



Published in final edited form as:

J Neurol Sci. 2023 January 15; 444: 120510. doi:10.1016/j.jns.2022.120510.

Unaltered T cell responses to common antigens in individuals with Parkinson's disease

Gregory P. Williams^{a,b}, Kaylin Muskat^a, April Frazier^{a,b}, Yaqian Xu^{b,g}, José Mateus^a, Alba Grifoni^a, Ricardo da Silva Antunes^a, Daniela Weiskopf^a, Amy W. Amara^{b,d}, David G. Standaert^{b,d}, Jennifer G. Goldman^e, Irene Litvan^f, Roy N. Alcalay^h, David Sulzer^{b,c,i}, Cecilia S. Lindestam Arlehamn^{a,b,*},¹, Alessandro Sette^{a,b,j,*},¹

^aCenter for Infectious Disease and Vaccine Research, La Jolla Institute for Immunology, La Jolla, CA 92037, USA

^bAligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, USA

^cDepartment of Psychiatry, Columbia University Irving Medical Center, New York State Psychiatric Institute, New York, NY 10032, USA

^dDepartment of Neurology, University of Alabama at Birmingham, Birmingham, AL 35233, USA

^eShirley Ryan AbilityLab, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA

^fDepartment of Neuroscience, University of California San Diego, La Jolla, CA 92093, USA

^gDepartment of Neurology, Columbia University Irving Medical Center, New York, NY 10032, USA

^hNeurological Institute, Tel Aviv Sourasky Medical Center, Tel Aviv 6423906, Israel

ⁱDepartments of Psychiatry and Pharmacology, Columbia University; New York State Psychiatric Institute, New York, NY 10032, USA

^jDepartment of Medicine, University of California San Diego, La Jolla, CA 92093, USA

Abstract

Background and objectives: Parkinson's disease (PD) is associated with a heightened inflammatory state, including activated T cells. However, it is unclear whether these PD T cell responses are antigen specific or more indicative of generalized hyperresponsiveness. Our

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

*Corresponding authors at: Center for Infectious Disease and Vaccine Research, La Jolla Institute for Immunology, La Jolla, CA 92037, USA, cecilia@lji.org (C.S. Lindestam Arlehamn), alex@lji.org (A. Sette).

¹Contributed equal effort.

Author contributions

KM performed the experiments, GPW, KM analyzed the data, AF, YX recruited participants and AWA, DGS, JGG, IL, RNA performed clinical evaluations. JM, AG, RA, and DW designed, characterized, and provided epitope pools. GPW wrote the manuscript, DS, CLA, ALS designed and discussed the data. All authors read, edited, and approved the manuscript before submission.

Disclosure

The authors report no relevant disclosures for this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jns.2022.120510>.

objective was to measure and compare antigen-specific T cell responses directed towards antigens derived from commonly encountered human pathogens/vaccines in patients with PD and age-matched healthy controls (HC).

Methods: Peripheral blood mononuclear cells (PBMCs) from 20 PD patients and 19 age-matched HCs were screened. Antigen specific T cell responses were measured by flow cytometry using a combination of the activation induced marker (AIM) assay and intracellular cytokine staining.

Results: Here we show that both PD patients and HCs show similar T cell activation levels to several antigens derived from commonly encountered human pathogens/vaccines in the general population. Similarly, we also observed no difference between HC and PD in the levels of CD4 and CD8 T cell derived cytokines produced in response to any of the common antigens tested. These antigens encompassed both viral (coronavirus, rhinovirus, respiratory syncytial virus, influenza, cytomegalovirus) and bacterial (pertussis, tetanus) targets.

Conclusions: These results suggest the T cell dysfunction observed in PD may not extend itself to abnormal responses to commonly encountered or vaccine-target antigens. Our study supports the notion that the targets of inflammatory T cell responses in PD may be more directed towards autoantigens like α -synuclein (α -syn) rather than common foreign antigens.

Keywords

Parkinson's disease; T cells; Inflammation; Neuroimmunology; Immune system; Viral immunity; Bacterial immunity; Neurodegeneration

1. Introduction

Parkinson's disease (PD), the second most common neurodegenerative disease behind Alzheimer's disease (AD), affects over 6 million people globally. PD symptomology is typically characterized by motor dysfunction including bradykinesia, tremor, and rigidity. However, non-motor symptoms often present long before clinical diagnosis and include loss of smell, disturbed sleep (e.g., REM sleep behavior disorder), constipation, and depression [1]. The major pathologies believed to underlie these PD symptoms are the degeneration of neurons (especially dopaminergic neurons in the substantia nigra) as well as the excessive accumulation and aggregation of the neuronal protein α -synuclein (α -syn) [2].

Alongside the hallmark neuronal pathology observed in PD is the observation of increased pro-inflammatory markers among several different cell types and spanning multiple tissues [3]. This includes both microgliosis and astrogliosis in the central nervous system (CNS) [4,5], as well as dysregulated monocyte populations in circulating blood [6,7]. However, the heightened PD inflammatory state is not limited to innate immune cells; increased CD4 and CD8 T cell infiltration in PD post-mortem brain and abnormal blood T cell frequencies, compared to healthy controls (HC), have also been observed in some studies [8–10]. More recent findings have expanded on the potential inflammatory nature of T cells in PD and shown that some PD patients possess expanded numbers of circulating, α -syn-specific T cells [11,12]. A follow-up study showed that α -syn-specific T cell responses in PD patients were associated with early PD and a longitudinal study of a single case showed that α -syn-

specific T cell responses were present years before the individual displayed symptoms [13]. Furthermore, the α -syn evoked T cell responses reached their highest magnitude around the same time as that individual acquired a varicella-zoster virus (shingles) infection. Potential connections between PD risk and infectious triggers have been investigated in the past and include associations with influenza [14], common cold coronavirus [15], and Epstein-Barr virus (EBV) [16]. Intriguingly, it has also been observed that individuals on anti-TNF treatment are at a lower risk of developing PD [17]. It is, however, unclear whether these results are reflective of a general heightened inflammatory state and altered global immune function in PD, or more the result of T cell responses specific to bacterial/viral derived antigens, α -syn, or other potential self-antigens.

