Laidlaw Scholars Undergraduate Leadership and Research Programme

Research Report

Cross-Species Transcriptomic and Tissue Evidence of Peripheral Immune Dysregulation in Parkinson's Disease

Viktoria Springer

Research Advisor: Dr. Olga Rojas

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Introduction

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease, and its prevalence is projected to double over the next 30 years (Dorsey, et. al, 2018). PD is characterized by the progressive loss of dopaminergic neurons in the substantia nigra, leading to motor symptoms such as tremors, rigidity, and bradykinesia. The main pathological feature is the accumulation of alpha-synuclein protein (Joers et.al, 2022) in the brain (Poewe, et. al, 2017).

Although traditionally considered a neurodegenerative disorder that affects peripheral organs as well as the central nervous system, PD is a multisystem disorder with a wide range of non-motor symptoms such as gastrointestinal dysfunction (Joers et.al, 2022). Growing evidence suggests that factors outside the brain may also contribute to its onset and progression. In particular, the immune system has emerged as a potential driver of PD pathogenesis and progression (Joers et. al, 2022).

Background

Although Parkinson's Disease (PD) has typically been studied through the lens of neuronal loss and α-synuclein pathology, a growing body of work has shifted attention to the immune system as a key player in disease pathogenesis. Neurohistological and neuroimaging studies support the presence of neuroinflammation in PD. Both innate and adaptive immune responses appear to be altered in PD. For instance, elevated levels of circulating inflammatory cytokines such as interleukin-2, interleukin-6, tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ) have been reported in PD patients, suggesting systemic immune activation (Qin et al., 2016). At the genetic level, genome-wide association studies have identified susceptibility loci within immune-related genes, further implicating immune dysfunction in PD (Pierce et. al, 2017).

Although the role of B cells is not as well understood, B cells are also altered in PD. Naïve B cells and plasma cells have been found to shift in abundance, which may indicate dysregulated antibody production or altered antigen presentation (Xiong et al., 2024). These findings suggest that PD involves broader adaptive immune remodeling beyond T cells alone.

Recent studies have highlighted that immune changes may extend beyond the circulation to lymphoid organs and CNS-adjacent tissues. The spleen and gut are major immunological sites that may reflect systemic dysregulation, and the dura mater has recently been recognized as an

immunologically active hub shielding the brain in health and disease (Alves de Lima et al., 2020). This discovery opens the possibility that immune remodeling in PD is not restricted to brain parenchyma but also involves peripheral and meningeal niches.

Despite these advances, many questions remain. It is unclear how transcriptional signatures observed in human PBMCs translate into functional immune remodeling in peripheral tissues. Furthermore, the relationship between cytotoxic $CD8^+$ T cells, B cell alterations, and lymphoid architecture in PD remains poorly defined. Addressing these gaps requires both computational analysis of human data and experimental validation in preclinical models.

Hypothesis:

Based on the existing evidence, I hypothesized that Parkinson's Disease (PD) involves peripheral immune dysregulation characterized by the expansion of cytotoxic $CD8^+$ T cells and alterations in B cell subsets, which can be detected not only in circulation but also within peripheral tissues such as lymphoid tissues and the gut.

Objectives

This project had two main objectives. First, to characterize immune cells in human PD vs controls using publicly available single-cell transcriptomic datasets of peripheral blood mononuclear cells (PBMCs). Second, to validate these findings in a preclinical PD mouse model using flow cytometry and immunofluorescence to examine the dura mater, spleen, and gut.

Methods

Transcriptomic analysis

Publicly available single-cell RNA sequencing (scRNA-seq) datasets of PBMCs from PD patients and healthy controls were analyzed (Xiong et al., 2024). Using Seurat R toolkit v5 (Stuart et al., 2019), quality control and normalization were performed before dimensionality reduction and clustering using UMAP. Clusters were annotated based on canonical markers of T cells, B cells, and plasma cells. Differential abundance testing identified clusters enriched in PD, while differential gene expression analysis determined genes differentially regulated in PD versus healthy control samples. The disease groups were determined using the Hoehn-Yahr scale and were stratified as Early (Stage I-II) and Late (Stage III-IV) PD. This disease classification

aligns with both clinical symptoms and disease burden. Importantly, Xiong et al. validated these categories in a larger 42-patient cohort, adding confidence to staging framework.

Preclinical mouse model and tissue collection

To investigate peripheral immune alterations in Parkinson's Disease (PD), a preclinical mouse model was utilized based on overexpression of human α-synuclein carrying the pathogenic A53T mutation. The A53T variant was delivered via an adeno-associated viral (AAV) vector directly into the substantia nigra of the mouse brain, a well-established approach to induce progressive α-synuclein aggregation and PD-like pathology in vivo (Luk et al., 2012; Thakur et al., 2017). Overexpression of mutant A53T α-synuclein in this model results in progressive neuronal dysfunction accompanied by immune alterations that resemble key features of human PD. Control animals were injected with a non-α-synuclein AAV construct (empty vector). All animal procedures were approved in accordance with established ethical animal guidelines at University Health Network.

