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Cerebrospinal fluid and serum cytokine profiles in narcolepsy with cataplexy: A case-control study

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ABSTRACT

Recent advances in the identification of susceptibility genes and environmental exposures provide strong support that narcolepsy–cataplexy is an immune-mediated disease. Only few serum cytokine studies with controversial results were performed in narcolepsy and none in the cerebrospinal fluid. We measured a panel of 12 cytokines by a proteomic approach in the serum of 35 patients with narcolepsy–cataplexy compared to 156 healthy controls, and in the cerebrospinal fluid of 34 patients with narcolepsy–cataplexy compared to 17 non-narcoleptic patients; and analyzed the effect of age, duration and severity of disease on the cytokine levels. After multiple adjustments we reported lower serum IL-2, IL-8, TNF-α, MCP-1 and EGF levels, and a tendency for higher IL-4 level in narcolepsy compared to controls. Significant differences were only found for IL-4 in cerebrospinal fluid, being higher in narcolepsy. Positive correlations were found in serum between IL-4, daytime sleepiness, and cataplexy frequency. The expression of some pro-inflammatory cytokines (MCP-1, VEGF, EGF, IL2, IL-1β, IFN-γ) in either serum or CSF was negatively correlated with disease severity and duration. No correlation was found for any specific cytokine in 18 of the patients with narcolepsy with peripheral and central samples collected the same day. Significant decreased pro/anti-inflammatory cytokine profiles were found at peripheral and central levels in narcolepsy, together with a T helper 2/Th1 serum cytokine secretion imbalance. To conclude, we showed some evidence for alterations in the cytokine profile in patients with narcolepsy–cataplexy compared to controls at peripheral and central levels, with the potential role of IL-4 and significant Th1/2 imbalance in the pathophysiology of narcolepsy.

Introduction

Narcolepsy with cataplexy (NC) is a disabling orphan disorder caused by a loss of hypothalamic hypocretin/orexin producing neurons (Dauvilliers et al., 2007). Recent advances in the identification of susceptibility genes and environmental exposures provide strong support that narcolepsy may be considered as an immune-mediated disease. A tight association has long been reported with the Human Leukocyte Antigen (HLA) DRB1*1501-DQB1*0602 haplotype with more recent associations with T-cell receptor alpha, purinergic receptor P2RY11 polymorphisms, Cathepsin H and Tumor necrosis factor (ligand) superfamily member 4 (Hallmayer et al., 2009; Kornum et al., 2011; Mignot et al., 2011; Hor et al., 2010; Faraco et al., 2013). All these findings underline the importance of antigen presentation by HLA Class II to T-cells in the pathophysiology of NC. Environmental factors may also be involved in NC, with streptococcus infections being frequently reported at disease onset with high titers of anti-streptolysin O (Aran et al., 2009). An association between H1N1 vaccination (mostly represented by Pandemrix®), and NC has been recently reported in children and adults (Dauvilliers et al., 2013; Partinen et al., 2012). However, unlike most of other autoimmune diseases, no antibodies against either nuclear proteins or hypocretin neurons have been reported in NC (Overeem et al., 2006), but elevated Tribbles homolog 2 antibodies were detected in some patients, close to disease onset (Cvetkovic-Lopes et al., 2010). Intrathecal synthesis of immunoglobulins is rarely found and autopsy studies failed to report any T lymphocytes infiltration

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or increased HLA Class II expression in the hypothalamus in NC (Fontana et al., 2010; Overeem et al., 2008; Peyron et al., 2000). However, the low sample size and the long time period between the disease onset and the study prevents any conclusion on the immune mechanisms involved in hypocretin-deficient NC.

Cytokines are small, short-lived proteins produced by blood leukocytes and glial cells that initiate, propagate or inhibit an inflammatory response. These signaling compounds form a complex network of inflammatory mediators with a central role in the immune system (Sokol et al., 2008). Even if cytokines and their receptors are involved in a variety of immunological and inflammatory diseases, only rare studies focused on their expression in NC. Previous studies failed to report on abnormalities of T-cell function in NC (Fontana et al., 2010; Overeem et al., 2008), except for higher interleukin 6 (IL-6) secretion by mitogen-stimulated blood monocytes (Hinze-Selch et al., 2008). A slight increase of IL-6 and Tumor Necrosis Factor-α (TNF-α) serum levels was later detected in NC compared to controls (Okun et al., 2004; Vgontzas et al., 1997). Himmerich and collaborators did not confirm these results but found higher soluble TNF receptor 2 levels in NC in comparison to gender-, age-, and BMI-matched controls (Himmerich et al., 2006). However, none of previous cytokine studies focused on cerebrospinal fluid (CSF) assessment in NC neither took into account the duration and severity of the disease while cytokine concentrations profile may vary depending on temporal factors, and the tissue or fluid they are measured in. Recently the technology offers the possibility to determine multiple cytokine expressions simultaneously, providing valuable information relating to each cytokine under test and possible associations between CSF and serum cytokines.