While previous work has mainly addressed non-specific, baseline PD T cell responses, we set out to test whether there is a general increased T cell responsiveness in individuals with PD compared to age-matched HC in regards to common bacterial/viral antigens. We utilized different cell-based assays to determine the magnitude, specificity, and type of T cell response to commonly encountered pathogen and vaccine antigens. These common antigens included common cold coronaviruses, rhinovirus, respiratory syncytial virus, influenza, cytomegalovirus, varicella-zoster virus, pertussis, and tetanus toxoid. Using the activation induced marker assay [18–20] and intracellular cytokine staining (ICS) as a readout for T cell activation, we exposed both PD patient and age-matched HC peripheral blood mononuclear cells (PBMCs) to pathogen-specific peptide pools that have been previously shown to be targets of T cell responses. We found no difference in antigen-specific T cell activation between HC and individuals with PD. These results suggest that the inflammatory profile associated with PD does not influence the immune response to commonly encountered pathogens and vaccine targets as circulating T cells specific to several bacterial/viral targets had equivalent responses across groups—supporting the notion that general T cell responses are similar between HC and individuals with PD.

2. Methods

2.1. Ethics statement

All participants provided written informed consent for participation in the study. Ethical approval was obtained from the Institutional Review Boards at La Jolla Institute for Immunology (LJI; Protocol Nos: VD-124 and VD-118), Columbia University Irving Medical Center (CUMC; protocol number IRB-AAAQ9714 and AAAS1669), University of California San Diego (UCSD; protocol number 161224), Rush University Medical Center (RUMC; Office of Research Affairs No.16042107-IRB01), Shirley Ryan AbilityLab Northwestern University (protocol number STU00209668-MOD0005), and the University of Alabama at Birmingham (UAB; protocol number IRB-300001297).

2.2. Study subjects

39 participants were recruited for this study, 20 individuals diagnosed with PD and 19 age-matched healthy subjects. These subjects were recruited across multiple sites which consisted of Columbia University Irving Medical Center (CUMC) (HC $n = 1$ and PD $n = 6$), La Jolla Institute for Immunology (LJI) (HC $n = 11$ and PD $n = 10$), University of

California San Diego (HC $n = 3$ and PD 2), Rush University Medical Center (RUMC) (HC $n = 1$), Shirley Ryan AbilityLab Northwestern University (HC $n = 1$ and PD $n = 2$), and the University of Alabama at Birmingham (UAB) (HC $n = 2$). The characteristics of the enrolled subjects are detailed in Table 1.

Subjects with idiopathic PD and HCs were recruited by the Movement Disorders Clinic at the Department of Neurology at CUMC, by the clinical core at LJI, by the Parkinson's and Other Movement Disorder Center at UCSD, by movement disorder specialists at the Parkinson's disease and Movement Disorders program at Shirley Ryan AbilityLab, and by movement disorder specialists at UAB Movement Disorders Clinic. Inclusion criteria for PD patients consisted of i) clinically diagnosed PD with the presence of bradykinesia and either resting tremor or rigidity ii) PD diagnosis between ages 35–80 iii) history establishing dopaminergic medication benefit, and iv) ability to provide informed consent. Exclusion criteria for PD were atypical parkinsonism or other neurological disorders, history of cancer within past 3 years, autoimmune disease, and chronic immune modulatory therapy. Age and sex matched HC were selected on the basis of i) age 45–85 and ii) ability to provide informed consent. Exclusion criteria for HC was the same as PD except for the addition of self-reported PD genetic risk factors (i.e., PD in first-degree blood relative). For the LJI cohort, PD was self-reported. Individuals with PD recruited at CUIMC, UCSD, and Northwestern all met the UK Parkinson's Disease Society Brain Bank criteria for PD.

2.3. PBMC isolation

Venous blood was collected from each participant in either heparin or EDTA containing blood bags/tubes. PBMCs were isolated from whole blood by density gradient centrifugation using Ficoll-Paque plus (GE #17144003). Blood was first spun at 1850 rpm for 15 mins with brakes off to remove plasma. Plasma depleted blood was then diluted with RPMI and 35 mL of blood was carefully layered on tubes containing 15 mL Ficoll-Paque plus. These tubes were then centrifuged at 1850 rpm for 25 mins with brakes off. The interphase cell layer resulting from this spin were collected, washed with RPMI, counted, and cryopreserved in 90% v/v FBS and 10% v/v DMSO and stored in liquid nitrogen until tested. The detailed protocol for PBMC isolation can be found at protocols.io (<https://dx.doi.org/10.17504/protocols.io.bw2ipgce>).

2.4. Antigen pools

Our lab routinely identifies CD4 and CD8 T cell epitopes, as well as uses existing data in the Immune Epitope Database and Analysis Resource (IEDB [21]) to develop peptide “megapools”. The utilization of “megapools” allows the ability to test a large number of epitopes spanning multiple HLA-types allowing a breadth of responses in immune-diverse populations. Peptides were synthesized commercially as crude material by TC Peptide Lab (San Diego, CA). Lyophilized peptide products were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 20 mg/mL and their quality was spot checked by mass spectrometry. Overlapping and peptides predicted to bind MHC class II molecules were combined to form antigen “pools” for all of the respective antigens tested. Composition of the peptide pools used in this study have been previously defined, characterized, and are

listed in Table 2. Note: Flu-Other references influenza antigens derived from flu proteins “other” than HA.

