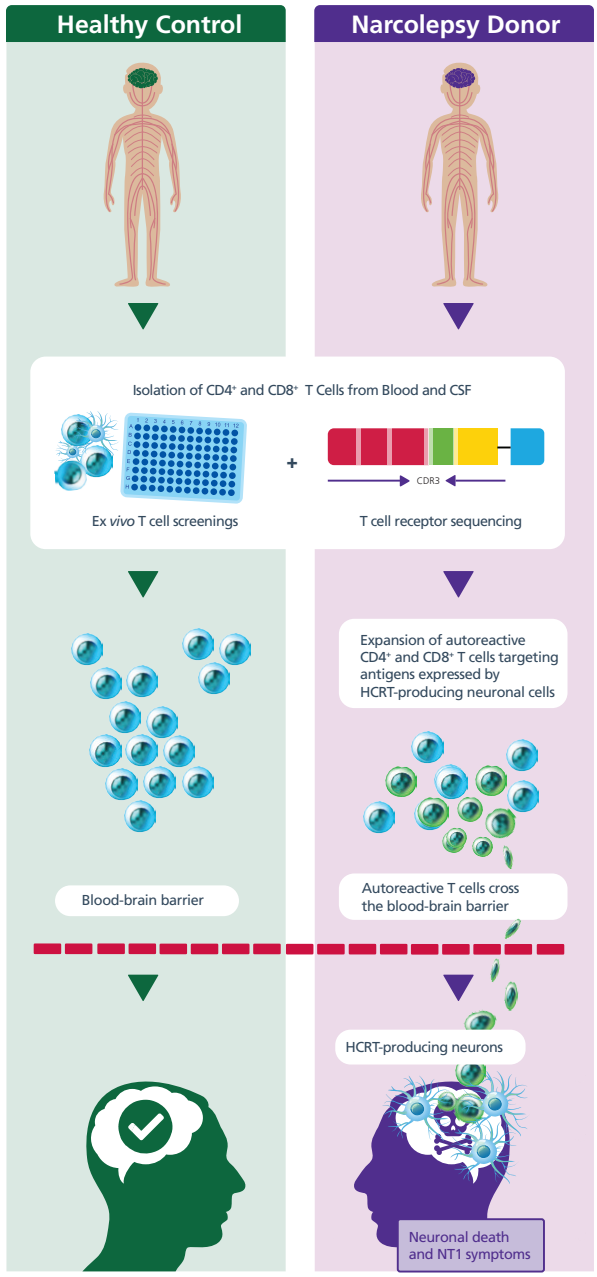
Spotlight research interview: Narcolepsy, a neurological autoimmune disorder

Narcolepsy is defined by the National Organization for Rare Disorders (NORD) as a rare, chronic neurological sleep disorder characterized by bouts of excessive drowsiness and is sometimes referred to as excessive daytime sleepiness (EDS)[1]. Research in the field suggests narcolepsy has its origins in autoimmunity, where the body's immune cells target and mediate the disruption of healthy tissues/cells. We had the pleasure of speaking with Dr. Daniela Latorre regarding her work published in *Nature* that was first to demonstrate direct evidence of autoreactive immune cells in narcolepsy patients and offer a highlight of her findings as well as her thoughts and insights on the work in this exciting field.

In narcolepsy, classic symptoms also include cataplexy – sudden extreme muscle weakness – hallucinations that may occur before or right after falling asleep, and episodes of paralysis upon awakening. It is thought to affect around 1 in 2,000 people, though many believe additional individuals may be undiagnosed or misdiagnosed and may not be accounted for in this figure[1].

The immune response is a vastly complex system that is diligently being decoded by scientists globally. The participation of immune cells, such as B cells as well as memory CD4⁺ and CD8⁺ T cells, helps in mounting an effective immune response to fight infections, while avoiding unintended attacks against healthy 'self' tissues of the body. However, there are clear aspects of heterogeneity and multifactorial causes that contribute to the dysregulation of the immune responses leading to autoimmune diseases, making it a very active field in need of breakthroughs with translational implications[2,3].



"The existence of autoreactive T cells in narcolepsy patients was still a matter of debate when our study started. In this research, we combined different experimental approaches, based on *ex vivo* T cell screenings, generation of single cell clones and deep sequencing of T cell receptors, which resulted particularly suitable to detect and characterize rare autoreactive T cells in the blood and cerebrospinal fluid of narcolepsy patients. Overall, our research revealed the existence of autoreactive T cells targeting hypocretin-producing neurons in narcolepsy patients, thus providing the first solid evidence of the autoimmune basis of this enigmatic disease." - Dr. Latorre on the main takeaways from her work

In the case of narcolepsy, researchers believe that immune cells attack brain hypocretin (HCRT)- producing neurons, leading to the selective loss of this cell population. This hypothesis is based on observations pointing to a strong association of the disease with HLA-DQB1*06:02 and immune dysregulation, as well as to an increased narcolepsy incidence upon environmental factors, such as influenza infections or vaccination. Until recently with the publication by *Latorre D. & Kallwait U* et al., however, a direct demonstration of this autoreactivity was not identified[4]. Her publication was the feature of numerous research highlight articles in Cell and Nature press as a crucial discovery in the field of narcolepsy, firmly placing autoimmunity as a hallmark of the disease[5-7].

