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Analysis of antibodies to surface epitopes of contactin-2 in multiple sclerosis

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Abstract

Contactin-2 was recently identified as an autoantigen targeted by T-cells and autoantibodies in multiple sclerosis (MS). Here we analyzed the frequency of antibodies to contactin-2 (contactin-2-ab) by a cell-based assay in the serum from 105 MS patients and at least 5 years of follow-up (19 clinically isolated syndromes, 51 relapsing–remitting, 20 secondary-progressive, and 15 primary-progressive). Contactin-2-ab were detected in 4 (7.8%) relapsing–remitting patients. Clinical and magnetic resonance imaging characteristics were not significantly different from the rest of relapsing–remitting patients. In conclusion, contactin-2-ab are identified in a minority of MS patients but their presence is not associated with a particular clinical-radiological profile.

Keywords

Multiple sclerosis; Contactin-2; Autoantibody; Cell-based assay; Magnetic resonance imaging

1. Introduction

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) with both clinical and pathological heterogeneity. In the past few years, the concept of disease suggesting that T-cell-mediated immunity is sufficient for full expression of MS has been challenged, and it is now evident that B cells and humoral immune mechanisms also play key roles (Lassmann et al., 2007; Breij et al., 2008; Franciotta et al., 2008; Hauser et al., 2008; Weiner, 2009).

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Pathological studies suggest that antibodies might contribute to plaque initiation and ongoing demyelination in MS patients with established disease (Breij et al., 2008). Although a wide range of candidate antigens and autoantibodies have been reported, the true relevance remains uncertain (Reindl et al., 2006; Racke, 2008). Recently, contactin-2 (and its rodent orthologue, transiently expressed axonal glycoprotein 1, TAG-1), expressed in the juxtaparanodal region of myelinated axons, was identified as an autoantigen targeted by T-cells and autoantibodies. Passive transfer of contactin-2-specific T-cells induced an experimental autoimmune encephalomyelitis (EAE) with preferential inflammation of gray matter (Derfuss et al., 2009). In that study, only a minority of antibodies to contactin-2 (contactin-2-ab) bound to the surface of the protein, a prerequisite to mediate a pathological effect, and a clinical-immunological correlation was not done. The aim of the present study was to analyze the frequency and clinical significance of antibodies to surface epitopes of contactin-2 using a sensitive cell-based assay in a larger series of patients with MS.

2. Patients and methods

2.1. Patients

We selected serum samples from 105 clinically definite MS patients (Poser et al., 1983), 40 of them with paired serum/CSF samples, and at least 5 years of follow-up [at the time of sample collection the clinical syndromes were as follows: clinically isolated syndrome (19 patients), relapsing–remitting MS (51), secondary-progressive MS (20), and primary progressive MS (15)]. Patients are routinely included in a database and followed every 3–6 months. Information on clinical relapses, disability (EDSS [Expanded Disability Status Scale]) and therapies are registered prospectively as previously reported (Llufriu et al., 2010). Demographics, clinical, and brain magnetic resonance imaging (MRI) characteristics of the patients at the time of sampling were reviewed blinded to the antibody results. As controls, we included serum samples from 20 healthy donors. The study was approved by the Ethics Committee of the Hospital Clinic and an informed consent was previously obtained.

2.2. Cell-based assay

The presence of contactin-2-ab was analyzed by indirect immunofluorescence on HEK293 cells transfected with a plasmid containing human contactin-2 cDNA clone (*Homo sapiens* contactin 2, axonal, CNTN2, SC303578, Origene Technologies, Rockville, MD 20850) as previously described (Boronat et al., 2011). Briefly, patients' sera (dilution 1:40) or CSF (dilution 1:2) and commercial rabbit antibody to contactin-2 (dilution 1:20; rabbit polyclonal to TAG-1, ab66252, Abcam, Cambridge, UK) were applied to live cells for 1 h at 37 °C, washed and fixed with 4% paraformaldehyde, followed by anti-human IgG (dilution 1:2000; fluorescein-conjugated goat F(ab')₂ fragment to human IgG (Fc), 55184, Cappel, MP Biomedicals, LLC, Solon, OH) and goat anti-rabbit IgG (dilution 1:1000; Alexa Fluor 555 F(ab)₂ fragment of goat anti rabbit IgG (H+L), A21430, Molecular Probes, Eugene, OR, USA) during 1 h at room temperature. Results were photographed under a fluorescence microscope using Zeiss Axiovision software (Zeiss, Thornwood, NY). The results were evaluated by 3 investigators (AB, LS, AS) blinded to the clinical data. Contactin-2-ab-positive samples were evaluated with a similar cell-based assay for the presence of

antibodies against proteins associated with contactin-2: leucine-rich glioma inactivated 1 (LGI1) and contactin associated protein-like 2 (Caspr2) (Lai et al., 2010).