2.5. Activation-induced marker (AIM) assay

PBMCs were thawed, washed, and counted for viability using the trypan blue exclusion method. A total of 1×10^6 cells per subject/condition were plated and cultured (after a 15 min pre-incubation with anti-CD40 (Miltenyi, clone HB14, RRID:AB_2660897), to allow subsequent staining with CD40L, in the presence of each of the previously described peptide pools (at a concentration of 1 $\mu\text{g}/\text{mL}$), PHA (10 $\mu\text{g}/\text{mL}$), or medium containing an equivalent amount of DMSO as what is present in the pool stimulation in a 96 well U-bottom plate for 24 h at 37 °C, 5% CO₂. After incubation, cells were then washed and stained with a cocktail of antibodies that included CXCR5-BUV395 (BD, clone RF8B2, RRID:AB_2740008) to capture T follicular helper cells, CD8-BUV496 (BD, clone RPA-T8, RRID:AB_2870223), CD3-BUV805 (BD, clone UCHT1, RRID:AB_2739277), CD45RA-BV421 (Biolegend, clone HI100, RRID:AB_10965547) to distinguish memory T cell subsets together with CCR7, Fixable Viability Dye eFluor506 (eBioscience, 65–0866–18), CD14-V500 (BD, clone M5E2, RRID:AB_10611856) to exclude CD14+ cells, CD19-V500 (BD, clone HIB19, RRID:AB_105623910) to exclude CD19+ cells, CD4-BV605 (BD, RPA-T4, RRID: AB_2744420), CD38-BV786 (BD, clone HIT2, RRID:AB_2738515) to capture recently activated cells together with HLA-DR, CCR7-FITC (Biolegend, clone G043H7, RRID:AB_10916386), CD40L-PerCP-eF710 (eBioscience, clone 24–31, RRID:AB_10670357), CD69-PE (BD, clone FN50, RRID:AB_395916), PD-1-PE-Dazzle594 (Biolegend, clone EH12.2H7, RRID:AB_2563659), OX40-PE-Cy7 (Biolegend, clone Ber-ACT35, RRID:AB_10901161), CD137-APC (Biolegend, clone 4B4–1, RRID:AB_830672), and HLA-DR-AF700 (eBioscience, clone LN3, RRID: AB_1907427) for 30 min at 4 °C. CD40L, CD69, OX40, and CD137 are all T cell activation markers. Afterwards, cells were washed and resuspended in 100 μL PBS for acquisition.

Cells were acquired on a Bio-Rad ZE5 cell analyzer, and further analysis was done using FlowJo software (FlowJo, RRID:SCR_008520, v10). CD40L staining is used as an additional activation marker for cytokine-producing T cells. CXCR5 combined with PD-1 staining allows for capturing T follicular helper populations. CD38 together with HLA-DR captures recently activated T cells as a result of recent reactivation. CD25, a commonly used marker for activation was excluded from our panel due to evidence that T regulatory cells upregulate this molecule as well upon antigen stimulation [20]. Per previous publications [18,19], quantification of live, single antigen-specific CD4⁺ and CD8⁺ T cells was determined as a percentage of their OX40/CD137 and CD69/CD137 double expression (AIM⁺), respectively. Antigen specific AIM⁺ CD4⁺ and CD8 T cell signals were background subtracted by their corresponding DMSO negative controls, with the minimal DMSO level set to 0.005%. The limit of detection (LOD) for the AIM assay was calculated by multiplying the upper confidence interval of the geometric mean of all AIM⁺ DMSO samples by 2. The threshold of positivity was calculated based on multiplying the standard deviation of DMSO control samples by 2, concordant with previous studies [18,31]. The detailed protocol for the AIM staining assay can be found at protocols.io (<https://dx.doi.org/10.17504/protocols.io.4r3l2o9w3v1y/v1>).

2.6. Intracellular cytokine assay

For the intracellular cytokine assay, PBMCs were processed and stimulated in the same way as the AIM assay (described above), but with the following modifications: After the 24 h incubation cells were then given a combination of Golgi-Stop (BD) and Golgi-Plug (BD) for 4 additional hours of culture. Afterwards the cells were surface and viability stained with CD3-BUV395 (BD, clone UCHT1, RRID: AB_2744387), CD8-BUV661 (BD, clone RPA-T8, RRID:AB_2871032), CD16-BV510 (Biolegend, clone 3G8, RRID:AB_2562085), CD14-BV510 (Biolegend, clone 63D3, RRID:AB_2716229), CD20-BV510 (Biolegend, clone 2H7, RRID:AB_2561941), CD45RA-BV750 (Biolegend, clone HI100, RRID:AB_2563813), CD4-BV711 (BD, clone RPA-T4, RRID: AB_2740432), CCR7-Pe-Cy7 (Biolegend, clone G0343H7, RRID:AB_11126145), and Fixable LIVE/DEAD Blue (Thermo Fisher) and incubated for 30 min at 4 °C. Cells were then washed, fixed (4% paraformaldehyde/PBS buffer), and then subsequently stained for intracellular cytokines with IL-4-BUV737 (BD, clone MP4-25D2, RRID: AB_2870157), IL-17-BV785 (Biolegend, clone BL168, RRID: AB_2566765), TNF-eFluor450 (eBioscience, clone Mab11, RRID: AB_2043889), IFN γ -FITC (eBioscience, clone 4S·B3, RRID:AB_465415), IL-2-BB700 (BD, clone MQ1-17H12, RRID:AB_2744488), IL-10-PE-Dazzle594 (Biolegend, clone JES3-19F1, RRID:AB_2632783), and CD40L-APC-eFluor780 (LifeTech, clone 24-31, RRID:AB_1603203) with saponin containing buffer (5 mg/mL) at room temperature for 30 mins. Afterwards, cells were washed and resuspended in 100 μ L PBS for acquisition.

Cells were acquired on a Bio-Rad ZE5 cell analyzer, and further analysis was done using FlowJo software. Similar to AIM analysis, quantification of live, singlet, cytokine producing CD4 cells were determined as a percentage of their CD40L⁺ Cytokine⁺ expression. CD40L/ Cytokine double positive signals were subtracted by their corresponding DMSO negative controls. The limit of detection for the ICS assay was calculated by multiplying the upper confidence interval of the geometric mean of all Cytokine⁺ DMSO samples by 2. The detailed protocol for the intracellular cytokine staining assay can be found at protocols.io ([dx.doi.org/10.17504/protocols.io.e6nvwkbwdvmk/v1](https://doi.org/10.17504/protocols.io.e6nvwkbwdvmk/v1)).

2.7. Data analysis

Lymphocytes were analyzed using a standardized gating method (see Supplemental Fig. 1 for detailed gating strategy) used within the lab. On top of the previously described signal background subtraction in both AIM and ICS assays, another quality-control measure employed in this study was the exclusion of samples that did not have a \geq 20% AIM/ Cytokine⁺ signal to the PHA-positive control stimuli.

Statistical analyses were performed and graphs created using GraphPad Prism's descriptive statistics and two-tailed Mann Whitney tests (GraphPad Prism, RRID:SCR_002798, v9).

Raw flow cytometric data for the AIM and ICS analyses can be found at (<https://flowrepository.org/id/FR-FCM-Z57F>) and (<https://flow-repository.org/id/FR-FCM-Z57Q>) respectively (donor key: <https://doi.org/10.5281/zenodo.7392339>).

3. Results

3.1. T cell reactivity to common antigens in HC and PD PBMCs

We utilized PBMCs from a cohort of age and sex-matched 19 HC and 20 PD individuals recruited from multiple sites (see Table 1 for detailed subject characteristics).

