. To find out whether antibodies directed against aquaporin 4 (Aqp-4) and neuronal surface antigens (ion channels and other neuronal membrane proteins) are involved in TDL pathogenesis, we screened a panel of well-characterized anti-neuronal antibodies and neuronal cell surface antibodies in relapsing remitting MS cases presenting with TDLs.

Seven relapsing remitting MS patient (6 women, 1 man; average age±standard deviation 42.2±11.7 year-old) presenting with neurological episodes characterized with TDLs, Controls included age/gender matched relapsing remitting MS patients without any history of TDLs (n=40), autoimmune limbic encephalitis and NMO patients with well-characterized antibodies (n=25) and healthy individuals (n=50).

None of the MS patients with or without TDLs and healthy controls showed antibodies directed against well-characterized neuronal surface antigens or any other cell membrane antigen expressed by cultured live neuronal cells. By contrast, control autoimmune encephalitis and NMO patients showed various serum anti-neuronal antibodies (5 Aqp-4, 3 CASPR2, 3 LGI1, 5 NMDAR, 2 AMPAR, 2 $GABA_BR$, 5 GAD antibody positivity), as expected.

Our study failed to reveal any association between TDL occurrence and neuronal surface antibodies. Our results imply that absence of serum anti-neuronal antibodies reacting with membrane antigens of cultured live neurons in TDL patients suggests that antibody-mediated mechanisms are not involved in TDL pathogenesis.

INTRODUCTION

Tumefactive demyelinating lesion (TDL) is a brain lesion with a diameter of 2 cm or more and is often associated with a mass effect, perilesional edema and ring enhancement (1). TDLs are occasionally encountered in multiple sclerosis (MS) and neuromyelitis optica (NMO) patients and aquaporin 4 (Aqp-4) antibody positive NMO patients with TDL have recently been described (2). Serum Aqp-4 antibody levels have been shown to be correlated with clinical deterioration in a patient with TDL (3), suggesting that Aqp-4 antibodies are actively involved in TDL formation.

In the last decade, several anti-neuronal antibodies reacting with the neuronal membrane antigens and capable of causing acute onset neurological symptoms (typically in the form of autoimmune encephalitis) have been described. These antibodies are identified by their characteristic reactivity with neuronal membrane antigens of cultured live neuronal cells and human embryonal kidney (HEK) cells transfected with plasmids encoding specific ion channel subunits (4,5). Patients with these antibodies may present with demyelinating white matter lesions and are infrequently detected in MS patients (6). NMO, autoimmune encephalitis and TDL attacks are all characterized with acute onset and favorable and prompt response to antibody depleting treatment methods (1-5), suggesting that antibody-mediated mechanisms play role in TDL generation.

To find out whether antibodies directed against Aqp-4 and neuronal surface antigens (ion channels and other neuronal membrane proteins) are involved in TDL pathogenesis, we screened a panel of well-characterized anti-neuronal antibodies and neuronal cell surface antibodies in relapsing remitting MS cases presenting with TDLs.

MATERIAL AND METHODS

Seven relapsing remitting MS patients (6 women, 1 man; average age±standard deviation 42.2±11.7 year-old) presenting with neurological episodes characterized with TDLs (Figure 1) were included and their sera were collected during TDL episodes before starting immunosuppressive treatment. All 7 patients had only one TDL attack characterized with a single subcortical hemispheric lesion (>2 cm diameter). Neurological examination findings included hemihypesthesia and hemiplegia in 5 and only hemihypesthesia in 2 patients. Aphasia and visual field defects were

detected in 1 and 2 patients, respectively. TDLs were located in parietotemporal region in 6 (4 right, 2 left) patients and right frontal lobe in 1 patient. Open ring-enhancement was observed in neuroimaging studies of 5 patients and cerebrospinal fluid (CSF) oligoclonal bands were detected in 4 patients. All patients responded favorably to pulse steroid treatment. Controls included age/gender matched relapsing remitting MS patients without any history of TDLs (n=40), autoimmune limbic encephalitis and NMO patients with well-characterized antibodies (n=25) and healthy individuals (n=50). Sera of all control patients were obtained during clinically active periods and before initiation of any immunosuppressive treatment. Consent forms were obtained from all participants and the study was approved by the Institutional Review Board.

Sera of patients and control subjects were tested for antibodies to Aqp-4, contactin-associated protein-like 2 (CASPR2), leucine-rich glioma inactivated 1 (LGI1), N-methyl-D-aspartat receptor (NMDAR), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), gamma-aminobutyric acid B receptor ($GABA_BR$) by cell-based assays using a kit containing HEK293 cells transfected with plasmids encoding relevant neuronal cell surface antigens (Euroimmun, Luebeck, Germany). GAD antibodies and antibodies to uncharacterized VGKC-complex antigens were investigated by ELISA and RIA kits (RSR Ltd, Cardiff, UK), respectively.

