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Hypocretin-1 CSF levels in anti-Ma2 associated encephalitis

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Abstract

Idiopathic narcolepsy is associated with deficient hypocretin transmission. Narcoleptic symptoms have recently been described in paraneoplastic encephalitis with anti-Ma2 antibodies. The authors measured CSF hypocretin-1 levels in six patients with anti-Ma2 encephalitis, and screened for anti-Ma antibodies in patients with ideopathic narcolepsy. Anti-Ma autoantibodies were not detected in patients with idiopathic narcolepsy. Four patients with anti-Ma2 encephalitis had excessive daytime sleepiness; hypocretin-1 was not detectable in their cerebrospinal fluid, suggesting an immune-mediated hypocretin dysfunction.

Most patients with narcolepsy have a deficient hypothalamic hypocretin (orexin) neurotransmission.[1–3] Patients with idiopathic narcolepsy typically have excessive daytime sleepiness (EDS) and cataplexy, associated with human leukocyte antigen (HLA) DQB1*0602, and an absence of hypocretin-1 in CSF.[4] Idiopathic narcolepsy is thought to be autoimmune because of the HLA association, supported by the peripubertal onset of the disease, the low concordance rate of only 25 to 30% in monozygotic twins, and the complex genetic susceptibility in families.[5] CNS involvement has been suggested in several cases.[6] Until now, however, no specific antibodies have been found to support the autoimmune hypothesis.[7]

A recently discovered symptomatic variant of narcolepsy with cataplexy occurred in patients with anti-Ma2-encephalitis, an uncommon autoimmune disorder.[8] Ma-2 is the major antigen of the Ma family of onconeuronal proteins that are targets of immune responses in paraneoplastic limbic, hypothalamic, and brainstem encephalitis.[8,9] The majority of cases occur with germ-cell tumors of the testis.[8] Clinical signs and symptoms depend on the localization and extent of the encephalitis.

These data suggested a possible causal link between anti-Ma2 antibodies and hypocretin deficiency. We investigated whether there is a hypocretin deficiency in patients with anti-Ma2 encephalitis with symptomatic narcolepsy and whether patients with idiopathic narcolepsy have anti-Ma antibodies.

Methods

CSF samples of six patients with anti-Ma2–associated encephalitis were collected by J.D. at the Department of Neurology, University of Arkansas for Medical Sciences (Little Rock, AR).

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Samples were obtained at the time of symptom presentation, and kept frozen until use. The sample selection was based only on the amount of CSF that was available for study. Clinical data were retrospectively obtained from clinical charts. This included the presence of EDS, but the presence or absence of cataplexy could not determined with certainty. Patients were considered to have EDS when this complaint had been documented in the clinical chart.

Hypocretin-1 levels were measured in unextracted samples, using a standardized radioimmunoassay with a detection limit of 100 pg/mL.[6] Investigators examining the hypocretin levels were blinded for clinical information.

Serum and CSF samples of 19 patients with narcolepsy (12 men) fulfilling the International Classification of Sleep Disorders criteria for narcolepsy were screened for anti-Ma antibodies. Their mean age was 35.8 ± 12.6 years, and the mean duration of illness was 9.7 ± 8.3 years. Eighteen were HLA DQB1*0602 positive; no typing had been performed in the remaining case. Samples were obtained at the Department of Neurology, Leiden University Medical Center (Leiden, the Netherlands) and kept frozen until use. Several were selected because of a recent onset of disease or an increased immunoglobulin G (IgG) index or oligoclonal bands in the CSF. The presence of anti-Ma antibodies was examined using immunoblot techniques. In brief, recombinant Ma1 and Ma2 fusion proteins were obtained as reported.[8] Immunoblot strips were preincubated with 10% normal goat serum for 1 hour, followed by an overnight incubation at 4 °C with patient serum or CSF (serum diluted 1:500; CSF diluted 1:10). Afterwards, strips were washed, incubated for 1 hour with biotinylated goat antihuman IgG, diluted 1:2,000 (Vector, Burlingame, CA), and developed with the avidin-biotin-peroxidase method (Vector). All incubations were performed at room temperature, unless otherwise stated.