PBMCs were stimulated with antigenic peptide pools specific for coronaviruses NL63, 229E, HKU1, OC43, influenza virus (FLU-HA, FLU-Other), rhinovirus, respiratory syncytial virus (RSV), cytomegalovirus (CMV), varicella-zoster virus (VZV), *Bordetella pertussis* (PT), and tetanus toxoid (TT) antigens —see Table 2 for more detailed information on peptide pool compositions. Antigen pools tested were grouped into general classifications to aid in data viewing (e.g., Common Coronavirus, Lower Respiratory Tract Viruses). For CMV and EBV pools we also measured CD8-specific reactivity in addition to CD4 T cell responses (as these pools contain both class I and class II peptides). The antigen-specific T cell responses were measured using a flow cytometry activation-induced marker (AIM) assay [18–20] and antigen-specificity was determined as percentage of CD4⁺ cells expressing both OX40⁺ and CD137^{+18,19}, or CD8⁺ cells expressing both CD69⁺ and CD137^{+18,19} (Fig. 1a-d and Supplemental Fig. 1 for AIM gating strategy). No significant difference in the percentage of antigen-specific CD4 T cells between HC and PD was observed for any of the antigens tested. Similarly, we observed no difference in the CMV or EBV-specific CD8 AIM response between HC and PD (Fig. 1e). Additionally, we also measured the upregulation of other combinations of activation-associated markers and found no differences in responses between HC and PD (Supplemental Fig. 2). All HC and PD donor T cells responded to PHA stimulation (positive control) and there was no significant difference between the HC and PD response (Supplemental Fig. 3).

3.2. T cell derived cytokine response to common antigens in HC and PD PBMCs

As described above, we did not observe any significant differences between antigen-specific T cell activation in HC and PD individuals. To expand this observation, we next utilized a different assay system and addressed whether there is a difference in the specific cytokines produced between the two cohorts. PBMCs were stimulated with the same antigenic peptide pools used in the previous experiment and then the specific cytokine production was measured using an intracellular cytokine staining assay where we measured IFN γ , TNF α , IL-2, IL-17a, IL-4, and IL-10 to represent the majority of Th subsets. In terms of Th1 associated cytokine responses, there was no statistical difference between HC and PD CD4⁺, CD40L⁺, IFN γ producing T cells after stimulation with any of the antigen pools tested (Fig. 2a). Some marginal reductions were seen for certain antigen/cytokine combinations, such as a lower CD4 TNF α -production in PD subjects in response to the 229E coronavirus and VZV antigen pool (Fig. 2b), and reduced IL-2 production against the same common cold corona virus 229E antigen pool (Fig. 2c). These decreases were however not significant after considering corrections for multiple comparisons (Bonferroni corrected critical value of 0.003). No detection or low levels of IL-4, IL-10, and IL-17a, along with CD8⁺ IFN γ or TNF α were noted, with no difference in levels between HC and PD individuals (data not shown). Overall, we observed a generalized trend of lower cytokine responses in PD

individuals and more specifically to the 229E common cold coronavirus strain, however these differences would not meet significance if corrected for multiple comparisons.

Taken together, these results indicate that there is no difference between HC and PD in regard to antigen-specific T cell reactivity nor the types of cytokines produced to several commonly encountered antigens.

4. Discussion

This study investigated whether individuals with PD have T cell responses that are different compared to age-matched healthy controls after stimulation with commonly encountered antigens. Multiple groups have observed and reported on dysregulated and pro-inflammatory T cell responses detected in individuals with PD. [9,10,32,33] These findings include observations of increased antigen experienced T cells [32] as well as increases in T cell derived IFN- γ [10], IL-4 [10], and IL-17 [9,10]. However, these reports measured baseline T cell frequencies or cytokine responses following polyclonal stimulation such as PMA/Ionomycin. While these methods are informative in establishing the steady state of PD T cell composition and activation potential, they fall short of uncovering what targets are seemingly driving the pro-inflammatory T cell responses observed in the peripheral blood of PD patients. The common antigens tested encompassed multiple types of pathogens including common cold coronavirus, influenza, and herpesvirus. Both T cell activation and corresponding cytokine output were considered and we found no significant differences between the two cohorts. Our findings suggest that the hyper-inflammatory state reported in PD does not influence antigen-specific T cell responses to common pathogens consistently encountered either through natural exposure or vaccination. This observation is in agreement with the notion that the targets of peripheral T cell inflammation observed in PD are more directed towards autoantigens such as α -synuclein [11–13,34].

A potential link between infectious disease and development of Parkinson's disease has long been reported on across multiple different pathogens. An outbreak of encephalitic lethargica was reported around the time of the 1918 flu pandemic and transient neurological sequelae driven PD symptoms were associated with early stages of influenza infection [35]. Links between influenza infection and subsequent risk of developing PD have been debated throughout the years [14,36–38]. Most recently, an observational study found an increase in PD incidence following clinical influenza infection [39]. Two potential mechanisms that could link infection and development of PD would be the direct insult of the infection to the nigrostriatal system or the infection leading to an over-activation of the immune system (a common feature of PD). In the case of influenza, it has been shown that some type A strains can be neurotrophic in mammals and lead to dopamine neuron loss, α -syn pathology, and motor deficits [40,41]. Alternatively, non-neurotrophic influenza strains have also been found (albeit rare) to be associated with neurological symptoms due to encephalitis [42]. In both instances, glia derived neuroinflammation is thought to be the major driver of the neuropathology/neurodegeneration. However, in terms of the adaptive immune response, the data presented here does not suggest an increased influenza-specific T cell response in PD patients compared to healthy controls. Another pathogen tested in this study, Epstein-Barr virus has previously been linked to PD [43–45]

and EBV has been heavily implicated in development of another CNS disease, multiple sclerosis [46]. Lastly, it has been hypothesized that coronavirus infection may be involved in PD pathogenesis as increased CSF coronavirus antibodies have been previously been observed in PD patients [15] along with the fact that a coronavirus strain in mice was observed to infect and deteriorate their basal ganglia [47]. To our knowledge, this is the first study of its kind to measure antigen-specific T cell responses in patients with PD and HC to pathogens previously linked to PD (influenza, coronavirus, and EBV), as well as previously unassociated infectious species (rhinovirus) and vaccine antigens (pertussis, tetanus).