In her influential study, Dr. Latorre and colleagues employed highly sensitive methodologies, including *ex vivo* cellular screening (Figure 1) and the T cell library method (Figure 2), to provide evidence that autoreactive $CD4^+$ and $CD8^+$ T cells directed against neuronal antigens (HCRT and TRIB2) are present in the blood and cerebrospinal fluid (CSF) of several patients suffering from narcolepsy type 1 (NT1) or type 2 (NT2) compared to healthy controls.

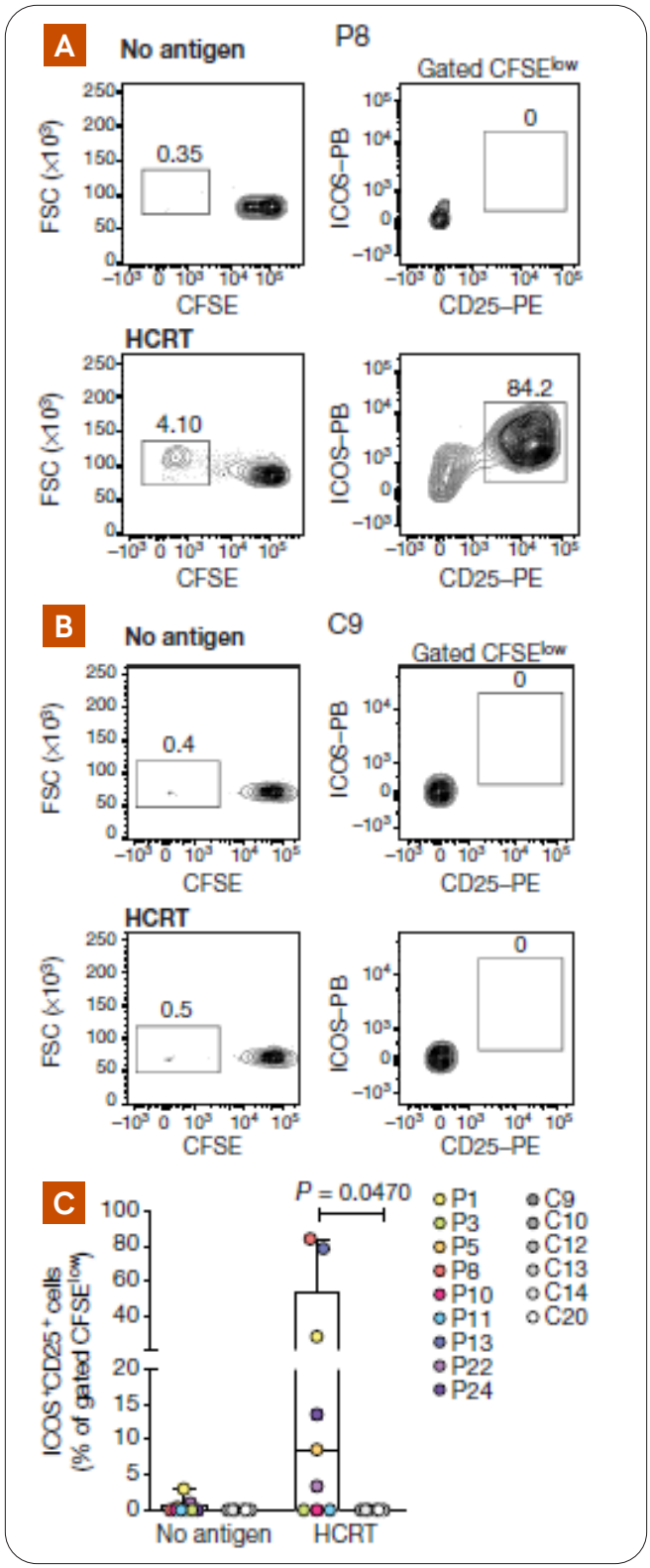


Figure 1: *Ex vivo* stimulation of memory $CD4^+$ T cells from narcolepsy patients (P) versus healthy controls (C) shows that those collected from narcolepsy patients displayed increase proliferation ($CFSE^{lo}$) and activation ($ICOS^+CD25^+$) when stimulated with autologous monocytes challenged with a HCRT peptide pool compared to control. Figure taken from Figure 1[4]; additional method details can be found in the method and figure legend of https://doi.org/10.1038/s41586-018-0540-1.

