Narcolepsy with hypocretin/orexin deficiency, infections and autoimmunity of the brain

Birgitte Rahbek Kornum, Juliette Faraco and Emmanuel Mignot

The loss of hypothalamic hypocretin/orexin (hcrt) producing neurons causes narcolepsy with cataplexy. An autoimmune basis for the disease has long been suspected and recent results have greatly strengthened this hypothesis. Narcolepsy with hcrt deficiency is now known to be associated with a Human Leukocyte Antigen (HLA) and T-cell receptor (TCR) polymorphisms, suggesting that an autoimmune process targets a single peptide unique to hcrt-cells via specific HLA-peptide–TCR interactions. Recent data have shown a robust seasonality of disease onset in children and associations with Streptococcus Pyogenes, and influenza A H1N1-infection and H1N1-vaccination, pointing towards processes such as molecular mimicry or bystander activation as crucial for disease development. We speculate that upper airway infections may be common precipitants of a whole host of CNS autoimmune complications including narcolepsy.

Address
Stanford Center for Sleep Sciences and Medicine, Stanford University School of Medicine, 1050 Arastradero Rd., Palo Alto, CA 94304, USA

Corresponding author: Mignot, Emmanuel (mignot@stanford.edu)

Narcolepsy with hypocretin deficiency
Narcolepsy with hypocretin deficiency occurs in approximately 1 out of 3000 individuals and is characterized by severe, irresistible daytime sleepiness and abnormal sleep–wake patterns. Most patients also have cataplexy, a specific sudden loss of muscle tone occurring with strong emotions such as laughter. In contrast to sleepiness and other symptoms, cataplexy is a very specific symptom of the disease.

In 1999, mutations in the hcrt receptor 2 or a selective loss of hcrt were shown to produce narcolepsy in dogs and mice [1,2]. This discovery was rapidly followed by the observation that hcrt is undetectable in the cerebrospinal fluid (CSF) of almost all patients with cataplexy [3,4]. Postmortem analysis of narcolepsy brain tissue found a 90% loss of hcrt-cells, explaining the loss of peptide in the CSF [5–7]. Interestingly, nearby melanin-concentrating hormone neurons are intact [5,6]. The very specific loss of the hcrt-cells causing narcolepsy with cataplexy are now thought to result from an autoimmune attack.

Parallel with the narcolepsy research, a substantial amount of basic research has concentrated on understanding the neurobiology and pharmacology of the hypocretin system. Hcrt neurons are thought to sustain wakefulness and suppress REM sleep, and besides a primary role in the regulation of the sleep–wake cycle, hert also affects several other functions such as feeding, cardiovascular regulation, pain, locomotion, stress, and addiction [8,9]. Despite growing knowledge on hypocretin signaling however, it is still uncertain which downstream circuits are responsible for the different aspects of the narcolepsy/cataplexy phenotype. Signaling through hert receptors in multiple brain regions such as the locus coeruleus, the tuberomammillary nuclei, the pontine inhibitory area, and the basal forebrain may be involved [10–13]. Further, brain imaging studies of patients have found abnormal activity in the reward circuits such as ventral–dorsal striatum and amygdala in response to winning [14]. Manipulation of the hert system has great clinical interest and antagonists as well as agonists are under development [15].

Autoimmune destruction of the hypocretin neurons
Multiple factors generally contribute to the development of autoimmune diseases including genotype differences notably at the level of the HLA locus, hormonal milieu, and environmental factors [16,17]. In narcolepsy with cataplexy, these key phenomena have been demonstrated. First, genetic associations with key components of the immune system are consistently found in narcolepsy with cataplexy [18,19,20]. Second, although there is no strong gender disparity in narcolepsy, onset is often around adolescence and may also be associated with premature puberty [21]. Finally, the role of environmental factors in narcolepsy with cataplexy is strongly implied as the disease concordance rate between monozygotic twins is only 20–35% [22,23]. Recent studies have reported associations with upper airway infections [24], Streptococcus Pyogenes [25], and influenza H1N1-infections and H1N1-vaccinations [26,27], a finding also supported by the robust seasonality of disease onset [28].
Narcolepsy/hypocretin deficiency is strongly associated with HLA DQB1*06:02 and other immune related polymorphisms

In humans, more than 20 polymorphic HLA genes encode multiple subtypes of Major Histocompatibility Complex (MHC) class I and II proteins, which present foreign peptides to T-cells during infections, triggering immune responses. In the case of autoimmunity, self-peptides are mistakenly seen as foreign, which causes tissue destruction. More than 60 autoimmune diseases affecting virtually every organ are known, almost all associated with specific HLA subtypes.

