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Letter to the Editor

LETTER TO THE EDITOR

Treatment of narcolepsy with natalizumab

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Introduction

Researchers have hypothesized that narcolepsy type 1 (NT1) is caused by an autoimmune process since the 1990s, when narcolepsy was first found to be associated with the human leukocyte antigen (HLA) DQB1*06:02/DQA1*01:02 (DQ0602). In 2000, NT1 was discovered to be due to the selective loss of the hypocretin (HCRT, also known as orexin)-producing neurons in the hypothalamus [1–3]; and in 2010, Pandemrix, an H1N1 flu vaccine used in northern Europe, triggered numerous new cases of narcolepsy, further supporting an autoimmune trigger [4].

In the last year, several groups have established that people with NT1 have autoreactive T cells targeting the prepro-hypocretin peptides [5–8]. In addition, T cells expressing specific T-cell receptor alpha (TCRα) J24 gene segments cross-react with the C-terminal ends of HCRT-1 and HCRT-2 when presented by DQ0602 [6, 7, 9]; specifically, NT1 is associated with an F to L polymorphism in the CDR3 region of J24 [6]. Considered together, these findings suggest that hypocretin neurons are directly injured by these T cells, resulting in NT1, and that medications that inhibit migration of T cells into the brain could halt the destruction of the hypocretin neurons.

Given these new discoveries, we treated a young woman soon after NT1 onset with natalizumab (Tysabri), a drug that inhibits T cells and other leukocytes from crossing the blood-brain barrier, and then tracked her clinical symptoms and cerebrospinal fluid (CSF) hypocretin over time.

Case Report

In November 2017, a 21-year-old woman received an influenza vaccine (Fluvirin) and also developed bronchitis and sinusitis, possibly due to mycoplasma pneumonia as her IgG and IgM antibodies were elevated when tested 2 months later (4.46 and 1204 U/mL, respectively). Antistreptolysin O titers were not elevated.

In mid-December 2017, she developed severe daytime sleepiness (Epworth Sleepiness score 18), fragmented sleep, sleep paralysis, and hypnagogic hallucinations. By January 2018, she had gained 5 kg and began having frequent, vivid dreams of danger and violence. Some dreams were so vivid that she would act on them (e.g. packing for a meeting upon awakening). At that time, she also developed cataplexy 5–10×/day which manifested as episodes of face, arm, and whole-body weakness triggered by laughter, orgasm, and other strong emotions. Her physical exam and brain MRI with and without contrast were unrevealing. She is HLA *DQB1*06:02* positive.

Her polysomnogram showed a sleep latency of 3.5 min and a short rapid eye movement (REM) sleep latency of 6.0 min. She had 440 min of sleep and a sleep efficiency of 86%. She had infrequent hypopneas in REM sleep, no REM sleep without atonia, and no periodic limb movements. Her Multiple Sleep Latency Test showed a mean sleep latency of 0.2 min and 2/5 sleep-onset REM sleep periods. In further support of NT1, her CSF hypocretin-1 was 70 pg/mL (detection limit 40 pg/mL), and CSF showed no leukocytosis or oligoclonal bands.

In late January 2018, she was treated with intravenous immunoglobulins (IVIG) 2 g/kg/day for 2 days, but this produced

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headache, stiff neck, and no improvement over the next 3 weeks.

As her CSF hypocretin was low but not absent, the patient opted to begin treatment with natalizumab, an α4 integrin antagonist in an attempt to prevent leukocyte trafficking into the central nervous system. The plan was to restrict treatment to 1 year to minimize risks such as progressive multifocal leukoencephalopathy. A single patient Investigational New Drug Application for treatment with natalizumab was obtained from the FDA and approved by the BIDMC Committee on Clinical Investigations. Informed consent was obtained.

From March 21, 2018 to January 28, 2019, the patient received monthly infusions of natalizumab (300 mg IV; 12 doses total). Natalizumab antibodies were not detected 5 and 10 months later. Her JC (John Cunningham) virus antibody index was initially high (1.65; >0.4 is positive), but 5 and 10 months later, it was only 0.14 and 0.07; we suspect the initial titer was elevated due to the recent infusion of IVIG which may have contained exogenous JC virus antibodies. MRI was repeated 6 and 20 months after starting natalizumab to screen for progressive multifocal leukoencephalopathy and was normal. Despite treatment with natalizumab, her CSF hypocretin further dropped to 13.6 pg/mL on September 4, 2018 and to 17.8 pg/mL on January 6, 2019 (Figure 1).

