The autoimmune hypothesis of narcolepsy and its unexplored clinical features

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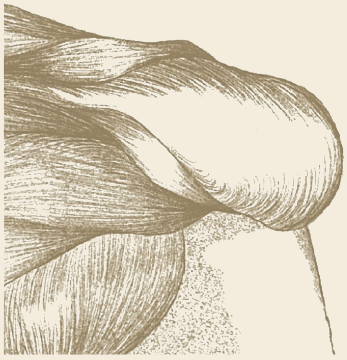

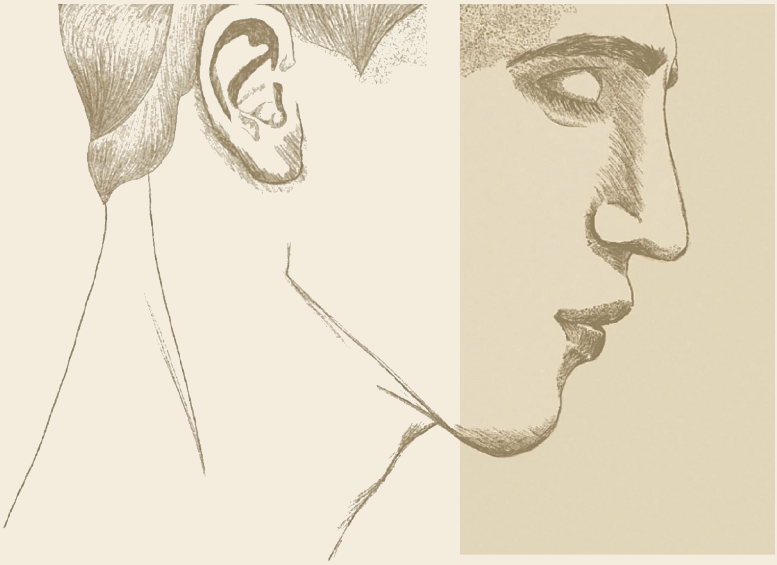

General introduction

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Narcolepsy is a sleep-wake disorder that can be subdivided in two types, diagnosed according to the criteria outlined in the Third Version of the International Classification of Sleep Disorders (ICSD, 2014). The distinction between type 1 and 2 is made based on the presence of cataplexy and/or hypocretin deficiency. This thesis will focus mainly on narcolepsy type 1 (NT1), since this disease entity is clearly distinct from other hypersomnolence disorders because of the presence of cataplexy and the absence of hypocretin in the cerebrospinal fluid (Table 1). NT1 is characterized by five core symptoms: excessive daytime sleepiness, cataplexy, sleep paralysis, hypnagogic hallucinations and disturbed night sleep. It is a rare disorder with a prevalence of around 1 in 3000 individuals (Wijnans et al., 2013).

Until the 1980s, not much was known about the cause of narcolepsy. During the 1980s the identification of a strong association of a human leukocyte antigen (HLA) molecule with narcolepsy gave researchers a direction where to look for the cause of the disease. This marked the starting point of the interest in the autoimmune hypothesis of narcolepsy.

Table 1. Diagnostic criteria for narcolepsy type 1 according to the Third Version of the International Classification of Sleep Disorders.

Criteria A and B must be met for making the diagnosis of narcolepsy type 1
• A. The patient has daily periods of irrepressible need to sleep or daytime lapses into sleep occurring for at least 3 months.
• B. The presence of one or both of the following:
• 1. Cataplexy and a mean sleep latency of ≤8 minutes and two or more sleep-onset REM periods (SOREMPs) on an multiple sleep latency test (MSLT) performed according to standard techniques. A SOREMP (within 15 minutes of sleep onset) on the preceding nocturnal polysomnogram may replace one of the SOREMPs on the MSLT.
• 2. CSF hypocretin-1 concentration, measured by immunoreactivity, is either ≤110 pg/mL or <1/3 of mean values obtained in normal subjects with the same standardized assay.

Pathophysiology

Discovery of hypocretin and its deficiency in narcolepsy

In 1998, the discovery of a novel peptide signaling system led to the unravelling of the cause of narcolepsy. Two groups separately, but simultaneously, identified two new peptide hormones. The one group called them orexins (Sakurai et al., 1998) after the Greek word for appetite, ὄρεξις, based on the role the hormones supposedly played in the regulation of appetite and metabolism based on their location in the hypothalamus); the other called them hypocretins based on their amino acid sequence that somewhat resembles that of the gut hormone secretin (de Lecea et al., 1998). Hypocretin-1 and hypocretin-2 are peptides that are derived from a common precursor protein called preprohypocretin that is produced solely in a group of around 80,000 neurons that are located in the lateral and posterior hypothalamus, henceforth in this thesis called hypocretin-producing neurons. One year later, in 1999, it was discovered that a mutation in one of the receptors for these hormones, the hypocretin receptor 2, was the cause of narcolepsy in narcoleptic dogs (Lin et al., 1999). Subsequently, the selective loss of hypocretin-producing neurons in the hypothalamus of narcolepsy patients was found to be the cause of narcolepsy in humans (Nishino et al., 2001, Peyron et al., 2000). This discovery directed research in narcolepsy to the question what causes the selective loss of these hypocretin-producing neurons. In addition, the discovery of the hypocretins allowed for using the concentration of hypocretin-1 in the cerebrospinal fluid as a biomarker for narcolepsy (Ripley et al., 2001, Mignot et al., 2002). **Chapter 1** highlights a case that underscores the role of the development of hypocretin deficiency as the cause of NT1 symptoms.