Considering the limitation of knowledge regarding the immune mechanisms involved in hypocretin deficiency NC, we aimed to (1) investigate peripheral (serum) and central (CSF) characteristic cytokine profile in patients with NC in comparison to controls, and to analyze (2) the effect of age, duration of disease, and symptoms severity on the cytokine levels.

Materials and methods

Participants

Sixty-eight unrelated patients (40 males and 28 females, 15 children < 18 y.o. and 53 adults, median age at 28.5 y [4–78]) were included. All patients had a clinical suspicion of central hypersomnia and underwent one night of polysomnography (PSG) followed by the multiple sleep latency test (MSLT) in the sleep laboratory as required in ICSD-2 (The International Classification of Sleep Disorders, 2005). Patients were not taking psychostimulants, anticataplectics, immune therapy, or any other medication known to influence sleep $\geqslant$2 weeks prior to sleep recording. Patients were systematically evaluated for clinical parameters including: disease duration, Epworth Sleepiness Scale (ESS) Johns, 1991 and its adapted version for children (AESS), cataplexy frequency scale (scale from 0 to 5) Dauvilliers et al., 2001, hypnagogic hallucinations, sleep paralysis, and body mass index (BMI). Sleep was scored based on standard method. Subjects with an index of respiratory events (apneas plus hypopneas) >10 or with periodic leg movement index during sleep associated with microarousal >10 were excluded from the study. None of the patients had any psychiatric disorder based on the DSM-IV criteria. Patients were further classified based on their primary ICSD diagnosis. None of the patients were exposed to H1N1 vaccination at time of study.

Among these patients, 51 were narcoleptics and 17 were classified as unspecified hypersomnia. Patients with narcolepsy (31 males and 20 females, 15 children, median age at 27 y [4–78]) had clear-cut cataplexy and positive HLA DQB1$*$0602 typing. They presented at least two sleep onset REM periods (SOREMP) and a mean sleep latency of less than 8 min on the MSLT, except for two patients with a mean sleep latency above 8 min and one without any SOREMP but with low CSF hypocretin-1 levels. Patients with an unspecified hypersomnia diagnosis (9 males and 8 females, median age at 29 y [18–55]) reported a complaint of constant EDS (ESS > 10) without any cataplexy; however results on polysomnography plus MSLT even if repeated, 24-h continuous PSG or both could not confirm any well-defined hypersomnia diagnosis (The International Classification of Sleep Disorders, 2005; Dauvilliers et al., 2012).

One hundred and fifty-six unrelated healthy Caucasian healthy controls (125 males and 31 females, median age at 53.2 y [19–72]) without any primary complaint of sleep disorders were included, being children or grandchildren of women recruited in a multicenter prospective study on hip fracture risk factors (Blain et al., 2012; Dargent-Molina et al., 1996). Subjects were excluded if they met any of the following criteria: (i) known metabolic bone or arthritis disease, (ii) ischemic heart disease, (iii) neurological or psychiatric disorder based on the DSM-IV criteria, (iv) malignant tumors, (v) infectious or autoimmune disease, or (vi) medication known to affect the musculoskeletal system or the immune system.

All subjects gave their written informed consent to participate in the study, which was approved by the Human Subjects Research Committee of our University Hospital. According to the French law, this collection was registered to the "Ministère de l'Enseignement Superieur et de la Recherche" (Number DC-2008- 417).

Sample collection

Fasted morning peripheral blood samples were collected from 35 patients with NC (21 males and 12 children) and 156 healthy control subjects. The blood was immediately centrifuged at 1000*g* for 10 min at 4 °C and serum aliquots were stored at −80 °C until use for cytokine measurements.

A lumbar puncture was performed prior to the study to measure CSF hypocretin-1 levels in 42 patients with NC and 17 patients with unspecified hypersomnia. CSF samples were collected and aliquots were frozen and stored immediately at −80 °C until use for cytokine measurements in 34 patients with NC and all patients with unspecified hypersomnia. Paired samples of serum and CSF were collected the same day from 18 patients with NC (including 7 children).

Hypocretin and cytokine measurements

Hypocretin-1 was determined in duplicate from CSF samples by direct ^{125}I radioimmunoassay (Phoenix Pharmaceuticals, Belmont, CA). CSF hypocretin-1 levels below 110 pg/ml were considered as low and normal over 200 pg/ml (Dauvilliers et al., 2007, 2012). All values were back-referenced to Stanford reference samples (HHMI Stanford University Center for Narcolepsy, Palo Alto CA).

A panel of cytokines/chemokines was determined on either frozen serum or CSF by a proteomic approach, a multianalyte biochip array using a charge-coupled camera and imaging system (Investigator Evidence®; Randox, Mauguio, France). Twelve cytokines were simultaneously measured with a single drop of 100 µl per sample on a biochip: Interleukin-1 α (IL-1α), IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, Epidermal Growth Factor (EGF), Interferon-γ (IFN-γ), Monocyte Chemotactic Protein-1 (MCP-1), TNF-α, and Vascular Endothelial Growth Factor (VEGF). The total intra-assay and the total interassay variation coefficients for all cytokines measured were <11% (ranging: 3.9–11%) and <13% (ranging: 4.2–13%) respectively (Dupuy et al., 2005; Fitzgerald et al., 2005).