Given her severe sleepiness, she was also treated with methylphenidate ER 20 twice each day plus sodium oxybate 4.5 g twice each night which reduced her Epworth score to 11. Natalizumab did not alter her stimulant requirement. For about 1 year, she also was treated with fluoxetine 20 mg each day for depression manifest as tearfulness, hopelessness, irritability, and loss of self-confidence which began in December 2018.

T-cell analysis

Blood was collected prior to starting natalizumab and 1 month later (February 1, 2018 and April 19, 2018). Peripheral blood mononuclear cells (PBMCs) were expanded by culturing them in the presence of hypocretin peptide fragments ($HCRT_{54–66-NH2}$ or $HCRT_{86–97-NH2}$, collectively referred to as $HCRT_{NH2}$) or two H1N1 peptide fragments ($pHA_{273–287}$ or $NP_{17–31}$ also present in Pandemrix), followed by single-cell sorting and TCR sequencing of cells positive for the corresponding peptide tetramers [6].

On February 1, 2018 and April 19, 2018, the patient's blood contained antigen-specific CD4+ T cells reactive to C-terminal amidated $HCRT_{54–66-NH2}$ and $HCRT_{86–97-NH2}$ fragments (Figure 2). PBMCs from February 1, 2018 were tested three times; partial results and detailed methods were reported previously [6]. A separate population of CD4+ T cells reacted with $pHA_{273–287}$ only in the April 19, 2018 sample, while the reaction to $NP_{17–31}$ was very weak. No clear change in the number of T cells recognizing these antigens was observed across the two time points.

Individual antigen-specific CD4+ T cells were isolated and sequenced for *TCRα* and *TCRβ*. For $HCRT_{NH2}$ reactivity in the blood sample drawn on February 1, 2018, the most enriched sequence (42/279 cells) was *TCRα* V26-1-CIVRSQGGSYIPTF-J6 paired with an unidentified *TCRβ* sequence. Two J24α-positive TCR clones, *TCRα* V24-CAFTTDSWGKFQF-J24 (containing the J24F narcolepsy associated allele, underlined) paired with *TCRα* V19-CASSILGGGSGYTF-J1-2 and the previously reported *TCRα* V2-CAVETDSWGKLQF-J24 (containing J24L, underlined) in association with various *TCRβ* sequences were also enriched (11/279 and 7/279 cells, respectively). These J24α-containing TCR clones were not found in the subsequent sample drawn on April 19, 2018 and are different from those reported by Jiang et al. [8]. For $pHA_{273–287}$, the most frequent *TCRα/β* clone isolated was *TCRβ*V7-8-CASNPAGAVSYNEQFF-J2-1/TCRα V4-CLVGAPSLYGGSQGNLIF-J42 (27/115 cells).

Discussion

People with NT1 have T cells targeting fragments of the prepro-hypocretin peptides [5–8], and as these T cells may be pathogenic, we treated a young woman with natalizumab soon after NT1 onset. This patient's blood contained CD4+ T cells reactive

