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Specific antibody index in cerebrospinal fluid from patients with central and peripheral paraneoplastic neurological syndromes

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Abstract

We evaluated the concentration of antineuronal antibodies in paired cerebrospinal fluid (CSF) and serum samples from 19 patients with central and peripheral paraneoplastic neurological syndromes (PNS), using an enzyme linked immunosorbent assay (ELISA) employing recombinant antineuronal antigens (HuD, Yo, Ri, CV2/CRMP5, amphiphysin, PNMA2/Ma2). The specific antibody index (AI) [Q_{spec}/Q_{IgG}] was calculated to estimate specific intrathecal antibody synthesis. An AI >1.3 was considered as evidence of intrathecal specific antibody synthesis. 14 (88%) of 16 patients with exclusive or predominant paraneoplastic involvement of the central nervous system (CNS) showed an AI>1.3, indicating a specific intrathecal antibody synthesis, while all three patients with isolated involvement of the peripheral nervous system showed an AI<0.8. All together, in 17 of 19 patients (89%) we found a significant association ($p<0.05$) between central or peripheral neurological manifestations on the one hand and presence or absence of specific intrathecal synthesis respectively on the other hand. These data support the hypothesis that autoimmunity is involved in the pathogenesis of PNS.

Keywords: Paraneoplastic neurological syndromes; Intrathecal antibody synthesis; Specific antibody index; Recombinant ELISA; Clinical-immunologic correlation

1. Introduction

Paraneoplastic neurological syndromes (PNS) are rare remote effects of cancer that can affect any part of the nervous system from cortex to peripheral nerves and neuromuscular junctions (Posner, 1995). Detection of antineuronal antibodies cross-reacting with neuronal tissue and tumour antigens is useful to prove paraneoplastic etiology of suspected neurological disorders. Furthermore, these antibodies provide an important marker with a high predictive value, for the diagnosis of an underlying cancer (Dalmau et al., 1992). Although antineuronal antibodies seem to reflect an underlying autoimmune process, with some exceptions their pathogenic role still remains unclear (Voltz, 2002). However, there is evidence of T-cell involvement in the pathogenesis of PNS (Roberts and Darnell, 2004; Pellkofer et al., 2004).

Paraneoplastic antineuronal antibodies could be detected both in patients' serum and cerebrospinal fluid (CSF) (Furneaux et al., 1990). In individual cases antibody concentrations in CSF may be several orders of magnitude higher than in serum. Vega and colleagues demonstrated a positive correlation between intrathecal anti-Hu synthesis and clinical involvement of the central nervous system (CNS), in contrast to patients with isolated paraneoplastic

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subacute sensory neuronopathy, who did not reveal specific intrathecal antibody synthesis (Vega et al., 1994).

Using ELISA employing recombinant antigens (HuD, Ri, Yo, CV2/CRMP5, amphiphysin and PNMA2/Ma2), we investigated paired CSF and serum samples of 19 patients with different PNS manifestations affecting both the central and peripheral nervous system. We studied the presence of antigen specific intrathecal synthesis of paraneoplastic antineuronal antibodies in relation to clinical presentation of symptoms.

2. Patients and methods

2.1. Patients and controls

Nineteen serum and CSF pairs, positive for antineuronal antibodies of various specificities, from patients with previously diagnosed definite PNS, according to established criteria (Graus et al., 2004), were simultaneously investigated. Routine laboratory detection of antineuronal antibodies in serum was previously done by immunohistochemical methods and confirmed by immunoblot using recombinant antigens. All PNS patients harboured antineuronal antibodies with a relevant high anti-neuronal reactivity against only one single onconeuronal antigen. Therefore, intrathecal synthesis of each subject was determined only for specific antineuronal antibodies that were previously tested positive. CSF/serum pairs of nine patients with multiple sclerosis and ten patients with normal pressure hydrocephalus served as controls. All samples were stored at −80 °C prior to investigation.

