NEUROIMMUNOLOGY

Disease mechanisms in narcolepsy remain elusive

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A pandemic influenza vaccine with a specific type of vaccine antigen has been linked to an increased incidence of narcolepsy in children from 2009–2010. However, the recent retraction of an article that reported a putative autoantigen means that the search for the mechanisms behind the vaccine–narcolepsy connection continues.

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In 2010, a sudden increase in the incidence of narcolepsy in young children and adolescents (4–20 years of age) was observed in Sweden and Finland. The increase coincided with immunization against the swine flu virus H1N1, using the pandemic influenza vaccine Pandemrix™ (GlaxoSmithKline, UK).[1–4] At the same time, an increased incidence of narcolepsy shortly after the influenza pandemic was found in China, where Pandemrix™ was not in use.[5]

Narcolepsy with cataplexy, also known as type 1 narcolepsy according to the Sleep Disorders Classification of the American Academy of Sleep Medicine,[1] is considered to be an autoimmune disorder in which the host defence systems are targeting hitherto unidentified autoantigens in hypothalamic neurons that secrete orexin (also known as hypocretin). This aberrant autoimmune response leads to degeneration of orexin-secreting neurons and subsequent loss of orexin production, which eventually results in development of clinical symptoms of narcolepsy, most importantly excessive daytime sleepiness and paroxysmal muscle weakness during wakefulness.[1,6]

A major step forward in the search for the putative autoantigen in narcolepsy apparently triggered by influenza vaccination was published in December 2013 in *Science Translational Medicine* by Emmanuel Mignot's group. The researchers, from Stanford University, CA, USA, identified an orexin epitope that resembled an epitope in haemagglutinin, an influenza virus surface glycoprotein. The authors found that in a subset of patients with narcolepsy, orexin protein and peptides induced a cross-reactive T-cell response that was specific to haemagglutinin of influenza type A—the category of viruses that includes H1N1. In further studies, however, the authors failed to confirm the specificity of the cross-reactive antigenic epitope shared by orexin and haemagglutinin, which led to retraction of the original publication in summer 2014.[7] This retraction is a major setback in identification of the putative autoantigen and the disease mechanisms in narcolepsy.

> "...we still lack detailed understanding of the disease mechanism in ... narcolepsy"

Type 1 narcolepsy is a chronic neurological disease characterized by excessive daytime sleepiness, unintended daytime sleep episodes, sudden loss of muscle tone associated with emotions (cataplexy), disturbed nocturnal sleep and, occasionally, hypnagogic hallucinations at sleep onset and various psychiatric symptoms.[1,6] Type 1 narcolepsy is associated with loss of orexin-producing neurons in the hypothalamus, which leads to disturbed control of the sleep–wake pattern.[1,6] In countries where the disease is well known, the prevalence of narcolepsy is around 30 per 100,000 people. Symptom onset in narcolepsy peaks in children and adolescents (aged between 12 and 17 years), but the symptoms can often be mild, leading to delayed diagnosis.

More than 20 years ago, Yutaka Honda and his co-workers[8] found that narcolepsy is strongly associated with the MHC class II-encoding gene *HLA-DQB1*. Type 1 narcolepsy was later shown to be strongly associated with *HLA-DQB1*06:02* allele, and other genetic factors, such as *HLA-DQA1*01:02* and genes encoding T-cell receptor α, P2Y purinoceptor 11, carnitine palmitoyltransferase 1B, choline kinase β, cathepsin H and tumour necrosis factor superfamily member 4, were also found to contribute to the disease.[1,6] Many of these molecules have immunoregulatory functions, and the association of *HLA-DQB1*06:02* with type 1 narcolepsy is perhaps the most robust association that is seen in any autoimmune disease. These findings strongly suggest that the disease mechanisms are related to T cells and cell-mediated immunity.

Despite these observations, a role for humoral immunity in the aetiopathogenesis of narcolepsy cannot be ruled out, because a recent study showed an association between antibodies against Tribbles homologue 2 and narcolepsy.[9] Another study reported that hypothalamus-specific autoantibodies were more commonly found in serum specimens among patients with narcolepsy than in control individuals; some of these antibodies were reactive against an epitope in hypothalamic α-melanocyte-stimulating hormone, an antipyretic neuropeptide involved in regulation of appetite and sleep.[10]

Clinicians are well aware that the onset and progression of some chronic neurological diseases, such as multiple sclerosis, are often associated with infections. Similarly, the onset of narcolepsy has been linked with infections such as *Streptococcus pyogenes* and respiratory viral pathogens, including influenza A virus.[1,6] The Pandemrix™ vaccine, which was used extensively from 2009–2011 to prevent infections caused by the H1N1 virus, was highly immunogenic and effective against infections caused by this virus.[1–4] However, first in Finland and Sweden and subsequently in Norway, France, Ireland and the UK, the incidence of narcolepsy was found to be increased 4–13-fold in children and adolescents and 2–4-fold in younger adults who had received Pandemrix™ vaccination, compared with unvaccinated individuals.[1–4]

