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Research Paper

Prostaglandin D2 Receptor DP1 Antibodies Predict Vaccine-induced and Spontaneous Narcolepsy Type 1: Large-scale Study of Antibody Profiling

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ABSTRACT

Background: Neuropathological findings support an autoimmune etiology as an underlying factor for loss of orexin-producing neurons in spontaneous narcolepsy type 1 (narcolepsy with cataplexy; sNT1) as well as in Pandemrix influenza vaccine-induced narcolepsy type 1 (Pdmx-NT1). The precise molecular target or antigens for the immune response have, however, remained elusive.
Methods: Here we have performed a comprehensive antigenic repertoire analysis of sera using the next-generation phage display method - mimotope variation analysis (MVA). Samples from 64 children and adolescents were analyzed: 10 with Pdmx-NT1, 6 with sNT1, 16 Pandemrix-vaccinated, 16 H1N1 infected, and 16 unvaccinated healthy individuals. The diagnosis of NT1 was defined by the American Academy of Sleep Medicine international criteria of sleep disorders v3.
Findings: Our data showed that although the immunoprofiles toward vaccination were generally similar in study groups, there were also striking differences in immunoprofiles between sNT1 and Pdmx-NT1 groups as compared with controls. Prominent immune response was observed to a peptide epitope derived from prostaglandin D2 receptor (DP1), as well as peptides homologous to B cell lymphoma 6 protein. Further validation confirmed that these can act as true antigenic targets in discriminating NT1 diseased along with a novel epitope of hemagglutinin of H1N1 to delineate exposure to H1N1.
Interpretation: We propose that DP1 is a novel molecular target of autoimmune response and presents a potential diagnostic biomarker for NT1. DP1 is involved in the regulation of non-rapid eye movement (NREM) sleep and thus alterations in its functions could contribute to the disturbed sleep regulation in NT1 that warrants further studies. Together our results also show that MVA is a helpful method for finding novel peptide antigens to classify human autoimmune diseases, possibly facilitating the design of better therapies.

1. Introduction

Narcolepsy type 1 (NT1) is a chronic neurological disease characterized by irresistible daytime sleepiness, disturbed nocturnal sleep, and cataplexy associated with the inadequate function of the hypothalamus (Peyron et al., 2000; Thannickal et al., 2000; Partinen et al., 2014). The major neuropathological features of NT1 are loss of orexinergic neurons and an increased gliosis in the posterior hypothalamic nuclei (Partinen et al., 2014). Increased levels of pro-inflammatory cytokines have been associated with (spontaneously occurring) idiopathic (sNT1) and Pandemrix vaccine-induced narcolepsy (Pdmx-NT1) close to disease onset (Lecendreux et al., 2015). Pandemrix (Pdmx) is an influenza vaccine used during the H1N1 2009 swine influenza A(H1N1) pandemic and was distributed to over 30 million people in EU/EEA countries

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during the A(H1N1) outbreak. As of January 2015, >1300 cases of vaccine-associated NT1 had been reported to the European Medicines Agency. Epidemiologic and clinical studies conducted in different countries including Finland, Sweden, Ireland, England, Norway, and France have confirmed the association of NT1 in children and adolescents with the AS03-adjuvanted Pdmx (Partinen et al., 2014; Sarkanen et al., 2017). Subsequently, wild-type influenza A(H1N1) infections in China were associated with narcolepsy (Han et al., 2013, 2011). Along with the pandemic A(H1N1) infection, seasonality and post-infectious priming by upper respiratory tract viruses and streptococci have been suggested as triggers of autoimmune response that leads to NT1 in genetically susceptible individuals (Aran et al., 2009; Longstreth Jr et al., 2009).

Genome-wide association studies have revealed a strong association of narcolepsy with the T-cell receptor alpha locus (Hallmayer et al., 2009) and especially with Major Histocompatibility Complex (MHC) class II *DQB1*06:02* alleles (Bonvalet et al., 2017; Tafti et al., 2014). *DQB1*06:02* is present in approximately 30% of Finnish and Swedish populations (Bomfim et al., 2017). In Finland, all patients with Pdmx-NT1 have been positive for *DQB1*06:02* (Partinen et al., 2014). The latter immune haplotype is also strongly associated with the Pdmx-NT1 in Sweden (Bomfim et al., 2017). In another series of 522 patients with narcolepsy and cataplexy from different countries, only 9 patients (1.7%) with low levels of orexin (OX) in cerebrospinal fluid (CSF) were *DQB1*06:02* negative (Han et al., 2014). It was also suggested that cross-reactive epitopes to Pdmx vaccine antigens may exist in NT1 diseased as a significant proportion of HLA-*DQB1*0602*–positive Finns diagnosed with NT1 and with a history of H1N1 vaccination were immunoreactive to OX receptors (Ahmed et al., 2015). However, it still is unclear whether OX-positive neurons and/or their neighboring cells express OX receptors that could be targets for the immune response in NT1 (Valko et al., 2013; Vassalli et al., 2015). The antibody levels to viral nucleoprotein (NP), a Pdmx vaccine antigen, were increased in NT1-diseased carrying the HLA *DQB1*06:02* allele (Vaarala et al., 2014), whereas the role of this and other circulating (including intrathecal) autoantibodies in NT1 pathogenesis is not fully understood (see list of previously identified antigens in Table S1). Although NT1-related autoantibodies are found in some patients, the clinical response to intravascular immunoglobulin (IVIG) has been hard to predict (Knudsen et al., 2012). Likewise, use of the drug rituximab might have only short-lasting beneficial effects in NT1 (Sarkanen et al., 2016).