2.3. Statistical analysis

Chi-squared or Fisher exact test were performed to compare categorical variables. Comparisons between continuous variables were performed using *t*-test. Kaplan–Meier analysis was used to estimate cumulative survival probabilities for time to reach an EDSS (Expanded Disability Status Scale) endpoint of 3.0, 4.0 and 6.0. All significant results were set at two-tailed *p*-value < 0.05. All statistical analysis was performed by software SPSS version 18.0.

3. Results

The demographics and clinical characteristics of the patients included in the study are shown in Table 1. Contactin-2-ab were found in the serum of 4 of the 51 (7.8%) relapsing–remitting patients (Fig. 1). The rest of the samples analyzed, including the available CSF of 3 of the positive patients and the control samples, were negative. Positive samples did not present antibodies against LGI1 or Caspr2. The clinical and MRI characteristics of the positive patients were heterogeneous but not significantly different from the rest of relapsing–remitting patients in terms of demographics, relapses, disability and years of follow-up (Table 2). There were also no significant differences in the analyzed MRI characteristics (available at the time of sampling in 37 negative patients and the 4 positive patients) (Table 2). A new serum sample of the 4 positive patients was analyzed after a median follow-up of 9 years (8–14 years), and the antibodies remained positive in all of them. During the follow-up, 1 (25%) of the positive patients and 11 (23.4%) of the negative patients developed the secondary progressive form of the disease (Table 2).

4. Discussion

This study confirms the existence of an autoantibody response to surface epitopes of contactin-2 in a minority of MS patients. The presence of contactin-2-ab was not consistently associated with a particular clinical profile at the time of detection or with a different evolution at long term.

Autoantibody response to contactin-2 was first identified in MS by proteomic approach (Dorfuss et al., 2009). Contactin-2-ab were detected by ELISA not only in MS patients but also in patients with other neurological diseases, and healthy controls. However, when antibodies against surface epitopes of contactin-2 were tested on cell lines transfected with contactin-2 by flow cytometry, only 2 of the 25 MS samples were positive (Dorfuss et al., 2009), a similar frequency to that found in the current study.

Contactin-2, a cell-adhesion molecule of the immunoglobulin superfamily, exists both as a glycosphosphatidylinositol-linked cell surface isoform and as a released form. At the juxtaparanodal region of myelinated axons it is expressed on the glial membrane and on the axon as a contactin-2/Caspr2 heterodimer (Poliak et al., 2003; Savvaki et al., 2008; Dorfuss et al., 2010). In this localization, the intact myelin sheath probably prevents antibodies to

gain access to contactin-2 in the juxtaparanodal region. In fact, contactin-2-ab failed to cause any additional damage when injected to experimental animals with EAE induced by transfer of contactin-2-specific T-cells (Derfuss et al., 2009, 2010). Similar results have been observed with antibodies to neurofascin, another recently identified autoantigen in MS (Mathey et al., 2007).

Antibodies to surface epitopes of contactin-2 were recently detected in 5 of the 96 patients who had antibodies previously attributed to voltage-gated potassium channel (VGKC) (Irani et al., 2010). Four of the 5 positive samples also had additional antibodies. Two of them had antibodies to Caspr2, one to LGI1, and one to Kv1.2. These results are not unexpected considering that contactin-2 is in the list of proteins that, like LGI1 and Caspr2, colocalizes or associates with VGKC (Poliak et al., 2003; Savvaki et al., 2008). In contrast, MS patients of the present study only presented contactin-2-ab.

Although contactin-2-ab do not seem to induce neuronal dysfunction of gray matter inflammation (Derfuss et al., 2009), the close association of antibodies against contactin-2-related proteins with disorders characterized by neuronal dysfunction (Lancaster et al., 2011), and the expression of contactin-2 in subsets of cortical neurons (Savvaki et al., 2008) supports the previous observation that contactin-2-specific T-cells could play a pathogenic role of in the cortical pathology of MS patients (Derfuss et al., 2009). This pathogenic effect would be relevant because in the progressive forms of the disease, both secondary and primary progressive MS, the cortex is severely affected with extensive cortical demyelination associated with inflammatory infiltrates in the leptomeninges (Lassmann et al., 2007). Moreover, cortical lesions can be present from the onset of the disease, and contribute to physical disability and cognitive impairment (Mike et al., 2011).

Finally, in our study contactin-2-ab were only detected in the serum, and it is similar to neuromyelitis optica and NMO-IgG in which the antibodies are less frequently detectable in CSF (Jarius et al., 2010). Although the persistence of contactin-2-ab in all 4 patients after a median follow-up of 9 years would suggest that they are not merely an epiphenomenon, it does not mean that contactin-2-ab are relevant for the disease as is suggested in our study. It is important to note, however, that the lack of significant differences between contactin-2-ab positive and negative patients could be related to the low number of positive patients. This is in contrast with autoantibodies to MOG in which their detection by cell-based assays seems to identify a subgroup of patients with acute disseminated encephalomyelitis (Di Pauli et al., 2011).

In conclusion, the frequency of contactin-2-ab in MS is low, mostly observed in relapsing forms and, although the antibodies persist for long time, their presence is not associated with a particular clinical-radiological profile. Our results would suggest that contactin-2-ab analysis would not be useful for identifying different disease subtypes or prognosis.