In conclusion, though there are multiple reports detailing a baseline hyper-inflammatory state in PD, our results shown here suggest that this hyper-responsiveness does not apply to antigen-specific T cell responses to many commonly encountered antigens. Given the fact that there have been multiple associations with PD and infectious triggers, it may be that other facets of the immune system (auto-antibodies, bystander damage due to general inflammation) could be of more importance to these epidemiological connections than antigen-specific T cell responses. The only increased antigen-specific T cell response observed thus far in PD has been to α -syn, a neuro-antigen. Interestingly, preclinical models of PD have also supported the notion that autoimmune T cell responses are involved in PD pathobiology [48–52]. More work is needed to explore if other relevant neuro-antigens, perhaps through molecular mimicry [45,53], might be the target of PD T cells and could better explain the sources of increased inflammation present in Parkinson's disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

The authors want to sincerely thank all of the generous individuals who donated samples for the study. We are also grateful to the La Jolla Institute for Immunology's clinical core for processing the blood samples.

Study funding

The study is funded by the joint efforts of The Michael J. FoX Foundation for Parkinson's Research (MJFF) and the Aligning Science Across Parkinson's (ASAP) initiative, the NIH T32AI125179 (GPW), P50NS108675 (DGS, AWA), and the JPB Foundation (DS). MJFF administers the grant ASAP-000375 (CLA, DS) on behalf of ASAP and itself. For the purpose of open access, the author has applied a CC BY public copyright license to all Author Accepted Manuscripts arising from this submission.

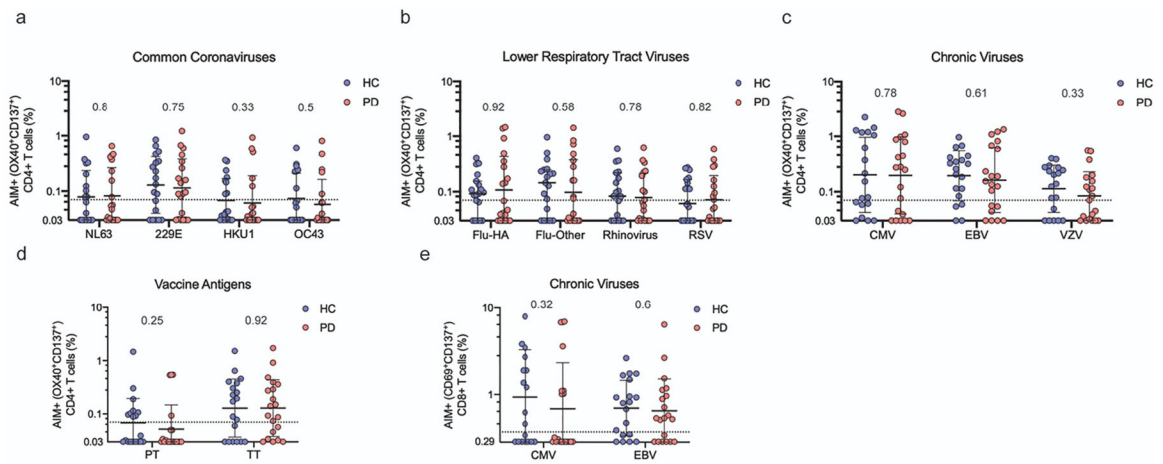
References

- [1]. Tolosa E, Garrido A, Scholz SW, Poewe W, Challenges in the diagnosis of Parkinson's disease, *Lancet Neurol* 20 (5) (May 2021) 385–397, 10.1016/S1474-4422(21)00030-2. [PubMed: 33894193]
- [2]. Kalia LV, Lang AE, Parkinson's disease, *Lancet* 386 (9996) (Aug 29 2015) 896–912, 10.1016/S0140-6736(14)61393-3. [PubMed: 25904081]
- [3]. Schonhoff AM, Williams GP, Wallen ZD, Standaert DG, Harms AS, Innate and adaptive immune responses in Parkinson's disease, *Prog. Brain Res* 252 (2020) 169–216, 10.1016/bs.pbr.2019.10.006. [PubMed: 32247364]