HLA

As mentioned, in the early 1980s, it was found that Japanese narcolepsy patients all carried the HLA class I subtype DR2 (Juji et al., 1984), that is used by antigen-presenting cells of the immune system to present antigens to CD4+ T cells. This strong association was reproduced and it was found that the HLA-DQ subtype DQB1*06:02 is the most frequent subtype in narcolepsy across all ethnic groups (Mignot et al., 1994). This HLA-DQ subtype forms a haplotype with HLA-DQA1*01:02. It was reported that 85-95% of all NT1 patients carry this specific haplotype, compared to a frequency in the general population of

20-30%. In recent studies, the reported association with HLA-DQB1*06:02 in NT1 was found to be closer to an almost perfect association with close to 100% of NT1 patients expressing the HLA-DQB1*06:02 allele (Tafti et al., 2014, van der Heide et al., 2015b). In addition, homozygosity for the HLA-DQB1*06:02 subtype was reported to constitute an even higher risk for the development of NT1 than the presence of one HLA-DQB1*06:02 already does (Pelin et al., 1998). As a result, HLA-DQB1*06:02 has been considered as a genetic factor that is necessary but not sufficient to develop NT1. Apart from the strong association with HLA- DQB1*06:02, there are both positive and negative associations between HLA-DQB1 alleles and NT1: the frequency of HLA-DQB1*03:01 was found to be increased, whereas HLA-DQB1*02:01 (HLA-DQ2), HLA-DQB1*05:01, HLA-DQB1*06:01, HLA-DQB1*06:03 and HLA-DQB1*06:09 were decreased in NT1 patients as compared with healthy controls (Hong et al., 2007, Ollila et al., 2015, Tafti et al., 2014). Apart from HLA-DQ, also positive and negative associations with other HLA subtypes, both HLA class I and class II, such as HLA-DR, HLA-A, HLA-B and HLA-C are described in NT1 (Juvodden et al., 2019b, Lind et al., 2019) albeit not nearly as strong as the association with HLA-DQB1*06:02.

2009 H1N1 influenza A pandemic

The 2009 H1N1 influenza A pandemic brought about a new surge in interest in the autoimmune hypothesis of NT1. This influenza strain, also called Mexican or swine flu, infected and killed many people around the world. The emergence of the pandemic was shortly followed by vaccination programs that had to be established in a shorter time and on a bigger scale than ever before. One year later, an increase in the incidence of NT1 was observed. In several (Northern) European countries, but also in China, a steep increase in narcolepsy incidence compared to the decade before the pandemic was described (Dauvilliers et al., 2013, Feltelius et al., 2015, Lind et al., 2014, Partinen et al., 2012, Han et al., 2011). The role that influenza vaccines played in this increased incidence of NT1 was discussed and investigated widely. Only Pandemrix, a vaccine predominantly used in Scandinavian countries, was suggested to constitute a small, but significant, risk factor for the development of NT1 in these countries (Sarkanen et al., 2018).

Following these epidemiological and laboratory reports, a discussion on the existence of a post-H1N1 NT1 variant has arisen. Several recent Scandinavian

studies have sparked the discussion whether different immunological mechanisms may be involved in post-H1N1 NT1 cases (Juvodden et al., 2019a, Lind et al., 2019). These results would test the hypothesis that sporadic and post-H1N1 NT1 should be regarded as separate entities based on different immunological mechanisms leading to the disease. **Chapter 2** adds new information to this discussion.

Association of NT1 with other infections

Also other infections are associated with the development of NT1. Most notably, the finding of elevated anti-streptococcal antibody titres in recent-onset NT1 patients suggested an association with other upper airway infections than only H1N1 influenza, particularly β-hemolytic streptococcal infections (Aran et al., 2009). Several other studies report associations of NT1 with streptococcal infections (Lopes et al., 2015, Natarajan et al., 2013), flu or other respiratory tract infections (Picchioni et al., 2007), tick-borne encephalitis virus vaccination (Hidalgo et al., 2016) or proxies of infections in the patient, such as month of diagnosis (Han et al., 2011). In **Chapter 1**, we describe a case that developed NT1 shortly after a gastrointestinal infection. The exact role of infections other than H1N1 influenza remains to be elucidated. This is partly due to the fact that NT1 is a rare disease, but also because documentation on the presence of especially viral infections is poor, because many people do not consult a doctor for these infections.