Results

Clinical and demographic data of the six patients with anti-Ma2–associated encephalitis are summarized in the table. Four patients had EDS. Of these, one had additional hypothalamic dysfunction (hypothyroidism and diabetes insipidus); the second had treatment-refractory partial and generalized seizures; the third had symptoms of limbic encephalopathy (short-term memory problems, depression, and partial complex seizures); and the fourth developed short-term memory problems associated with diplopia and downgaze paresis. Two patients did not report EDS; one of these had diplopia, bursts of opsoclonus, gait difficulty, anxiety, and panic attacks, and the other parkinsonism, dementia, vertical gaze limitation, and gait difficulty. Brain biopsies had been performed in three patients with EDS (two medial temporal and one thalamic). These showed perivascular and interstitial inflammatory infiltration, with variable gliosis and neuronal degeneration. Hypocretin-1 could not be detected in CSF of any of the four patients with EDS, but it was present, with normal levels, in the CSF of the two patients without sleep complaints (see the table).

Of the 19 patients with idiopathic narcolepsy, 16 were hypocretin-deficient. All except two had cataplexy. Autoantibodies against Ma1 or Ma2 could not be detected in serum or CSF from any patient.

Discussion

We hypothesized that a link between anti-Ma2 and hypocretin deficiency might work in two directions. Our data suggest that the association works in one direction, but not the other.

Hypocretin-1 was undetectable in the four patients with anti-Ma2 encephalitis with EDS, whereas levels were normal in the two patients without EDS. This indicates that hypocretin deficiency can have an autoimmune-mediated cause. Several other cases of symptomatic narcolepsy with low or undetectable levels of hypocretin have been reported,[10] differing from

the present cases in that they were caused by relatively common disorders (tumors or ischemia) that incidentally involved the hypothalamus, in contrast to anti-Ma–associated encephalitis, which preferentially affects diencephalic and upper brainstem regions.

Pathologic studies in anti-Ma2–associated encephalitis suggested that T-cell mechanisms play a pathogenic role.[8,9] In some cases, the neurologic dysfunction may improve dramatically with treatment of the tumor and with immunosuppression. Neurologic improvement or stabilization occurred in only two of the patients reported here, suggesting that the damage was irreversible in most instances.

The results do not support a role of anti-Ma2 antibodies in idiopathic narcolepsy, although an immune-mediated cause is still possible. Anti-Ma encephalitis provides an example of how the immune system might be involved in narcolepsy: the immune-mediated response may itself be temporary in nature, but cause permanent damage. Furthermore, anti-Ma encephalitis affects specific brain regions, although Ma antigens are present in all neurons.

If narcolepsy is an autoimmune disorder, it is likely to exhibit the same features: hypocretin neurons may be preferentially damaged by a temporary attack against a ubiquitous antigen, which has not been identified so far.[2,3,7]

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Table

Clinical characteristics and laboratory findings in patients with anti-Ma associated encephalitis

Patient no.	Sex	Age, y	Neurologic symptoms	Tumor type	Radiology	Hcrt-1, pg/mL*
1	M	45	EDS (altered sleep habits), depression, memory loss, seizures, abnormal weight gain	Non-seminomatous germ cell tumor of the testis	MRI: T2-weighted lesions in both mesiotemporal regions, ventricular enlargement, temporal lobe atrophy	<100
2	M	22	Severe EDS, lethargy, depression, partial complex seizures	Mixed germ-cell tumor of the testis	MRI: left temporal enhancing abnormalities	<100
3†	F	82	Short-term memory problems, diplopia, opsoclonus, gait difficulty, personality change	Poorly differentiated lung cancer	MRI: Abnormalities in brainstem, periventricular region, and basal ganglia	237
4	F	67	EDS (altered sleep habits), diplopia, downgaze paresis, short-term memory problems, balance difficulties	Adenocarcinoma of the Lung	MRI: nodular enhancing abnormalities in the thalamus and superior collicular region; T2-weighted medial temporal lesions	<100
5	M	38	Severe EDS, lethargy, abulia, hypothalamic dysfunction(hypothyroidism, diabetes insipidus, episodes of hypertermia)	Germ-cell tumor of the testis (seminoma)	MRI: lesions hippocampus, midbrain	<100
6	F	53	Parkinsonism, dementia, vertical gaze limitation, gait difficulty	Adenocarcinoma of the Ovary	MRI: normal	218

*Hcrt-1 <100: concentration below detection limit of assay.

†In addition to Ma2, this patient also had Ma1 antibodies.

EDS = excessive daytime sleepiness.

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