Narcolepsy/hypocretin deficiency has one of the strongest HLA associations known, with more than 99% of patients carrying HLA DQB1*06:02, as well as HLA DQA1*01:02 carried in nearly complete linkage disequilibrium [29]. The genetic influence of HLA on narcolepsy predisposition is however not mediated solely through DQB1*06:02. Indeed, DQB1*06:02 homozygotes, or DQB1*06:02/03:01 heterozygotes are, for example, at higher risk for narcolepsy compared to DQB1*06:02 heterozygotes in general. Conversely, carriers of DQB1*06:02/06:01, DQB1*06:02/05:01 and DQB1*06:02/06:03 are at decreased risk [18,19,30]. Heterodimerization of DQA1*01:02 and DQB1*06:02 with other DQA1 and DQB1 alleles of the DQ1 group may explain these protective effects, by reducing abundance of the disease susceptibility DQA1*01:02/DQB1*06:02 heterodimer [30] (Figure 1).

Unique to narcolepsy is the genetic association with a polymorphism in the T-cell receptor alpha (TCRα) gene

Figure 1

Possible pathways for a role of influenza or streptococcus infections in the development of autoimmunity towards hypocretin (hcrt) cells. A peripheral H1N1 influenza or streptococcus pyogenes infection could stimulate autoreactive T-cells or B-cells via several different mechanisms. Selected resting autoreactive T-cells and B-cells may have reactivity towards hcrt cells, having escaped negative selection in the thymus. These could be activated in the following ways: (i) Molecular mimicry; T-cells. Antigens from the virus or bacteria are presented by, for example, MHC-DQA1*01:02-DQB1*06:02 on an antigen presenting cell (APC). A T-cell recognizes the antigen and is activated. The same T-cell (or a clone) migrates to the brain, where it recognizes an hcrt-cell specific antigen (cross-reactivity) inducing the autoimmune attack. (ii) Molecular mimicry, B-cells. An autoreactive B-cell can be activated if it also recognizes an antigen from the pathogen. This process requires signals from activated T-cells (T-cell help). (iii) Superantigens from streptococcus cross-link the MHC and TCR molecules independent of antigen specificity activating the autoreactive T-cell. (iv) Bystander activation. Resting autoreactive cells are activated as a result of general immune activation independent of specific antigens. (v) Lymphocyte migration to CNS. Once activated, the T-cells can migrate to the brain. Depending on the type of T-cell a variety of mechanisms could account for the autoimmune attack. MHC class II expression is restricted to microglia, but MHC class I are expressed in various brain cells including neurons [33]. (vi) Opening of the blood brain barrier. The general immune response to infection also normally includes fever and other factors that can make the blood brain barrier more penetrant to lymphocytes and also will allow antibodies to access CNS. (vii) Production of autoantibodies can also occur as a secondary response to hcrt-cell death via antigen presenting cells from the brain that have phagocytosed the dead neurons. Red dots indicate processes where release of cytokines or cytotoxic substances plays an important role. APC: Antigen presenting cell; H1N1: H1N1 influenza A virus or epitopes from adjuvanted vaccines; Hcrt: hypocretin; MHC: Major histocompatibility complex; Strep: Streptococcus Pyogenes; TCR: T-cell receptor.
that increases predisposition [20**]. The TCR is expressed in T-cells and consists of an alpha and a beta chain. Similarly to the immunoglobulin loci, DNA recombination occurs within the TCR loci to create an immense diversity in the receptors expressed. This somatic recombination leads to the generation of a diverse repertoire of unique TCR bearing T-cells [31]. Together with the strong association of narcolepsy with DQA1*01:02/ DQB1*06:02, the TCR association strongly suggests the existence of an autoimmune basis for narcolepsy, and further points towards the possibility that a single peptide unique to hcrt-cells is targeted by the autoimmune process through HLA presentation to specific T-cells. More recently, a genetic association of narcolepsy with the purinergic receptor subtype 2Y11 (P2RY11) was published [32*], and since this receptor is highly expressed in cytotoxic lymphocytes this also suggests an involvement of the immune system in the etiology of the disease. Of note, however, HLA, TCR, and P2Y11 are also expressed in the brain [33–35], although functional TCR molecules have not been demonstrated, thus a direct effect in the brain cannot be ruled out. For HLA in particular, whereas MHC class II molecules are not known to be expressed in neurons, MHC class I molecules have an established role in brain development and the regulation of synaptic plasticity [36,37]. It is thus possible that the cause of narcolepsy will involve novel functions for a growing numbers of immune-like molecules that are now shown to have functional effects in the central nervous system [38,39].