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Fig. 1.

Detection of antibodies to contactin-2 using a cell-based assay. Binding of antibodies from a positive patient to the surface of HEK293 cells transfected with human contactin-2 cDNA clone (A; green); the cells were coincubated with a rabbit antibody against contactin-2 (A; red); the reactivity of the patient's serum colocalizes with the reactivity of the rabbit antibody specific for contactin-2 (A; merge, yellow); HEK293 transfected cells did not show reactivity with the serum of a healthy control (B).

Table 1

Demographic and clinical characteristics of the patients included in the study.

	Clinically isolated syndrome (<i>n</i> = 19)	Relapsing– remitting MS (<i>n</i> = 51)	Secondary progressive MS (<i>n</i> = 20)	Primary progressive MS (<i>n</i> = 15)
Gender: female/male	14/4	31/20	13/8	9/5
Age at onset (y); mean ± SD	32 ± 10	31 ± 11	32 ± 11	43 ± 7
Age at sampling (y); mean ± SD	32 ± 10	36 ± 11	41 ± 12	51 ± 6
Disease duration at sampling (y); mean ± SD	0.3 ± 0.4	5.5 ± 6.5	8.9 ± 5.7	7.6 ± 5.3
Disease duration (y); mean ± SD	10.5 ± 2.0	16.1 ± 8.2	18.1 ± 5.7	16.3 ± 6.3
EDSS at sampling; mean ± SD	1.8 ± 1.1	2.2 ± 1.4	5.0 ± 1.6	4.4 ± 1.3
EDSS at last follow-up; mean ± SD	2.1 ± 1.6	3.5 ± 2.1	6.9 ± 1.6	6.1 ± 1.3
Follow-up from sampling (y); mean ± SD	10.2 ± 1.9	10.6 ± 3.1	9.2 ± 3.6	8.7 ± 4.1

y—Years; SD—standard deviation; *n*—number; EDSS—Expanded Disability Status Scale.

Table 2

Demographic, clinical and MRI characteristics of relapsing–remitting patients.

	Contactin-2-ab negative (<i>n</i> = 47)	Contactin-2-ab positive (<i>n</i> = 4)	<i>p</i> -Value
Gender: female/male	28/19	3/1	n.s.
Age at onset (y); mean ± SD	30 ± 11	33 ± 3	n.s.
Age at sampling (y); mean ± SD	36 ± 11	39 ± 7	n.s.
Disease duration at sampling (y); mean ± SD	5.4 ± 6.4	6.6 ± 9.1	n.s.
Disease duration (y); mean ± SD	16.1 ± 8.0	16.5 ± 11.8	n.s.
<i>n</i> relapses first 2 y; mean ± SD	2.3 ± 1.5	2.0 ± 0.8	n.s.
<i>n</i> relapses first 5 y; mean ± SD	4.6 ± 3.6	3.8 ± 2.2	n.s.
Annualized relapse rate; mean ± SD	0.56 ± 0.35	0.43 ± 0.24	n.s.
EDSS at sampling; mean ± SD	2.2 ± 1.4	2.0 ± 1.9	n.s.
EDSS at last follow-up; mean ± SD	3.5 ± 2.1	4.4 ± 3.1	n.s.
Time to EDSS 3.0 (y); mean (95% CI)	18.2 (14.8–21.7)	10.2 (3.82–16.7)	n.s.
Time to EDSS 4.0 (y); mean (95% CI)	21.2 (17.7–24.6)	14.5 (6.1–22.9)	n.s.
Time to EDSS 6.0 (y); mean (95% CI)	26.6 (23.1–30.2)	22	n.s.
Develop of SPMS; <i>n</i> (%)	11 (23.4)	1 (25)	n.s.
Time to SPMS (y); mean ± SD	18.6 ± 9	23	n.s.
Follow-up from sampling (y); mean ± SD	10.6 ± 3.2	9.9 ± 2.8	n.s.
Brain MRI at sampling (<i>n</i>):	37	4	
<i>n</i> Of T2 lesions; mean ± SD	22.37 ± 17.9	17.67 ± 16.2	n.s.
<i>n</i> Of periventricular lesions; mean ± SD	7.7 ± 5.4	5 ± 3.6	n.s.
<i>n</i> Of juxtacortical lesions; mean ± SD	4.43 ± 5.2	2 ± 2	n.s.
<i>n</i> Of infratentorial lesions; mean ± SD	1.9 ± 2.7	3.25 ± 3.3	n.s.
<i>n</i> Of cortical lesions; mean ± SD	0.17 ± 0.38	0.25 ± 0.5	n.s.
<i>n</i> Of enhancing lesions; mean ± SD	6.9 ± 16.6	1.0 ± 0	n.s.

Contactin-2-ab—antibodies to contactin-2; y—years; SD—standard deviation; *n*—number; EDSS—Expanded Disability Status Scale; SPMS—secondary progressive multiple sclerosis; CI—confidence interval; MRI: magnetic resonance images.