Furthermore, antibodies to uncharacterized neuronal surface antigens were investigated by using cultured hippocampal neurons of P1 rat pups, as described (4,5). The cultured neurons were incubated with patients' sera (1:50 to 1:200 dilutions) for one hour at room temperature, followed by 3% formaldehyde fixation and by incubation with Alexa Fluor 488-conjugated anti-human immunoglobulin (IgG) (Invitrogen, Paisley, UK) for 45 minutes. Images were photographed under a Zeiss fluorescence microscope with a digital camera using the Zeiss Axiovision software. In all assays, sera of previously diagnosed antibody positive (2-5 patients for each antibody) autoimmune limbic encephalitis patients were used as positive controls.

RESULTS

None of the MS patients with or without TDLs and healthy controls showed antibodies directed against well-characterized neuronal surface antigens or any other cell membrane antigen expressed by cultured live neuronal cells. By contrast, control autoimmune encephalitis and NMO patients showed various serum anti-neuronal antibodies (5 Aqp-4, 3 CASPR2, 3 LGI1, 5 NMDAR, 2 AMPAR, 2 $GABA_BR$, 5 GAD antibody positivity), as expected. Six autoimmune encephalitis patients with CASPR2 or LGI1 antibodies were also positive in the RIA assay used for detection of VGKC-complex antibodies (830-1760 pM). Eighteen autoimmune encephalitis patients showed immunoreactivity with the membrane antigens of cultured live neuronal cells (Figure 2).

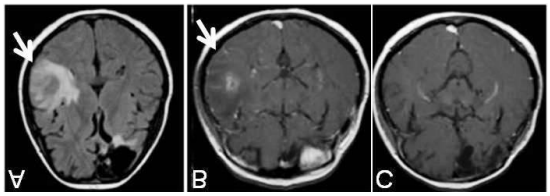

Figure 1. Representative axial cranial MR images (A, FLAIR-weighted; B and C, contrast enhanced T1-weighted) of a relapsing remitting multiple sclerosis patient presenting with a right parietotemporal tumefactive demyelinating lesion (TDL, arrows) showing central contrast enhancement (B). The TDL promptly regressed following one course of pulse methylprednisolone treatment (C).

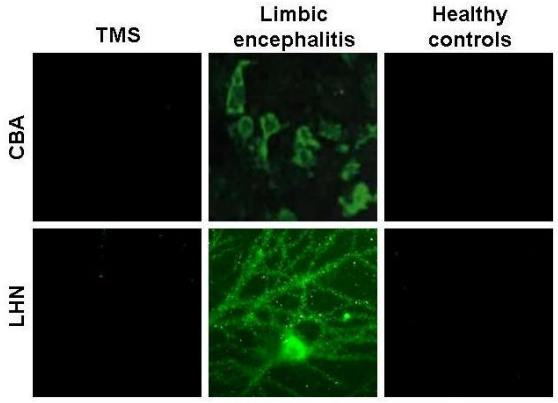

Figure 2. Representative images of antibody assays performed using sera of tumefactive multiple sclerosis (TMS) patients, control autoimmune limbic encephalitis patients and healthy controls. The cell based assay (CBA) shows that an autoimmune limbic encephalitis patient's serum antibodies react (green) with human embryonic kidney (HEK) 293 cells expressing NR1/NR2 heteromers of the N-methyl-D-aspartate receptor (NMDAR). By contrast serum IgGs of TMS patients and healthy controls do not show any immunoreactivity (upper row). Likewise, cultured live hippocampal neurons (LHN) incubated with the NMDAR-antibody positive limbic encephalitis patient's serum demonstrate intense immunolabeling (green) of neuronal membrane and processes, whereas serum antibodies of TMS patients and healthy controls do not show any reactivity with LHN (lower row). Original magnification in upper panels (400x) and middle-lower panels (800x).

DISCUSSION

Our study failed to reveal any association between TDL occurrence and neuronal surface antibodies. Some autoimmune disorders might coexist in the same patient and MS patients may often develop other autoimmune disorders during their disease course (7). Thus, TDL could hypothetically have been a form of autoimmune encephalitis tending to occur in MS patients. However, our results imply that this is probably not the case and thus routine screening of well-characterized antibodies in TDL patients is not recommended. Absence of serum anti-neuronal antibodies reacting with membrane antigens of cultured live neurons in TDL patients suggests that antibody-mediated mechanisms are not involved in TDL pathogenesis. A negative aspect of our study was absence of CSF samples and thus in future studies, antibody measurements are recommended to be performed in CSF samples of TDL patients. Also, potential T cell-mediated autoimmune pathogenic mechanisms and presence of antibodies to non-neuronal brain cells such as oligodendrocytes are warranted to be analyzed in patients with TDL.

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