Recent advances in proteomics (immunomics) have made it possible to study the adaptive immune response in various diseases in great detail and at a high resolution (lately reviewed in: (Ayoglu et al., 2016, Wu et al., 2016). We and others have suggested a strategy of high-throughput sequencing-assisted epitope mapping directly on serum for biomarker discovery and disease detection based on the idea that self- and environmental (exposome) antigens are reflected in the immune response profiles (immunoprofiles) (Anastasina et al., 2017; Christiansen et al., 2015; Ionov, 2010; Xu et al., 2015). Hence, the profiling of antibody response repertoire with high-density random peptide/polypeptide display methods could be a novel mean to characterize and classify human diseases in an unbiased manner according to the molecular/cellular targets relevant for the disease.

In the present study, we have used the mimotope-variation analysis (MVA) method to immunoprofile autoantibody repertoires in patients afflicted by NT1 and in controls. We had access to the clinical cohorts composed of 16 NT1 (sNT1 ($n = 6$) and Pdmx-NT1 ($n = 10$)) cases, where all NT1-diseased subjects carried the HLA *DQB1*06:02* allele, and apart from 2 sNT1 patients, all had been vaccinated with Pdmx. For reference, we used three well-defined control groups: 16 Pandemrix-vaccinated healthy controls (Pdmx-HC), 16 H1N1-infected Finnish subjects (H1N1-HC), and 16 healthy Estonian donors (HC – healthy controls) (Table 1). Our data revealed complex patterns of immune response in all patient groups including novel epitope sequences present in sera of Pdmx-NT1 and H1N1-HC. One such peptide epitope was identified as belonging to the prostaglandin D2 receptor (DP1) that together with its ligand prostaglandin D2 (PGD2) is involved in sleep regulation in humans and experimental animal models (see ref. in Urade and Hayaishi (2011)).

2. Materials and Methods

2.1. Vaccines

Pandemrix vaccine is derived from X-179A, a reassortant of hemagglutinin (HA), neuraminidase (NA) and polymerase acidic protein (PA) of A/California/07/2009 and X-157 H3N2 in a PR8 backbone (Jacob et al., 2015; Nicolson et al., 2012; Robertson et al., 2011). The vaccine composition can be found summarized by European Medicines Agency and GlaxoSmithKline plc (European Medicines Agency, 2009).

2.2. Study Population

The present study comprises a total of 64 individuals (Table 1). Altogether, 16 serum samples of H1N1-infected military servicemen (H1N1-HC), 16 serum samples of age/sex-matched Pandemrix-vaccinated healthy controls (Pdmx-HC) were kindly provided by National Institute of Health and Welfare, Finland. 16 serum samples were collected from patients with H1N1-induced (Pdmx-NT1) and sporadic narcolepsy (sNT1). Four out of 6 sNT1 patients were vaccinated with Pdmx after they had been diagnosed with NT1. Narcolepsy patients were diagnosed at the

Table 1
Description of samples studied.

Characteristics	Narcolepsy (NT1) patients		Healthy controls (HC)		
	Pdmx-NT1	sNT1	Pdmx-HC	H1N1-HC	Other HC
Group size (*n*)	10	6	16	16	16
Gender (female/male)	5/5	5/1	12/2[a]	0/16	10/6
Pandemrix vaccination	11/2009–1/2010	11/2009-1/2010[b]	11/2009-1/2010	–	–
Sample collection	2011	2011	2011	2011	2009
Median age at onset (y)	13	18	–	–	–
Median age at sampling (y)	14	22	NA	21	34.5
Unambiguous cataplexy	10/10 (100%)	6/6 (100%)	–	–	–
MSLT mean SL (range)	2.0 (0.4–4.3)	2.6 (0–7.5)	NA	NA	NA
SOREMPS mean (range)	3.7 (2–5)	2.7 (2–4)	NA	NA	NA
HLA *DQB1*0602* (%)	10/10 (100%)	6/6 (100%)	NA	NA	NA
CSF-orexin < 150 pg/mL (lower 1/3 limit in Finland)	7/7 (100%)	5/5 (100%)	NA	NA	NA

HC- healthy control, *H1N1-HC* – H1N1 infected, *Pdmx-HC*– Pandemrix-vaccinated, *NT1*- narcolepsy type 1 (including 10 Pdmx-induced NT1 samples (Pdmx-NT1) and 6 sporadic NT1 (sNT1) samples), *NA* – not available, *SL* – sleep latency, *MSLT* – Multiple sleep latency test, *SOREMPS* – Sleep onset REM periods as defined by the American Academy of Sleep Medicine.
[a] Gender of two Pdmx-HC is unknown.
[b] Four out of 6 sNT1 patients were vaccinated after they had been diagnosed with NT1.