Evaluation of Hypothalamic-Specific Autoimmunity in Patients With Narcolepsy

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Abstract: An autoimmune-mediated mechanism is considered the most probable etiology for narcolepsy. However, this hypothesis remains unproven. Since narcolepsy is characterized by dysfunction of the hypothalamic hypocretinergic (orexinergic) system, we evaluated the presence of hypothalamic-specific antibodies in sera and CSF of 25 hypocretin-deficient and 6 non-deficient narcoleptic patients by immunohistochemistry and analyzing a screening of a rat cDNA expression hypothalamic library. There was no hypothalamic-specific reactivity in serum or CSF by immunohistochemistry. The screening of the hypothalamic library detected some reactive clones but not a common reactivity. Our study did not find any evidence of hypothalamic-specific autoimmunity in narcolepsy.

Keywords: Narcolepsy, autoimmunity, hypothalamus, hypocretin, orexin


INTRODUCTION

AN AUTOIMMUNE ETIOLOGY IS HYPOTHETICALLY INVOLVED IN THE PHYSIOPATHOLOGY OF NARCOLEPSY, ALTHOUGH THERE IS CONFLICTING EVIDENCE. This disorder is related to abnormalities in the hypothalamic hypocretin (orexin) system with a characteristic deficiency in CSF Hypocretin-1 (Hcrt-1) levels in narcoleptic patients. The aim of our study was to investigate the presence of hypothalamic-specific antibodies in the serum and CSF of narcoleptic patients, previously tested for CSF Hcrt-1 status, by using immunohistochemistry and the screening of a hypothalamic cDNA expression library. The latter is a systematic and unbiased approach method widely used for the detection of onconeural antigens eliciting specific immune responses.

METHODS

Patients were evaluated with a complete clinical assessment, a standard nocturnal polysomnographic study followed by a Mean Sleep Latency Test (MSLT), and HLA typing for the presence of DQB1*0602. After written informed consent, a lumbar puncture was performed to determine CSF Hcrt-1 levels using a direct RIA with internal controls for validation of the results as previously reported. CSF Hcrt-1 levels lower than 110 pg/mL were considered low. The study was approved by the ethics committee of our hospital.

Immunohistochemistry

Serum and CSF immunoreactivity from 26 narcolepsy-cataplexy and 5 narcolepsy without cataplexy patients were analyzed by immunohistochemistry using an avidin-biotin technique as previously described. Serial hypothalamic sections of 5 µm obtained from paraformaldehyde-fixed frozen of Wistar rats brain were incubated with serum or CSF for 3 hours at 37°C at different dilutions (1:100, 1:200, and 1:500 for serum, 1:1 for CSF). Sections were then incubated with biotinylated goat anti-human antibody and developed with the avidin-biotin immunoperoxidase technique. Adjacent hypothalamic sections were incubated with rabbit hypocretin-2 polyclonal antibody (Chemicon International) to confirm that the area studied was representative for the hypocretinergic neuron cumulus. Sera from 4 patients with anti-Ma2 antibody related paraneoplastic syndrome (2 with low CSF-Hcrt-1 levels, 1 with intermediate levels, and 1 with normal levels, none with cataplexy) were analyzed in the immunohistochemistry process as positive controls due to the known hypothalamic involvement of this syndrome. CSF from one anti-Ma2 patient with hypersomnia and low CSF Hcrt-1 levels was also analyzed by immunohistochemistry.

Screening of a Hypothalamic cDNA Expression Library

A lambda ZAP-II Library (Stratagene, La Jolla, CA) from rat hypothalamus was immunoscreened at optimal density with a pool of sera from 5 representative hypocretin-deficient narcoleptic patients with cataplexy (each diluted 1/1000) as previously reported. Several rounds of antibody screening were performed to reach a yield of 100% positive plaques. Phage clones were subcloned in pBluescript plasmid using the in vivo phage rescue protocol (Stratagene). Plasmid DNA was purified with the QIAprep Spin Miniprep Kit (Qiagen, Santa Clarita, CA) and sequenced with the ABI3100 DNA sequencer (Applied Biosystems, Foster City, CA) using the ABI PRISM dRhodamine Terminator cycle sequencing kit. The BLAST program (National Center for Biotechnology Information; National Institutes of Health Bethesda, MD) was used to search for sequence homologies.

