**Project Title:** Dissecting autoreactive T cell immunity in distinct Guillain-Barré syndrome variants.

**1-Year Progress Report:** Guillain-Barré syndrome (GBS) is a rare disorder affecting the peripheral nervous system (PNS), often resulting in severe disability. Despite its classification as an autoimmune disease, the specific mechanisms driving its various clinical presentations remain unclear, making early diagnosis and treatment challenging. With the support of the GBS CIDP Foundation International (2023 Elevation grant), our recent discovery of autoreactive T cell immunity directed against PNS-myelin proteins (namely P0, P2 and PMP22) in acute inflammatory demyelinating polyneuropathy (AIDP), has offered significant new insights into the disease immunopathology, opening new perspectives for advancing our understanding of inflammatory peripheral neuropathies more broadly (Súkeníková et al, Nature 2024 and Ripellino et al, EJI, 2024).

Building on these findings, this project seeks to comprehensively characterize the autoimmune processes in distinct disease subtypes. Specifically, to investigate the potential presence of autoreactive T cells that recognize PNS-myelin antigens in patients with chronic inflammatory demyelinating polyneuropathy (CIDP) or the axonal disease variant (AMAN), we employed a sensitive *in vitro* stimulation assay memory CD4$^+$ T cells isolated from patients' peripheral blood. Preliminary data from this analysis revealed that memory CD4$^+$ T cells specific to at least one PNS-myelin antigen were detectable in 4 out of 6 CIDP patients, whereas no such responses were observed in AMAN patients at disease onset (Figure 1). From CIDP patients showing an autoreactive CD4$^+$ T cell response to PNS-myelin antigens, autoreactive T cells were further studied at single cell resolution by generating single T cell clones from proliferating T cell fraction from *in vitro* screenings, according to established protocols. Using this approach, a total of 284 single CD4$^+$ T cell clones specific for P0 (n = 109), P2 (n = 129) or PMP22 antigens (n = 46) were isolated from 4 CIDP patients and further studied for their MHC restriction and T cell receptor (TCR) sequences, indicating a preferential HLA-DR restriction and polyclonal TCR Vβ repertoire (preliminary data not shown). Importantly, within this research we have identified T cell clones in CIDP patients that exhibit cross-reactivity between PNS-myelin and viral antigens, particularly *Cytomegalovirus* (CMV) (preliminary data not shown). These findings build on our recent discovery of cross-reactivity between PNS-myelin and CMV antigens in AIDP patients with primary CMV infection (Súkeníková et al, Nature 2024), further indicating molecular mimicry as an underpinning mechanism.

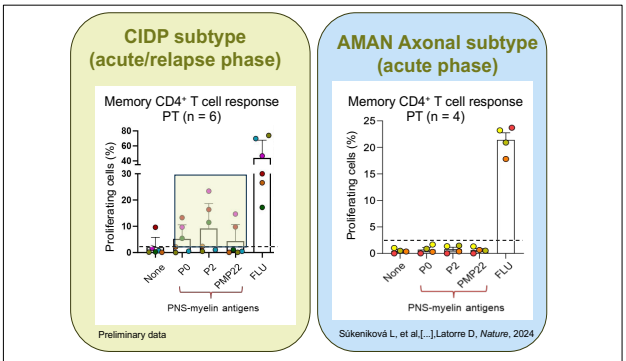


**Figure 1. *In vitro* screening for autoreactive memory CD4$^+$ T cells in CIDP and AMAN patients.** On day 6 from stimulation, autoreactive CFSE$^{low}$ T cells are detected by flow cytometry. Pooled data showing results from *in vitro* screening with the indicated PNS-myelin antigens of total memory CD4$^+$ T cells from CIDP patients (n = 6) and AMAN patients (n = 4).

*Overall, these preliminary findings suggest that autoreactive T cells may contribute to AIDP and CIDP subtypes, but not to axonal variants like AMAN. Our preliminary results provide a basis for further investigation into the antigens these T cells recognize and how their features varies across disease subtypes and stages. This work aims to identify immune patterns that could help explain disease heterogeneity and inform the development of targeted therapeutic strategies. In addition, it may offer broader insights into infection-associated autoimmunity and the role of T cells in related disorders.*