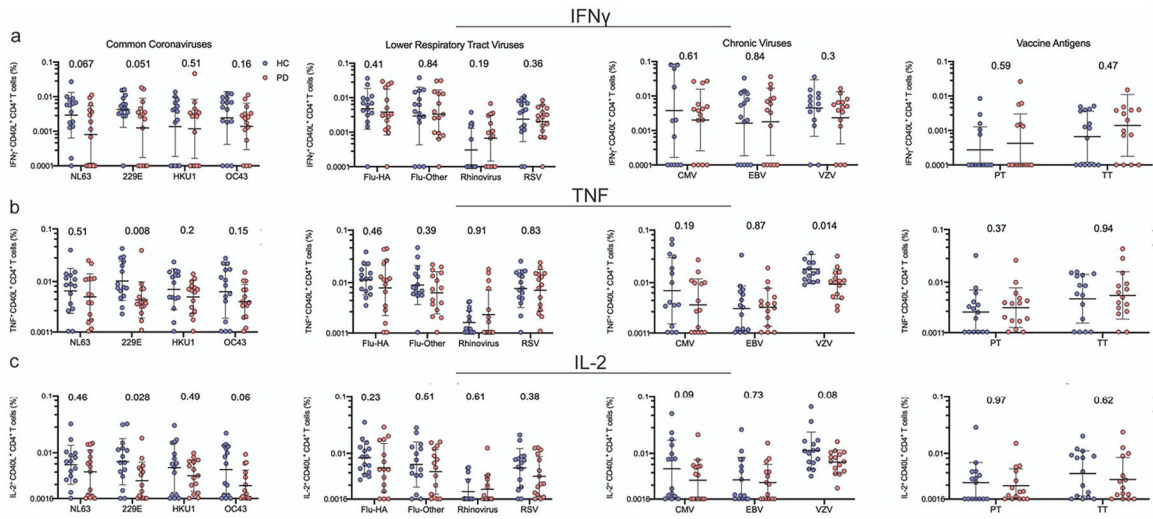
- [4]. Liddel SA, Guttenplan KA, Clarke LE, et al. , Neurotoxic reactive astrocytes are induced by activated microglia, *Nature* 541 (7638) (Jan 26 2017) 481–487, 10.1038/nature21029. [PubMed: 28099414]
- [5]. McGeer PL, Itagaki S, Boyes BE, McGeer EG, Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains, *Neurology* 38 (8) (Aug 1988) 1285–1291, 10.1212/wnl.38.8.1285. [PubMed: 3399080]
- [6]. Carlisle SM, Qin H, Hendrickson RC, et al. , Sex-based differences in the activation of peripheral blood monocytes in early Parkinson disease, *NPJ Parkinsons Dis* 7 (1) (Apr 13 2021) 36, 10.1038/s41531-021-00180-z. [PubMed: 33850148]
- [7]. Nissen SK, Shrivastava K, Schulte C, et al. , Alterations in blood monocyte functions in Parkinson's disease, *Mov. Disord* 34 (11) (Nov 2019) 1711–1721, 10.1002/mds.27815. [PubMed: 31449711]
- [8]. Brochard V, Combadiere B, Prigent A, et al. , Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease, *J. Clin. Invest* 119 (1) (Jan 2009) 182–192, 10.1172/JCI36470. [PubMed: 19104149]
- [9]. Sommer A, Marxreiter F, Krach F, et al. , Th17 lymphocytes induce neuronal cell death in a human iPSC-based model of Parkinson's disease, *Cell Stem Cell* 23 (1) (Jul 5 2018) 123–131, e6, 10.1016/j.stem.2018.06.015. [PubMed: 29979986]
- [10]. Yan Z, Yang W, Wei H, et al. , Dysregulation of the adaptive immune system in patients with early-stage Parkinson disease, *Neurol. Neuroimmunol. Neuroinflamm.* 8 (5) (Jul 2021), 10.1212/NXI.0000000000001036.
- [11]. Lodygin D, Hermann M, Schweingruber N, et al. , beta-Synuclein-reactive T cells induce autoimmune CNS grey matter degeneration, *Nature* 566 (7745) (Feb 2019) 503–508, 10.1038/s41586-019-0964-2. [PubMed: 30787438]
- [12]. Sulzer D, Alcalay RN, Garrett F, et al. , T cells from patients with Parkinson's disease recognize alpha-synuclein peptides, *Nature* 546 (7660) (Jun 29 2017) 656–661, 10.1038/nature22815. [PubMed: 28636593]
- [13]. Lindestam Arlehamn CS, Dhanwani R, Pham J, et al. , alpha-Synuclein-specific T cell reactivity is associated with preclinical and early Parkinson's disease, *Nat. Commun* 11 (1) (Apr 20 2020) 1875, 10.1038/s41467-020-15626-w. [PubMed: 32313102]
- [14]. Toovey S, Jick SS, Meier CR, Parkinson's disease or Parkinson symptoms following seasonal influenza, *Influenza Other Respir. Viruses* 5 (5) (Sep 2011) 328–333, 10.1111/j.1750-2659.2011.00232.x. [PubMed: 21668692]
- [15]. Fazzini E, Fleming J, Fahn S, Cerebrospinal fluid antibodies to coronavirus in patients with Parkinson's disease, *Mov. Disord* 7 (2) (1992) 153–158, 10.1002/mds.870070210. [PubMed: 1316552]
- [16]. Woulfe J, Gray MT, Ganesh MS, Middeldorp JM, Human serum antibodies against EBV latent membrane protein 1 cross-react with α -synuclein, *Neurol. Neuroimmunol. Neuroinflamm* 3 (4) (Aug 2016), e239, 10.1212/nxi.0000000000000239.
- [17]. Peter I, Dubinsky M, Bressman S, et al. , Anti-tumor necrosis factor therapy and incidence of Parkinson disease among patients with inflammatory bowel disease, *JAMA Neurol* 75 (8) (Aug 1 2018) 939–946, 10.1001/jamaneurol.2018.0605. [PubMed: 29710331]
- [18]. Dan JM, Mateus J, Kato Y, et al. , Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection, *Science* 371 (6529) (Feb 5 2021), 10.1126/science.abf4063.
- [19]. Grifoni A, Weiskopf D, Ramirez SI, et al. , Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals, *Cell* 181 (7) (Jun 25 2020) 1489–1501, e15, 10.1016/j.cell.2020.05.015. [PubMed: 32473127]
- [20]. Reiss S, Baxter AE, Cirelli KM, et al. , Comparative analysis of activation induced marker (AIM) assays for sensitive identification of antigen-specific CD4 T cells, *PLoS One* 12 (10) (2017), e0186998, 10.1371/journal.pone.0186998. [PubMed: 29065175]
- [21]. Grifoni A, Sidney J, Zhang Y, Scheuermann RH, Peters B, Sette A, A Sequence homology and bioinformatic approach can predict candidate targets for immune responses to SARS-CoV-2, *Cell Host Microbe* 27 (4) (Apr 8 2020) 671–680, e2, 10.1016/j.chom.2020.03.002. [PubMed: 32183941]