RESULTS

Reactivity of Narcoleptic Sera and CSF in Rat Hypothalamic Tissue

Narcolepsy-cataplexy patients (n = 26; 25 HLA positive, 24 low CSF Hcrt-1 levels) had a mean disease duration of 22.35 ± 15.45 years (range 2-50 years). Narcolepsy without cataplexy patients (n = 5; 2 HLA positive, 1 low CSF Hcrt-1 levels) had a mean disease duration time of 11.8 ± 5.32 years (range 4-18 years). No reactivity was found in any sera or CSF from narcoleptic patients.
in contrast with the finding of mild diffuse hypothalamic reactivity in the nuclei of neurones of the anti-Ma2 samples (3 sera and 1 CSF).

Antigen Analysis

The screening of the hypothalamic library produced 4 reactive clones coding for 3 proteins: Inositol 1,4,5-trisphosphate 3-kinase B isoform, copine I and prosaposin (2 clones). However, a common reactivity against these proteins from the 5 sera of the pool could not be found.

DISCUSSION

The association of narcolepsy with the HLA type II, the disease onset in young adulthood, and the anecdotal description of clinical improvement with immunotherapy at early stages suggest an autoimmune etiology. The hypocretinergic neurons are selectively reduced in the posterior hypothalamus of narcoleptic patients, so the hypocretin system might be a possible target of an autoimmune attack. It is a common finding in autoimmune diseases that the autoimmune attack is not primarily directed against the specific deficient protein of the disorder. Instead, the main target might be other non-identified proteins of the primary organs. Supporting this finding in narcolepsy, previous studies have showed no antibodies for preprohypocretin and its derivatives.1

We evaluated the presence of antibodies against the posterior hypothalamus in the serum and CSF from narcoleptic patients. However, we could not find any reactivity against the hypothalamus by immunohistochemistry nor could common target antigens be identified using a hypothalamic expression library. This result contrasts with a recent report of CSF IgG binding to rat hypothalamic tissue homogenate evaluated by enzyme linked immunosorbent assay.4 Our negative result does not completely exclude the presence of autoantibodies since a limitation of the serological screening of cDNA expression libraries is the recognition of proteins that undergo posttranslational modifications, conformational epitopes, and multimeric proteins.6 The long time delay after clinical onset makes possible that an antibody level in the sera and CSF could have decreased to a range undetectable by conventional immunohistochemistry, as occurs in other autoimmune disorders.4 However, although low antibody levels may also limit their detection by the screening of cDNA expression library, this method has a higher sensitivity for detecting specific antibodies against linear epitopes than immunohistochemistry or ELISA.8 Although the proteins identified in the screening have important functions in synaptic plasticity and in the lipid storage process that is probably altered in Niemann-Pick type C disease (a disorder that shares with narcolepsy the presence of cataplexy), the unsuccessful attempt to define these antigens as targets in the final process of the screening does not allow us to relate them with the physiopathology of narcolepsy.

An alternative hypothesis to explain this negative result is an heterogeneous autoimmune response that limited the detection of a common reactivity, as occurs in other autoimmune disorders such as opsoclonus-myoclonus, although this physiopathology would be less plausible in narcolepsy due to the common clinical phenotype and biological characteristics (HLA and hypocretin deficiency). The lack of positive results in the search for specific autoantibodies is also observed in some paraneoplastic disorders in which more sophisticated techniques might allow the detection of antibody titles.6,10

In summary, an hypothalamic-specific autoimmune hypothesis in narcolepsy, although not excluded, is not supported by our study.

REFERENCES