2.2. ELISA

Detection of antineuronal antibodies in CSF and serum was performed by an ELISA described elsewhere (Rauer and Kaiser, 2001). The ELISA employed six well characterised recombinant antigens (HuD, Ri, Yo, CV2/CRMP5, amphiphysin and PNMA2/Ma2) and has been previously evaluated (submitted elsewhere) showing an overall sensitivity and specificity of 100% and 96%, respectively. Recombinant antigens were provided by ravo-Diagnostika (Freiburg, Germany). Briefly, 96-well flat-bottomed ELISA plates (Falcon, Germany) were coated with 100 µl/well of recombinant antigens for 24 h at 4 °C (HuD, PNMA2/Ma2: 0.8 µg/ml, Yo: 0.4 µg/ml, CV2/CRMP5, Ri, amphiphysin: 0.2 µg/ml). The concentrations of total IgG in CSF and sera were measured by routine nephelometry (BN 100). CSF and sera were adjusted to equal concentrations of total IgG (2.5 mg/l). The diluted CSF and serum samples of each patient were incubated in duplicate for 30 min at 37 °C on the same plates. Bound antibodies were detected by peroxidase-conjugated goat anti-human IgG antibodies (Dianova), diluted 1:5000. As substrate solution orthophenylendiamine 0.4 mg/ml (DAKO) was added. The reaction was stopped with 2 M H2SO4 and the optical density (OD) was read at 410 nm in an ELISA reader (Dynatech MR 4000). Mean ODs of the blanks were subtracted from the samples. The diagnostic cut-off OD reading was set four standard deviations above the mean of nineteen CSF and serum control samples.

Table 1
Demographic data, antibody findings and antibody index (AI) results from nineteen patients with various paraneoplastic neurological syndromes

No.	AB	Gender, age	Neoplasm	Neurological manifestation		Specific AI	IgG-index >0.8 or OCBs of total IgG in CSF
				Group 1 CNS, CNS/PNS	Group 2 PNS		
1	HuD	F, 64	n.d.	PCD, neuropathy		1.4	Positive
2		F, 54	SCLC		Neuropathy	0.8	Negative
3		M, 66	SCLC		SN	0.7	Identical OCBs in serum and CSF
4		F, 66	SCLC	LE		9.1	Negative
5		F, 45	BC		Cranial neuropathy	0.6	Negative
6	Ri	F, 66	BC	Myelitis, jaw dystonia, ophthalmoplegia		4.6	Positive
7		F, 43	BC	PCD, stiff person like syndrome		4.9	Negative*
8		F, 65	n.d.	BE, myelitis		>1.3	Positive
9		M, 68	Carcinoid of the lung	LE		0.7	Negative
10	Yo	F, 43	BC	PCD		1.4	Positive
11		F, 61	Sigma Ca	PCD		2.0	Positive
12		F, 66	Metastasis of adeno-Ca	PCD		1.6	Positive
13		F, 56	OC	PCD		1.5	Positive
14		F, 66	OC	PCD		10.3	Positive
15	CV2/CRMP5	F, 73	Mediastinal tumour	PCD, neuropathy		2.9	Positive
16		F, 61	Lung cancer	PCD		0.6	Positive
17		M, 47	SCLC	Myelitis, neuropathy		9.5	Positive
18	amphiphysin	M, 67	SCLC	Dysphagia, visual impairment, SN		1.4	Negative
19	Ma2	M, 38	Suspected testicular cancer	LE		2.9	Negative

AB: antibodies; AI: antibody index; BC: breast cancer; BE: brainstem encephalitis; CNS: central nervous system; F: female; LE: limbic encephalitis; M: male; n.d. not detected; OC: ovarian cancer; PCD: paraneoplastic cerebellar degeneration, PNS: peripheral nervous system; SCLC: small cell lung cancer; SN: sensory neuropathy; * previously positive OCBs in CSF.