From narcolepsy patients that showed an autoreactive T cell response in the first screenings, the authors were able to generate and characterize in-depth a total of 214 autoreactive single CD4⁺ T cell clones. Epitope mapping screenings revealed that the majority of these clones targeted multiple HCRT or TRIB2 protein epitopes mostly on HLA-DR molecules, as demonstrated by MHC-class II inhibition experiments. Since there is a strong genetic association of the HLA-DQB1*06:02 molecule with narcolepsy, this was an unexpected result that may have different explanations in the context of an autoimmune response[5].

Additionally, autoreactive clones failed to respond to self-neuronal antigens when provided in the form of whole proteins, which requires processing by antigen presenting cells (APCs). This finding supports the idea that the mode of antigen processing may be critical for antigen recognition by autoreactive T cells and for escape from tolerance in human autoimmunity[8]. Notably, their study also demonstrated no T cell cross-reactivity with influenza antigens, suggesting that the hypothesized similarity of epitopes of influenza antigens vs HCRT may not be the causal agent for vaccination/infection-spurred narcolepsy incidences; that is, at least not in the patient cohort analyzed, though this point is still under scientific debate and further epidemiological studies are needed[9].

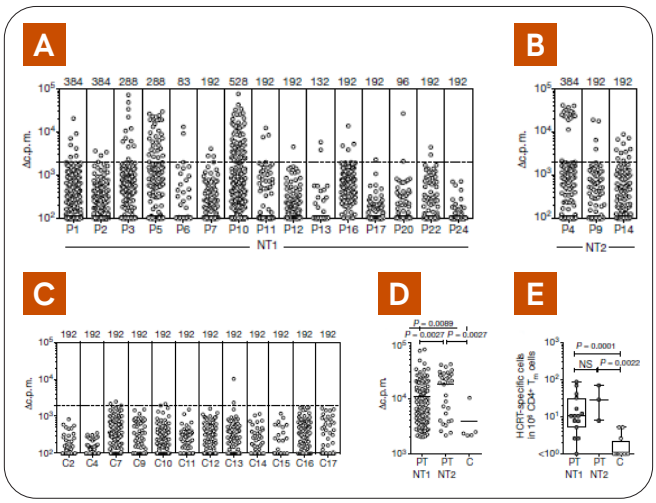


Figure 2: T cell library screening of memory CD4⁺ T cells in narcolepsy patients (NT1 and NT2) compared to healthy controls (C) challenged with HCRT and assessed for proliferation showed strong response in all patients except one (P24) compared to only a few proliferative lines in 3 out of 12 healthy controls. Figure taken from Figure 2[4]; additional method details can be found in the method and figure legend of https://doi.org/10.1038/s41586-018-0540-1.

The authors proceeded to characterize the TCR Vβ gene repertoire of autoreactive T cells by sequencing both HCRT-specific and TRIB2-specific CD4⁺ T cells. This analysis showed the presence of expanded autoreactive CD4⁺ T cell clones in the blood of the same as well as, in some cases, different patients, opening the door to the existence of potential pathogenic clones that are shared among narcolepsy patients. Lastly, they investigated the presence of autoreactive T cells in the CSF of narcolepsy patients and isolated two HCRT-specific CD8⁺ T cell clones with different TCRs from a NT2 patient with recent disease onset, normal levels of HCRT and lack of clinical symptoms for NT1 at time of sampling (Figure 3). Interestingly, the patient developed cataplexy almost one year later thus fulfilling the clinical criteria for NT1; thus suggesting that since time of sampling, an ongoing neurological autoimmune attack may have led to an NT1 disease progression. These data are in line with observations from animal studies showing that CD8⁺ T cells are able to mediate neuronal disruption with consequent narcolepsy-like symptom induction[10].

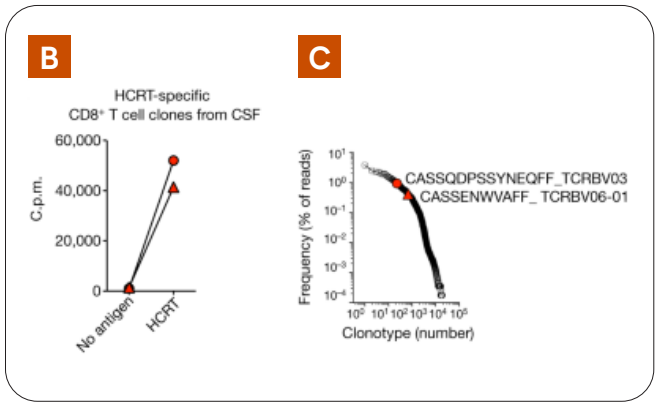


Figure 3: Isolation of 2 CD8⁺ T single cell clones from the CSF of a NT2 patient. These CD8⁺ T cell clones proliferate when stimulated with autologous monocytes challenged with a HCRT peptide pool (B) and carry 2 different TCR Vβ CDR3 sequences (C). Figure taken from Figure 4[4]; additional method details can be found in the method and figure legend of https://doi.org/10.1038/s41586-018-0540-1.