Interestingly, although this has been studied for over 30 years, it remains unclear how specific HLA alleles predispose to autoimmune diseases. One hypothesis is that the HLA associations observed in autoimmune diseases are a result of different disease-causing peptides having stronger affinity for certain HLA alleles, and thus a higher likelihood of being presented to autoreactive T-cells and inducing the autoimmune attack in individuals with these HLA alleles. Alternatively or additionally, disease-associated HLA alleles could bias the TCR repertoire generated during T-cell development in the thymus towards the selection of potentially pathogenic autoreactive clones. Finally, it has also been proposed that inappropriately high levels of HLA expression of specific alleles in diseased tissues might promote autoimmunity [40].

**Autoantibodies against hypocretin neurons**

The ultimate proof for autoimmunity would be the demonstration of T cell reactivity or auto-antibodies directed towards hypocretin neurons. Despite extensive research, findings have been either controversial or negative. Older studies typically searched for serum or cerebrospinal fluid autoantibodies against pre-prohypocretin, or against hcrt-cells using immunohistochemistry on brain tissue sections. Overall these studies have had a negative outcome [41]. Two recent studies have reported altered levels of the main subclasses of total IgG in narcolepsy with cataplexy and idiopathic hypersomnia [42] and increased serum levels of total but not free IgG and IgM autoantibodies against hcrt-1 in narcolepsy with cataplexy and also in narcolepsy without cataplexy and idiopathic hypersomnia [43]. Because these findings were not specific to cases with hcrt deficiency their significance remains unclear.

Recently, Cvetkovic-Lopes et al. [44*] engineered a strain of transgenic mice expressing a flag-tagged poly(A)-binding protein driven by the hcrt promoter with the purpose of capturing hcrt-neuron specific mRNAs. The resulting proteins were systematically screened for autoantibodies using human narcolepsy sera, resulting in the detection of autoantibodies against tribbles homologue 2 (TRIB2) in 14% of patients versus 5% of controls, a finding replicated in other patient populations [45,46], most notably in subjects with a recent onset of the disease. However, TRIB2 is expressed not only by hypocretin neurons, but also present in many other cell populations both in CNS and in the periphery, including immune cells [47,48]. Thus, TRIB2 autoantibodies are unlikely to be causative of the specific hcrt-cell destruction but are rather a downstream effect of the cell loss. The same holds true for many of the anti-neuronal antibodies that characterize neurological paraneoplastic disorders [49]. Intriguingly, in a recent paper describing 16 cases of narcolepsy with cataplexy very close to disease onset and following H1N1 infections or vaccinations (see below), all cases were TRIB2 antibody negative [50], suggesting that something unique occurred in cases studied preceding the TRIB2 finding, for example the activity of a specific virus strain that has since disappeared.

**Narcolepsy and winter-related infections**

Infections are increasingly recognized as playing a role in the pathophysiology of autoimmune diseases [17]. In the case of narcolepsy, two types of upper airway infections have been suggested to have an effect on narcolepsy susceptibility, influenza A and streptococcal infections. In a case-control study, unexplained fevers and flu infections in the year preceding onset were associated with a 3.9-fold and 1.8-fold increased risk, respectively [51]. Onset was also shown to be highly seasonal in children, with a 6-fold higher incidence in China in April versus December, suggesting occurrence most typically 5–6 months following the winter [28**]. Further, recent epidemiological population studies have shown that the risk of narcolepsy is increased 5.4 fold in subjects with a history of a physician-diagnosed streptococcal throat infection [24**], and in association with passive smoking in childhood [52]; the latter finding also points towards upper airway infections. Finally the involvement of streptococcal infections in development of the disease, is further supported by the presence of anti-streptococcal antibodies in 65% of narcoleptic patients.
within 1 year of onset compared to 26% in age matched controls [25*].

**Neurologic disorders and streptococcal infections**

Neurologic disorders with a hypothesized autoimmune etiology such as Sydenham Chorea, and more controversially Obsessive Compulsive Disorders, Tics and some autoimmune encephalitis have been long known to be associated with streptococcal infections [53,54]. Although the mechanism by which this could occur is unknown, streptococcal infections can stimulate autoimmunity by cross-linking TCR molecules and MHC molecules independently of antigen presentation (i.e. molecules from the pathogen act as superantigens). Most of the known superantigens bind primarily to the beta chain of the TCR and the alpha chain of MHC [55]. However, there are also known superantigens that bind to alpha chains of the TCR [56], and it has also been demonstrated that the TCR alpha chain is required for maximum stabilization of the TCR-superantigen–MHC complex [57]. It might thus be possible that the observed narcolepsy associated TCR alpha polymorphism may reflect involvement of streptococcal superantigens in narcolepsy. Streptococcal infections may also increase narcolepsy risk through non-specific effects such as a general activation of immunity (know as bystander activation) or an increased permeability of the blood brain barrier to autoreactive T cells, caused by inflammatory agents or fever [58,59].