- [22]. Antunes R da Silva, S. Pallikkuth, E. Williams, et al. , Differential T-cell reactivity to endemic coronaviruses and SARS-CoV-2 in community and health care workers, *J. Infect. Dis* 224 (1) (Jul 2 2021) 70–80, 10.1093/infdis/jiab176. [PubMed: 33822097]
- [23]. Meckiff BJ, Ramírez-Suástegui C, Fajardo V, et al. , Imbalance of regulatory and cytotoxic SARS-CoV-2-reactive CD4(+) T cells in COVID-19, *Cell* 183 (5) (Nov 25 2020) 1340–1353, e16, 10.1016/j.cell.2020.10.001. [PubMed: 33096020]
- [24]. Grifoni A, Mahajan S, Sidney J, et al. , A survey of known immune epitopes in the enteroviruses strains associated with acute flaccid myelitis, *Hum. Immunol* 80 (11) (Nov 2019) 923–929, 10.1016/j.humimm.2019.08.004. [PubMed: 31451291]
- [25]. Dhanda SK, Mahajan S, Paul S, et al. , IEDB-AR: immune epitope database-analysis resource in 2019, *Nucleic Acids Res* 47 (W1) (Jul 2 2019) W502–w506, 10.1093/nar/gkz452. [PubMed: 31114900]
- [26]. Carrasco Pro S, Sidney J, Paul S, et al. , Automatic generation of validated specific epitope sets, *J Immunol Res* 2015 (2015), 763461, 10.1155/2015/763461. [PubMed: 26568965]
- [27]. Dan JM, Lindestam Arlehamn CS, Weiskopf D, et al. , A cytokine-independent approach to identify antigen-specific human germinal center T follicular helper cells and rare antigen-specific CD4+ T cells in blood, *J. Immunol* 197 (3) (Aug 1 2016) 983–993, 10.4049/jimmunol.1600318. [PubMed: 27342848]
- [28]. Voic H, de Vries RD, Sidney J, et al. , Identification and characterization of CD4(+) T cell epitopes after Shingrix vaccination, *J. Virol* 94 (24) (Nov 23 2020), 10.1128/jvi.01641-20.
- [29]. da Silva Antunes R., Quiambao LG, Sutherland A, et al. , Development and validation of a *Bordetella pertussis* whole-genome screening strategy, *J Immunol Res* 2020 (2020) 8202067, 10.1155/2020/8202067. [PubMed: 32322598]
- [30]. da Silva Antunes R., Paul S, Sidney J, et al. , Definition of human epitopes recognized in tetanus toxoid and development of an assay strategy to detect ex vivo tetanus CD4+ T cell responses, *PLoS One* 12 (1) (2017), e0169086, 10.1371/journal.pone.0169086. [PubMed: 28081174]
- [31]. Mateus J, Grifoni A, Tarke A, et al. , Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans, *Science* 370 (6512) (Oct 2 2020) 89–94, 10.1126/science.abd3871. [PubMed: 32753554]
- [32]. Bas J, Calopa M, Mestre M, et al. , Lymphocyte populations in Parkinson’s disease and in rat models of parkinsonism, *J. Neuroimmunol* 113 (1) (Feb 1 2001) 146–152, 10.1016/s0165-5728(00)00422-7. [PubMed: 11137586]
- [33]. Saunders JA, Estes KA, Kosloski LM, et al. , CD4+ regulatory and effector/memory T cell subsets profile motor dysfunction in Parkinson’s disease, *J. NeuroImmune Pharmacol* 7 (4) (Dec 2012) 927–938, 10.1007/s11481-012-9402-z. [PubMed: 23054369]
- [34]. Gate D, Tapp E, Leventhal O, et al. , CD4(+) T cells contribute to neurodegeneration in Lewy body dementia, *Science* 374 (6569) (Nov 12 2021) 868–874, 10.1126/science.abf7266. [PubMed: 34648304]
- [35]. Limphaibool N, Iwanowski P, Holstad MJV, Kobylarek D, Kozubski W, Infectious etiologies of parkinsonism: Pathomechanisms and clinical implications, *Front. Neurol* 10 (2019) 652, 10.3389/fneur.2019.00652. [PubMed: 31275235]
- [36]. Estupinan D, Nathoo S, Okun MS, The demise of Poskanzer and Schwab’s influenza theory on the pathogenesis of Parkinson’s disease, *Parkinsons Dis* 2013 (2013), 167843, 10.1155/2013/167843. [PubMed: 23853734]
- [37]. Harris MA, Tsui JK, Marion SA, Shen H, Teschke K, Association of Parkinson’s disease with infections and occupational exposure to possible vectors, *Mov. Disord* 27 (9) (Aug 2012) 1111–1117, 10.1002/mds.25077. [PubMed: 22753266]
- [38]. Poskanzer DC, Schwab RS, Cohort analysis of Parkinson’s syndrome: evidence for a single etiology related to subclinical infection about 1920, *J. Chronic Dis* 16 (Sep 1963) 961–973, 10.1016/0021-9681(63)90098-5.
- [39]. Carrasco E, Gomez MM Las Heras de, Gabande-Rodriguez E, Desdin-Mico G, Aranda JF, Mittelbrunn M, The role of T cells in age-related diseases, *Nat. Rev. Immunol* 22 (2) (Feb 2022) 97–111, 10.1038/s41577-021-00557-4. [PubMed: 34099898]

- [40]. Hosseini S, Wilk E, Michaelsen-Preusse K, et al. , Long-term neuroinflammation induced by influenza A virus infection and the impact on hippocampal neuron morphology and function, *J. Neurosci* 38 (12) (Mar 21 2018) 3060–3080, 10.1523/jneurosci.1740-17.2018. [PubMed: 29487124]
- [41]. Jang H, Boltz D, McClaren J, et al. , Inflammatory effects of highly pathogenic H5N1 influenza virus infection in the CNS of mice, *J. Neurosci* 32 (5) (Feb 1 2012) 1545–1559, 10.1523/jneurosci.5123-11.2012. [PubMed: 22302798]
- [42]. Surana P, Tang S, McDougall M, Tong CY, Menson E, Lim M, Neurological complications of pandemic influenza a H1N1 2009 infection: European case series and review, *Eur. J. Pediatr* 170 (8) (Aug 2011) 1007–1015, 10.1007/s00431-010-1392-3.
- [43]. Espay AJ, Henderson KK, Postencephalitic parkinsonism and basal ganglia necrosis due to Epstein-Barr virus infection, *Neurology* 76 (17) (Apr 26 2011) 1529–1530, 10.1212/WNL.0b013e318217e7dd. [PubMed: 21519006]
- [44]. Woulfe J, Gray MT, Ganesh MS, Middeldorp JM, Human serum antibodies against EBV latent membrane protein 1 cross-react with alpha-synuclein, *Neurol. Neuroimmunol. Neuroinflamm* 3 (4) (Aug 2016), e239, 10.1212/NXI.0000000000000239. [PubMed: 27218119]
- [45]. Woulfe JM, Gray MT, Gray DA, Munoz DG, Middeldorp JM, Hypothesis: a role for EBV-induced molecular mimicry in Parkinson’s disease, *Parkinsonism Relat. Disord* 20 (7) (Jul 2014) 685–694, 10.1016/j.parkreldis.2014.02.031. [PubMed: 24726430]
- [46]. Bjornevik K, Cortese M, Healy BC, et al. , Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis, *Science* 375 (6578) (Jan 21 2022) 296–301, 10.1126/science.abj8222. [PubMed: 35025605]
- [47]. Fishman PS, Gass JS, Swoveland PT, Lavi E, Highkin MK, Weiss SR, Infection of the basal ganglia by a murine coronavirus, *Science* 229 (4716) (Aug 30 1985) 877–879, 10.1126/science.2992088. [PubMed: 2992088]
- [48]. Reynolds AD, Stone DK, Hutter JA, Benner EJ, Mosley RL, Gendelman HE, Regulatory T cells attenuate Th17 cell-mediated nigrostriatal dopaminergic neurodegeneration in a model of Parkinson’s disease, *J. Immunol* 184 (5) (Mar 1 2010) 2261–2271, 10.4049/jimmunol.0901852. [PubMed: 20118279]
- [49]. Cebriá C, Zucca FA, Mauri P, et al. , MHC-I expression renders catecholaminergic neurons susceptible to T-cell-mediated degeneration, *Nat. Commun* 5 (Apr 16 2014) 3633, 10.1038/ncomms4633. [PubMed: 24736453]
- [50]. Matheoud D, Cannon T, Voisin A, et al. , Intestinal infection triggers Parkinson’s disease-like symptoms in *Pink1*($-/-$) mice, *Nature* 571 (7766) (Jul 2019) 565–569, 10.1038/s41586-019-1405-y. [PubMed: 31316206]
- [51]. Matheoud D, Sugiura A, Bellemare-Pelletier A, et al. , Parkinson’s disease-related proteins PINK1 and Parkin repress mitochondrial antigen presentation, *Cell* 166 (2) (Jul 14 2016) 314–327, 10.1016/j.cell.2016.05.039. [PubMed: 27345367]
- [52]. Williams GP, Schonhoff AM, Jurkuvenaite A, Gallups NJ, Standaert DG, Harms AS, CD4 T cells mediate brain inflammation and neurodegeneration in a mouse model of Parkinson’s disease, *Brain* 144 (7) (Aug 17 2021) 2047–2059, 10.1093/brain/awab103. [PubMed: 33704423]
- [53]. Lanz TV, Brewer RC, Ho PP, et al. , Clonally expanded B cells in multiple sclerosis bind EBV EBNA1 and GlialCAM, *Nature* 603 (7900) (Mar 2022) 321–327, 10.1038/s41586-022-04432-7. [PubMed: 35073561]