"Understanding how the immune system plays a role in narcolepsy disease initiation and/or progression still remains poorly understood. Incomplete and evolving clinical manifestations of narcolepsy - the so-called "narcolepsy borderland" (NBL) - which affect about 1-2% of the population, currently represent the major clinical challenge. Our study has also revealed the existence of autoreactive T cells in patients with narcolepsy type 2 (NT2), a NBL disorder that, in some cases, can progress to NT1 with cataplexy and HCRT deficiency. This evidence has opened new avenues for basic immunological and clinical studies in the field of sleep-related disorders. We have recently started the Swiss Primary Hypersomnolence and Narcolepsy Cohort Study (SPHYNCS) addressing the hypothesis that NBL disorders may represent an intermediate stage of narcolepsy disease progression in which an autoimmune attack that has not yet led to a complete loss of HCRT-producing neurons is ongoing. The aim of this study is to identify new biomarkers of narcolepsy establishment and progression that may lead to the development of more precise diagnostic tools as well as effective clinical intervention[11]." – Dr. Latorre on the potential translational implications to NT diagnosis/ monitoring and treatment intervention stemming from her work

In summary, the authors proved the existence of circulating HCRT- and TRIB2-specific T cells in narcolepsy patients, thus providing insight into potential mechanisms of these autoreactive T cells in mounting an autoimmune response that may underlie the selective loss of HCRT-producing neurons. Overall, this study may help further our understanding of the biology behind narcolepsy and potential treatment targets or intervention/management strategies.

"These findings are the outcome of a successful collaborative effort between basic researchers and expert neurologists that provided the necessary complementary expertise to make important advances in the field of narcolepsy and sleep-related disorders. In particular, this research was performed in the laboratory of Prof. Federica Sallusto at Institute for Research in Biomedicine (IRB) and ETH Zurich in collaboration with clinicians from several Sleep centers in Switzerland, including Prof. Kallweit and Prof. Bassetti at the Insespital, University Hospital in Bern; Dr. Manconi at Ente Ospedaliero Cantonale in Ticino and Prof. Khatami at Klinik Barmelweid." – Dr. Latorre on the importance of scientific collaboration to accelerate research for rare disorders

About the scientist and institutions involved

Credits to Ph. Stefan Weiss and ETH Zurich

Dr. Daniela Latorre

Dr. Latorre obtained her PhD in Immunology at "Sapienza" University of Rome in Italy. Afterwards, she moved first to the Institute for Research in Biomedicine in Bellinzona, Switzerland and later to the Institute of Microbiology, ETH Zurich to perform her post-doctoral studies under the supervision of Prof. Federica Sallusto, a world renewed scientist in the field of human immunology. During these years, besides her study on narcolepsy that earned her a first author publication as a full article in Nature[4] and several awards, she also contributed to provide new insights into basic aspects of the immune response against microbial pathogens and vaccines in physiological and pathological conditions[4, 12-18].

Dr. Latorre's work has earned her several prizes, including the European Narcolepsy Network (EU-NN) Young Scientist award, the Best International Young Researcher on Narcolepsy Prize by AIN, and the Pfizer Prize for Research 2020. She has recently been awarded the PRIMA Grant, a highly competitive grant funded by the Swiss National Science Foundation, which allowed her to start an independent career as Group leader of the Human Neuroimmunology lab at the Institute of Microbiology, ETH Zurich. The primary objective of her present and future research is to study the role of autoreactive T cells in immune-mediated neurological diseases in order to shed light on basic aspects of human T cell biology in health and autoimmunity, and then translate those findings into biomedical applications. Her research is also supported by the Swiss Foundation for Research on Muscle Diseases (FSRMM) and the ETH Zurich. Find out More

Istituto di Ricerca in Biomedicina

Founded in 2000, the Institute for Research in Biomedicine (IRB) has gained an international reputation as a leading center of excellence in immunology research. In addition to immunology, the institute hosts researchers active in the field of DNA repair, rare disease, structural biology, and cell biology. Since 2009, the IRB is affiliated with the Università della Svizzera italiana (USI) and is now fully part of the newly created Faculty of Biomedical Sciences, which is currently setting up a Master of Medicine program.

University Hospital in Bern, Neurology Department at Inselspital

The Neurology Department at Inselspital, University Hospital in Bern, is the largest neurology center in Switzerland. Its international reputation is due to the excellent work in clinical assistance, teaching, and research being conducted on several neurological disorders.

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