The autoimmune hypothesis of narcolepsy

Figure 1 provides an overview of the autoimmune hypothesis of narcolepsy. The hypothesis is that antigens from outside the body (e.g. H1N1 influenza, *Streptococcus* species, vaccines) trigger the immune system. In people susceptible for developing NT1, these antigens are presented to CD4+ T cells by HLA-DQB1*06:02 on antigen-presenting cells. In these people, cross-reactive CD4+ T cells are present that are able to mount an immune response to both the foreign peptide and the hypocretin peptide which closely resembles this foreign peptide. When activated, these cross-reactive CD4+ T cells elicit an autoimmune response that via multiple suggested pathways (e.g. hypocretin peptide-specific cytotoxic CD8+ T cells or autoantibodies targeting hypocretin-producing neurons) eventually lead to the destruction of hypocretin-producing neurons in the lateral hypothalamus.

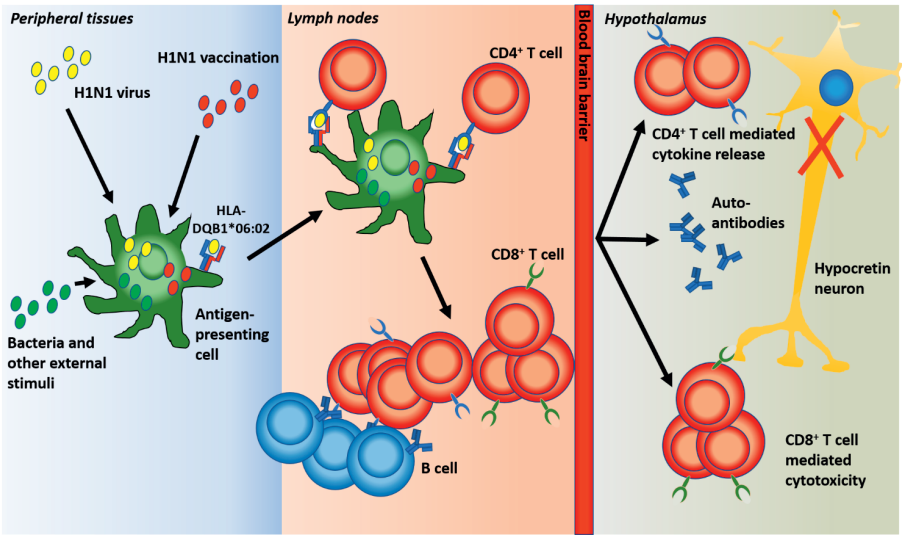

Figure 1. A depiction of the autoimmune hypothesis of narcolepsy, as described in the paragraph above.

Autoreactive immune cells

The hypothesis that cross-reactivity to the 2009 H1N1 influenza virus and hypocretin peptide has led to the development of NT1 in people at risk sparked the search for components of the immune system that are capable of mounting this cross-reactive immune response.

Since many autoimmune disease in the brain are mediated by autoantibodies, these seemed the most promising suspect to target hypocretin-producing neurons, leading to their destruction. However, no studies have been able to find an enrichment of autoantibodies specific for hypocretin or its precursor in NT1 (Giannoccaro et al., 2017, Luo et al., 2017, van der Heide et al., 2015a). Some studies report higher titres of autoantibodies that target antigens moderately specific for hypocretin-producing neurons or specific for peptides in the 2009 H1N1 influenza A virus in NT1 patients (Cvetkovic-Lopes et al., 2010, Lind et al., 2017, Saariaho et al., 2015, Thebault et al., 2015). Another study focusing on the human OX2 receptor reported the presence of a mimic peptide from the influenza nucleoprotein A, used in the 2009 H1N1 influenza A vaccination campaign, that shared protein residues with a fragment of the extracellular part of the OX2 receptor (Ahmed et al., 2015). Furthermore, this study demonstrates that antibodies present in NT1 patient serum cross-react with both the influenza nucleoprotein A and the extracellular part of the OX2 receptor. However, the

results of this study have been disputed by another group claiming that the OX2 receptor is not present on the surface of hypocretin-producing neurons and is therefore not a plausible target antigen for the autoimmune response leading to the development of NT1 (Vassalli et al., 2015).
Reports on T cells recognizing both H1N1 and hypocretin peptides have been published (De la Herran-Arita et al., 2013, Luo et al., 2018), but the first of these studies has subsequently been retracted (De la Herran-Arita and Garcia-Garcia, 2014). In **Chapter 3**, our experiments on cross-reactive T cells are presented. Recent studies have identified autoreactive T cells against hypocretin peptides (Latorre et al., 2018, Pedersen et al., 2019), but these cells were not HLA-DQB1*06:02 restricted. One study showed HLA-DQB1*06:02-restricted autoreactive T cells against hypocretin peptides, but they were found both in NT1 patients and in healthy controls (Jiang et al., 2019).