**Fig. 1.**

Antigen specific T cell responses to common antigens in HC and PD patient's PBMCs. a-d) AIM responses (OX40⁺ CD137⁺) in CD4 T cells and e) CD8 T cells (CD69⁺ CD137⁺) after stimulation to previously defined antigenic peptide pools. PBMCs from HC (blue circles) and PD (red circles) were stimulated with 1 μ g/mL of NL63, 229E, HKU1, OC43, Flu-HA, Flu-Other, Rhinovirus, RSV, CMV, EBV, VZV, PT, or TT for 24 h, stained, and run on a Bio-Rad ZE5 flow cytometer. e) AIM responses (CD69⁺ CD137⁺) in CD8 T cells. Each dot represents an individual subject, HC $n = 19$, PD $n = 20$. Two-tailed Mann-Whitney test; geometric mean with standard deviation is displayed. Dashed line indicates threshold of positivity (TP). Numbers above groups indicate the test values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Fig. 2.**

T cell derived cytokine responses to common antigens in HC and PD patients PBMCs.

a-d) Intracellular cytokine responses in CD4⁺ T cells after stimulation to previously defined antigenic peptide pools. PBMCs from HC (blue circles) and PD (red circles) were stimulated with 1 μ g/mL of NL63, 228E, HKU1, OC43, Flu-HA, Flu-Other, Rhinovirus, RSV, CMV, EBV, VZV, PT, or TT for 24 h, stained, and run on a Bio-Rad ZE5 flow cytometer. Cells were gated on live, singlet CD4⁺ populations and then on different cytokine populations (a: IFN γ /CD40L⁺ b: TNF α /CD40L⁺, c: IL-2/CD40L⁺). Each dot represents an individual subject, PD $n = 20$, HC $n = 19$. Two-tailed Mann-Whitney test; geometric mean with standard deviation is displayed. Numbers above groups indicate the test values.

Table 1

Cohort general information.

	HC	PD	<i>p</i> value
	n = 19	n = 20	
Sex (male/female)			
Male	9	10	N/A
Female	10	10	N/A
Age (mean, standard deviation-SD):			
Total	65.00 ± 5.98	65.40 ± 5.96	0.83
Male	64.11 ± 5.15	65.10 ± 5.78	0.7
Female	65.80 ± 6.81	65.70 ± 6.47	0.97
Caucasian (%):	89 (17/19)	85 (17/20)	
Time Since PD Diagnosis (mean, range):			
Total	N/A	8.05 (1–28)	
Male	N/A	7.90 (1–18)	
Female	N/A	8.20 (2–28)	
Unified Parkinson's Disease Rating Scale (UPDRS) Part III ^a (mean, SD):			
Total	N/A	18.5 ± 5.73	
Male	N/A	17 ± 6.96	
Female	N/A	21 ± 1.73	
Montreal Cognitive Assessment (MoCA) Score ^b (mean, SD):			
Total	N/A	25.36 ± 2.46	
Male	N/A	24.08 ± 2.06	
Female	N/A	25.36 ± 3.27	

N/A: Not applicable.

^aUPDRS Part III collected at CUMC, UAB, RUMC, and UCSD.^bMoCA collected for PD at CUMC, LJI, and UCSD.

Table 2

Antigen pools compositions.

Peptide pool	Group	No. of peptides	Reference
Coronavirus (NL63)	Common Coronavirus	270	[22]
Coronavirus (229E)	Common Coronavirus	233	[22]
Coronavirus (HKU1)	Common Coronavirus	270	[22]
Coronavirus (OC43)	Common Coronavirus	271	[22]
Influenza, Hemagglutinin (Flu-HA)	Lower Respiratory Tract Virus	161	[23]
Influenza, non-HA (Flu-Other)	Lower Respiratory Tract Virus	169	[23]
Rhinovirus	Tract Virus Lower Respiratory	136	[24]
Respiratory Syncytial Virus (RSV)	Lower Respiratory Tract Virus	216	[25]
Cytomegalovirus (CMV) *	Chronic Virus	313	[26]
Epstein-Barr Virus (EBV) *	Chronic Virus	355	[27]
Varicella Zoster Virus (VZV)	Chronic Virus	424	[28]
<i>Bordetella Pertussis</i> (PT)	Vaccine Antigen	132	[29]
Tetanus Toxoid (TT)	Vaccine Antigen	125	[30]

* Contains both class I and II peptides.