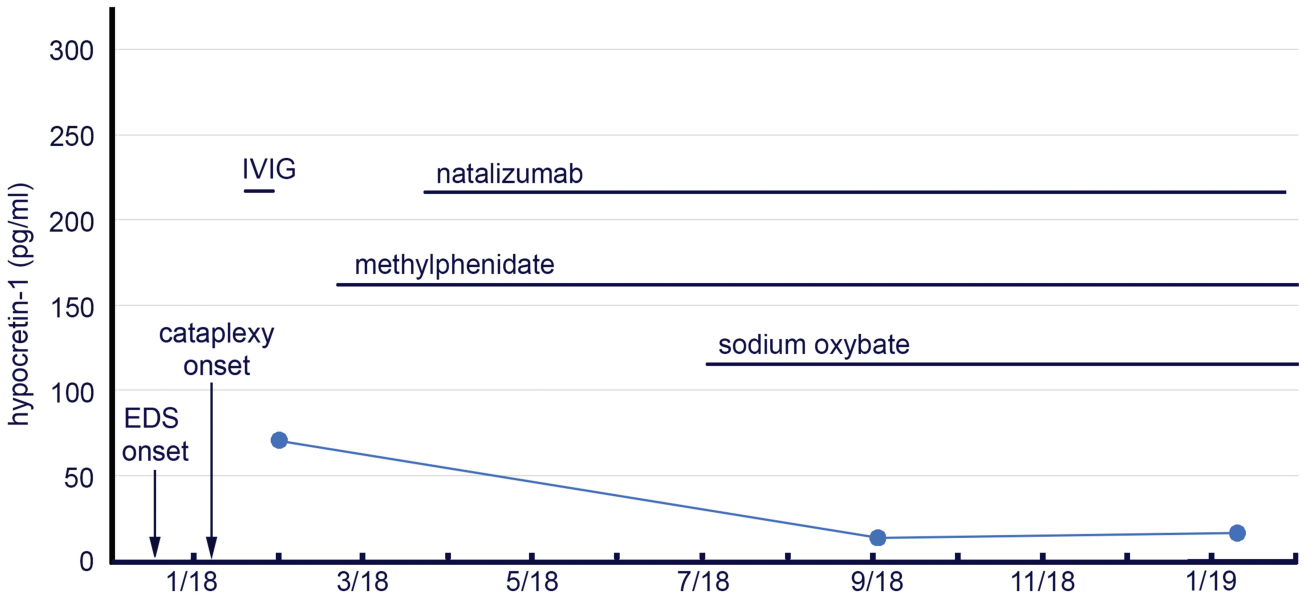

Figure 1. Time course of CSF hypocretin level and treatments in relation to symptom onset. IVIG, intravenous immunoglobulins; EDS, excessive daytime sleepiness.