Interestingly, the incidence of narcolepsy was not increased in Canada, where a similar—but not identical—pandemic influenza vaccine, Arepanrix™ (GlaxoSmithKline, UK), was used. Epidemiological data suggest that an increased risk of narcolepsy points to the viral antigen component of the Pandemrix™ vaccine, because both Pandemrix™ and Arepanrix™ had the same adjuvant, Adjuvant System 03 (AS03), which was produced in a factory in Belgium, whereas the viral antigen for Pandemrix™ was produced in European factories, and the antigen for Arepanrix™ in Canadian factories, with certain differences in vaccine virus purification and inactivation protocols.[2] It must be pointed out that seasonal influenza vaccines, which are given yearly to hundreds of millions of people, have not been associated with narcolepsy. Furthermore, even though the *HLA-DQB1*06:02* allele is very common in white populations (13–28%), the risk of narcolepsy in Pandemrix™-vaccinated children carrying this allele is low (about 1 in 1,600 vaccinated individuals).[3,4]

To understand the aetiopathogenetic mechanisms of narcolepsy, we need to know how the genetic and environmental factors and cell-mediated and humoral immunity of the host contribute to the development of the condition. Also of utmost importance is to identify the mechanisms through which influenza A virus infection and/or Pandemrix™ vaccination can trigger narcolepsy.

We can speculate that environmental factors, such as influenza A virus infection (or other microbial infections) or vaccination with inflammation-inducing vaccines, such as Pandemrix™ might stimulate pre-existing autoimmunity through a non-specific bystander activation mechanism (Figure 1). $CD8^+$ T cells could initiate a cytotoxic cascade, or activated $CD4^+$ T cells could induce cellulart cytotoxicity and production of inflammatory cytokines. *HLA-DQB1*06:02*-positive individuals might be at particular risk of crossreactive T-cell-mediated immune responses that could predispose them to the degeneration of orexin-producing hypothalamic neurons. Alternatively, B-cell activation and antigen presentation could result in development of crossreactive autoantibodies against hypothalamic cell structures.

Because the observation of molecular mimicry between orexin and the influenza haemagglutinin molecule could not be verified in confirmatory analyses, leading to the retraction of the original observation,[7] we still lack detailed understanding of the disease mechanism in Pandemrix™-associated narcolepsy. To our knowledge, only the parts of the retracted paper that were based on enzyme-linked immunospot analysis were not reproducible—the rest of the data seem solid, meaning that the hypothesis of possible molecular mimicry remains plausible. Improved understanding of the disease mechanisms in narcolepsy is required for development of more-efficient means to prevent and treat this disease.

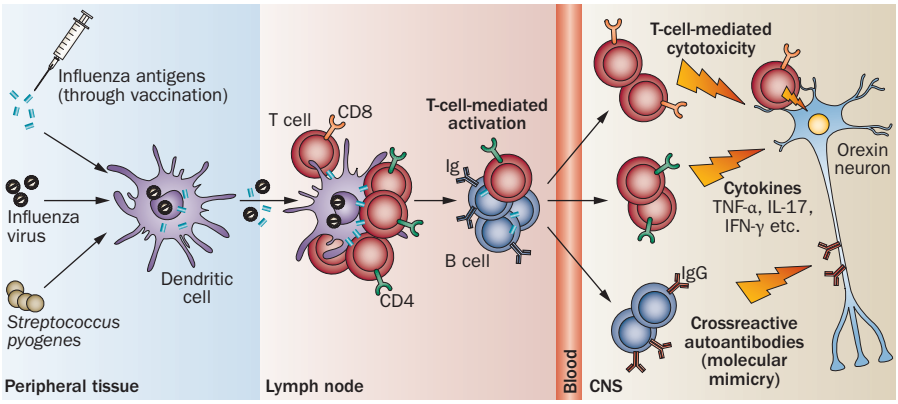

Figure 1 | Hypothetical model of autoimmunity in narcolepsy. Influenza A virus infection, *Streptococcus pyogenes* infection or immunization with AS03-adjuvanted pandemic influenza vaccine might trigger an infection or uptake of antigens by dendritic cells in peripheral tissues. This leads to maturation and migration of infected or antigen-loaded dendritic cells to local lymph nodes, where they present influenza-specific epitopes to T cells (red) that express CD4 or CD8. $CD4^+$ helper T cells costimulate B cells (blue) that have been exposed to soluble viral antigens. Activated T cells and B cells migrate via blood into the CNS. In the brain, an autoimmune response mediated by activated T cells and/or B cells contributes to the destruction of orexin-producing neurons in the hypothalamus.

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Competing interests
The authors declare no competing interests.

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