Genetics

As mentioned before, NT1 is strongly associated with the gene encoding HLA-DQB1*06:02 and other HLA type II genes. Also HLA type I genes, of which the gene product present antigens to cytotoxic CD8+ T cells, are associated with NT1, albeit these associations are considerably weaker than those with HLA type II genes (Ollila et al., 2015, Tafti et al., 2016). In addition to these HLA-associations, genome-wide association and gene sequencing studies have identified several other genes involved in the immune system that are associated with NT1: CTSH, that encodes pro-cathepsin H; P2RY11, that encodes a modulator of the autoimmune response to infection, p2Y purinoreceptor 11; and TNFSF4, that encodes tumor necrosis factor ligand superfamily member 4 (Faraco et al., 2013, Kornum et al., 2011, Tafti et al., 2014, Han et al., 2013). A recent study was the first to RNA sequence hypocretin-producing neurons in late embryonic mice, identifying the transcription of genes that distinguish hypocretin-producing neurons from adjacent melanin-concentrating hormone-producing neurons (Seifinejad et al., 2019). This could be a first step to identifying genes that, like hypocretin, are transcribed only in the hypocretin-producing neurons, and are therefore a plausible alternative target for an autoimmune response leading to the destruction of hypocretin-producing neurons leading to NT1.

Composition of the immune system in NT1

Another approach to identifying the components involved in the autoimmune response that destroys the hypocretin-producing neurons is to identify populations of immune cells that are enriched in NT1 patients compared with healthy controls. Preferably, this must be done in a way that is independent of the presumed autoantigen to focus solely on the immune cell populations that are present in NT1 patients and healthy controls. The composition of the immune system can either be tested in peripheral blood mononuclear cells or in cerebrospinal fluid. Several studies have compared the composition of the immune system in NT1 with that of healthy controls using flow cytometry in which a global T cell activation in peripheral blood of NT1 patients (Lecendreux et al., 2017, Moresco et al., 2018) and an even stronger T cell activation was seen in the cerebrospinal fluid compared with healthy controls (Moresco et al., 2018). In **Chapter 4**, we apply mass cytometry, a technique that is able to delineate immune cell subsets in the peripheral blood with unprecedented resolution, to assess the differences in immune cell composition between NT1 patients with recent symptom onset and HLA-DQB1*06:02-matched healthy controls.

Clinical features of NT1

NT1 is traditionally characterized by its four core symptoms: excessive daytime sleepiness, cataplexy, sleep paralysis, hypnagogic hallucinations. Disturbed nocturnal sleep is generally considered to be the fifth core symptom (Black et al., 2017, Bassetti et al., 2019, Kornum et al., 2017b). Although this summary of core symptoms is widespread and acceptable for many sleep specialists, it frequently does not describe the burden of the disorder for a NT1 patient very well. A better picture of the disorder can be generated by focusing on the everyday life of a NT1 patient. With the development of the disorder, frequently in adolescence or shortly after, patients experience an inability to stay awake and concentrated for longer periods of time, interfering with social interaction, education and professional life throughout the years. This is often combined with the inability to stay asleep during the night. In addition, partial expressions of (non-)REM sleep phenomena during the day, such as automatic behavior (non-REM), cataplexy, hypnagogic hallucinations and sleep paralysis (REM), further complicate effective participation in societal life. Also, comorbidities and symptoms such as mood and anxiety disorders (Lopez et al., 2017, Ruoff et al., 2017), autonomic disturbances (Plazzi et al., 2011), apathy, fatigue (Nordstrand

et al., 2019b) and weight gain (Fronczek et al., 2008, Poli et al., 2009), that are frequently reported in NT1 add to the difficulties these patients experience. In **Chapter 5 and 6**, two of these frequently overlooked symptoms in NT1 patients are described.

Outline of the thesis

As can be deducted from the Chapter topics, the focus of this thesis is two-fold. The lion share is about unravelling the autoimmune hypothesis of narcolepsy, which comprises a case report that links the disappearance of hypocretin-1 in cerebrospinal fluid to the emergence of NT1 symptoms (**Chapter 1**), a study on the role of HLA (**Chapter 2**) and cross-reactive T cells (**Chapter 3**) in the autoimmune response leading to NT1 and a study on which immune cells are unique to NT1 patients (**Chapter 4**). The second part of this thesis focuses on symptoms of NT1 that are highly relevant in NT1 patient's everyday life: weight gain (**Chapter 5**) and daytime sleep state misperception (**Chapter 6**).