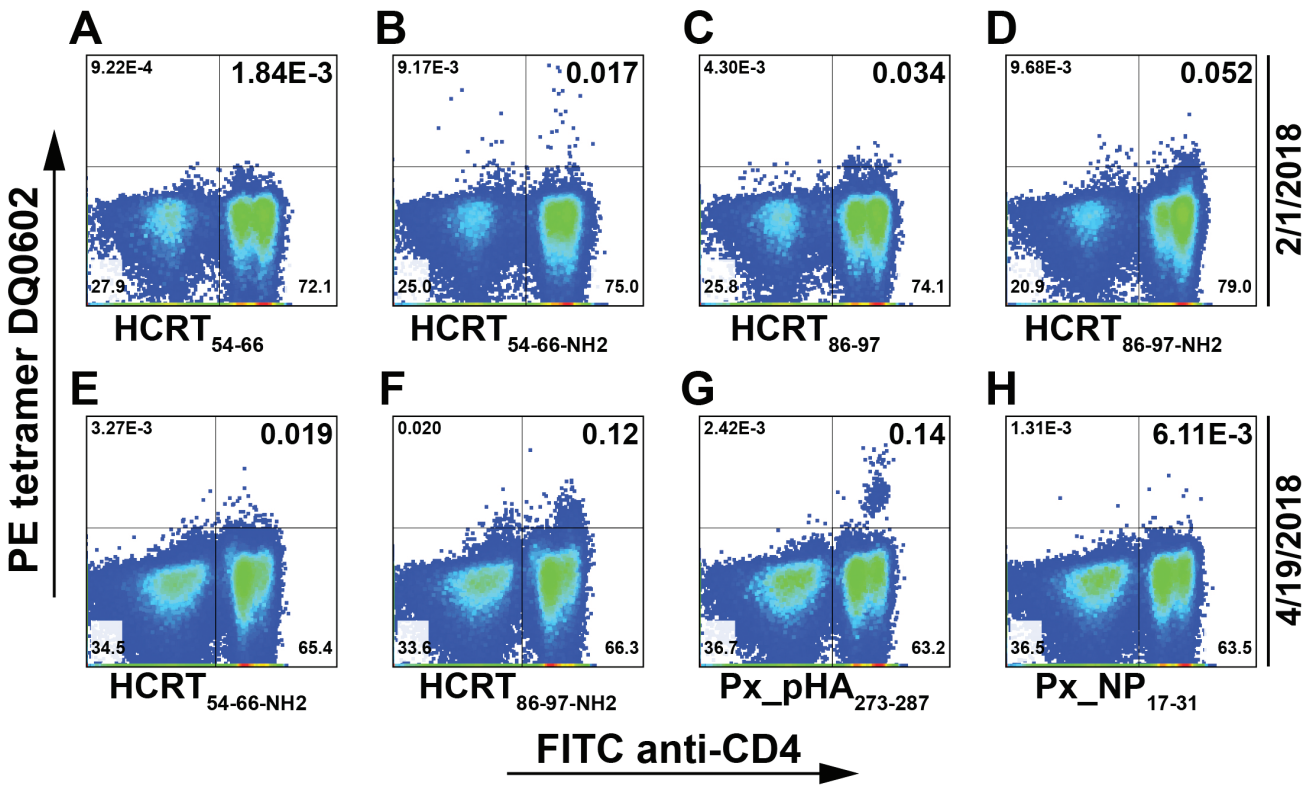

Figure 2. Detection of antigen-specific CD4+ T cells in recent-onset narcolepsy before and after treatment with natalizumab. PBMCs from February 1, 2018 and April 19, 2018 were cultured with hypocretin (HCRT) peptides (A–F) or Pandemrix (Px) (G and H) for 10 days and subsequently stained with DQ0602 tetramers loaded with peptides and labeled with R-phycoerythrin (PE)- and fluorescein isothiocyanate (FITC)-conjugated anti-CD4 antibody, followed by analysis with flow cytometry. Live T cells (labeled with Brilliant Violet 421 conjugated to anti-CD3 antibody) are shown in each panel, divided by a quadrant gate with frequency.

to hypocretin and H1N1, but treatment with natalizumab did not improve her clinical symptoms or CSF hypocretin.

We think it is unlikely that NT1 developed in this patient as a consequence of receiving Fluvirin. Fluvirin is a trivalent, non-adjuvanted vaccine that contains pandemic 2009 pH1N1 sequences. Though these sequences include some of those found in Pandemrix that likely triggered NT1 in Europe, the use of non-adjuvanted preparations containing these sequences in the United States has not been shown to be associated with an increased risk of narcolepsy [10].

In the sample closest to NT1 onset, the patient's blood contained T cells recognizing $HCRT_{NH2}$ and containing specific *TCRβ* J24 sequences. These may be involved in the pathological process and were not detectable in subsequent samples following natalizumab therapy. Testing the reactivity of TCR transfected in Jurkat T cells would be needed to confirm if these TCRs can actually be activated by $HCRT_{NH2}$ when presented by DQ0602.

Natalizumab is a monoclonal antibody directed against the alpha-4 subunit of integrin molecules, a protein expressed on lymphocytes and monocytes. Natalizumab is thought to inhibit the binding of immune cells to vascular endothelium and thus suppress the migration of these cells into the brain. As NT1 is likely caused by pathogenic T cells, blocking the migration of T cells into the brain should suppress the autoimmune attack on the hypocretin neurons.

Several factors may account for why natalizumab failed to produce a clinical improvement or increase in CSF hypocretin in this patient. First, the patient had a very rapid onset of NT1 with severe cataplexy soon after disease onset. As cataplexy probably requires more than 80% loss of hypocretin tone [11–14], the remaining number of hypocretin neurons was probably already very low even before natalizumab was begun. Not all patients with NT1 have such rapid onset, and some may develop cataplexy many years after the onset of sleepiness [15]; therefore, some patients may have a larger window of opportunity. Second, natalizumab and similar drugs can reduce the migration of T cells into the brain, but pathogenic T cells were likely already in the hypothalamus when the drug was started. Future research may determine how long these pathogenic cells remain in the brain, and whether ongoing entry of new T cells contributes to hypocretin neuron loss. Last, because an IND is required to use natalizumab off-label, it took about 1 month to initiate treatment, and the drug may have been started too late.

Do the benefits of an integrin inhibitor outweigh the risks in NT1? Drugs such as natalizumab that inhibit migration of T cells into the CNS are generally well tolerated, but they are associated with increased risk of progressive multifocal leukoencephalopathy (PML) [16]. Many healthy people have latent JC virus infection that is suppressed by T cells, but JC virus can produce PML in people with T cell dysfunction or reduced trafficking of T cells into the brain. If researchers opt to treat NT1 patients with natalizumab, we propose treatment for a maximum of 1 or 2 years. The risk of PML is quite low in the first year, and the risk is not elevated in the second year if JC virus antibodies are negative. As PML is a life-threatening disease, longer treatments may carry too much risk in NT1.

Although natalizumab did not improve this patient's clinical symptoms or CSF hypocretin, we believe larger studies of

T-cell-modifying medications should be pursued. Starting the medication very soon after the first symptoms is likely crucial, yet this will be a substantial challenge as many patients, especially children, are often not diagnosed until years after disease onset. In addition, the use of natalizumab in NT1 requires FDA approval. Still, large sleep centers occasionally see NT1 patients close to disease onset, and we feel the utility of T-cell-modulating medications could be tested by having protocols in place in advance at multiple institutions and measuring key endpoints with objective measures such as CSF hypocretin and the Maintenance of Wakefulness Test. Our hope is that inhibiting migration of T cells into the brain could halt the destruction of the hypocretin neurons, preventing the worsening of NT1 symptoms, and possibly enabling some clinical improvement driven by the remaining hypocretin neurons.

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