**Narcolepsy, H1N1 influenza vaccinations and infections**

Following the 2009–2010 H1N1 influenza pandemics, Finnish investigators and the Swedish Medical Product agencies reported a significant 6–9 fold increase in the risk of developing narcolepsy after pandemic H1N1 (pH1N1) flu vaccination in Scandinavian children [26**,27**]. The association was noticed following an unusually high coverage of vaccination in these countries (~50%) with Pandemrix, a pH1N1 vaccination formulation containing the adjuvant AS03, a combination of squalene and alphatocopherol [60*]. Case reports also came out of France and Canada where a similar vaccine was used [50]. Interestingly, the Pandemrix association was primarily found in children and not adults. Only a few cases were reported following other H1N1 vaccines, such as those containing only squalene used in Europe, or following non-adjuvanted pH1N1 vaccines used in the United States [50,61]. Finally, in a recent study in China, the occurrence of childhood cases was found to increase 3 fold following the winter of 2009–2010, independent of vaccination, suggesting that H1N1 infections may also by themselves increase susceptibility in children [28**].

Although much remains to be answered regarding the Pandemrix–narcolepsy association, two possible mechanisms could be involved, a specific immune response to H1N1 (and subsequent molecular mimicry) or a generalized stimulation of the immune system mediated by the...
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vaccine, as AS03 adjuvanted vaccines have been shown to induce a somewhat stronger immune response [60,62,63]. The fact that narcolepsy onset is seasonal, and associated with both streptococcus and H1N1 infections could also argue in favor of non-specific immune activation precipitating the disease in predisposed individuals. In this model, lifelong risk for narcolepsy may not even be increased with vaccination, and Pandemrix could only be involved in precipitating onset, for example via the activation of preexisting autoreactive T-cell clones.

The vast unexplored terrain of brain autoimmune disorders

Historically, other neurological disturbances including narcolepsy-like symptoms have been associated with H1N1 infections, most notably in the context of the 1918 Spanish flu pandemic. Coincident with this epidemic that killed over 100 million individuals, a smaller epidemic, that of Encephalitis Lethargica (EL), a disease characterized by hypersonmolence and posterior hypothalamic lesions (Figure 2), occurred, affecting tens of thousands of individuals worldwide as described by von Economo [64,65]. Although the causal relationship of EL with the Spanish flu H1N1 pandemic is still debated, the relationship was probably not coincidental, as a similar smaller encephalitis epidemic was also observed following the 1890 flu epidemic [66]. EL was a very polymorphic disorder, associated not only with somnolence but also more occasionally with psychosis, insomnia or movement disorders (reminiscent of Sydenham Chorea) and frequently resulting in residual Parkinson’s disease presentation (best illustrated in ‘Awakenings’ by Oliver Sacks).

Although EL has largely disappeared, cases are still occasionally reported, and interestingly were found to be associated with high ASO titers [67,68]. It is therefore possible that CNS disorders as diverse as schizophrenia, movement disorders, or Parkinson’s disease may occasionally be precipitated or caused by upper airway infections and subsequent autoimmune reactions, an hypothesis also supported by recently reported HLA associations in Parkinson’s disease [69] and schizophrenia [70]. In our opinion, upper airway infections such as influenza and streptococcal sur-infections may be common precipitants of a whole host of neuropsychiatric autoimmune complications including narcolepsy.

Conclusion

Until recently, multiple sclerosis was thought to be the only major autoimmune CNS disease, and autoimmunity directed towards neurons a very rare event. As narcolepsy affects hort neurons selectively, this idea is now challenged and the disease may offer a unique model to study tolerance towards neuron specific antigens. Further, with the ever-increasing appreciation of the complexities of T cell regulation, immune cell access to brain, and immune surveillance of neurons, much will be learned when this knowledge is combined with results from research in narcolepsy and other autoimmune CNS disorders.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest
• of outstanding interest


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26. A case–control study evaluating whether Streptococcus Pyogenes and Helicobacter pylori infections are triggers for narcolepsy using blood markers of infection. Antibodies against streptococcal proteins were significantly elevated in narcoleptic patients close to onset.


30. This study shows using clinical reports from China a strong seasonality in the onset of narcolepsy, and further demonstrates a sharp increase in the number of new cases following the H1N1 pandemic.


35. In a genome wide association study of narcolepsy with cataplexy an association is found with SNPs in the purinergic receptor subtype 2Y11 gene. An effect of the polymorphism on regulation of lymphocytes is also shown.


49. Sung HY, Francis SE, Crossman DC, Kiss-Toth E: Regulation of expression and signalling modulator function of mammalian associating is cell-type specific. Immunology Letters 2008, 104:171-177.


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