At experimental endpoints, mice were deeply anesthetized and perfused transcardially with ice-cold phosphate-buffered saline (PBS) to clear circulating blood cells and reduce nonspecific immune background. Following perfusion, the dura mater, spleen, and gut were carefully dissected. Dura mater samples were enzymatically digested using collagenase and DNase to obtain single-cell suspensions for downstream flow cytometry. The spleen and gut were embedded in optimal cutting temperature (OCT) compound, rapidly frozen in isopentane on dry ice, and stored at −80 °C. Cryosections 9 µm thick were cut, mounted on charged glass slides, and prepared for immunofluorescence staining. These approaches allowed for interrogation of immune cell phenotypes in CNS-adjacent tissue (dura mater) as well as in secondary lymphoid and mucosal organs (spleen and gut).

Flow cytometry

Cells isolated from the dura mater were stained with fluorophore-conjugated antibodies against CD3, CD4, and CD8 to identify total T cells, helper T cells, and cytotoxic T cells, respectively. Flow cytometry was performed on a Beckman Coulter CytoFLEX S (5-laser configuration), and data were analyzed using FlowJo v11 (Tree Star). Frequencies of $CD4^+$ and $CD8^+$ T cell subsets were quantified and compared between control and α-synuclein–injected animals.

Immunofluorescence

Immunofluorescence staining was performed to visualize immune architecture in spleen and gut sections. In the spleen, antibodies against B220, CD4, CD138 were used to label B cell follicles, T helper cells, plasma cells, and germinal center B cells. In the gut, CD8 and B220 were used to examine lymphocyte distribution in the small and large intestines. Nuclei were counterstained with DAPI. Slides were imaged using a ZEISS LSM 880 confocal microscope (Carl Zeiss, Oberkochen, Germany) and processed using ZEN Blue software. Differences between PD and control tissues were qualitatively assessed with a focus on follicular organization and lymphocyte localization.

Table 1. Antibodies used for flow cytometry and immunofluorescence staining.

Marker	Clone	Fluorophore	Company	Catalog #
CD3	17A2	APC	BioLegend	100236
B220	RA3-6B2	AF647	BioLegend	103226
CD4	GK1.5	AF488	BioLegend	100423
CD138	281-2	PE	BioLegend	142504
IgD	11-26c.2a	APC	BioLegend	405714
B220	RA3-6B2	PE	BioLegend	103207
GL7	GL7	AF488	BioLegend	144611
IgA	11-44-2	FITC	SouthernBiotech	1165-02
CD8	53-6.7	AF647	BioLegend	100724
IgG	SB77e	FITC	SouthernBiotech	1144-02

Results

Transcriptomic Analysis of PBMCs

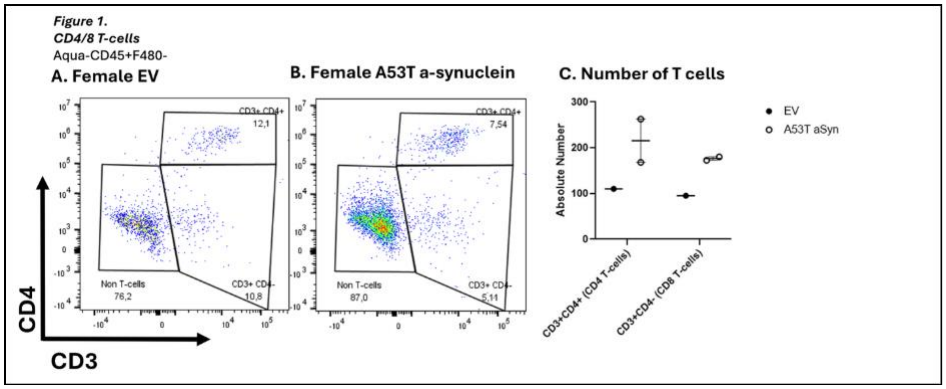
Single-cell transcriptomic analysis of PBMCs revealed distinct immune populations across PD and control samples. In PD, there was a significant enrichment of $CD8^+$ T cell subclusters with cytotoxic transcriptional profiles. $CD8^+$ T cells are specialized immune cells sometimes referred to as “killer T cells” because they destroy infected or damaged cells. While this function is normally protective, excessive activation of these cells can drive chronic inflammation. Differential expression analysis demonstrated upregulation of *CD8B*, *GZMA*,

GZMH, and *FGFBP2*. These genes are hallmarks of cytotoxic activity: *GZMA* and *GZMH* encode granzymes, enzymes that CD8+ T cells use to kill target cells, while *FGFBP2* is associated with highly active and proliferative CD8+ T cells that have been linked to neuroinflammatory states (Xiong et al., 2024).

In addition to T cells, alterations were observed in B cell compartments. In early PD patients (using advanced PD patients as the reference group), distinct shifts were identified across B cell subsets. Specifically, two subsets of naïve B cells (naïve B1 and naïve B2) were reduced in frequency, while plasma cells were significantly increased. B cells typically play a central role in producing antibodies and shaping long-term immune memory, and such redistribution of B cell subsets suggests a skewing toward terminal differentiation. The observed increase in plasma cells may indicate heightened antibody production, while the reduction in naïve B cells may reflect impaired replenishment of the B cell pool. Together, these findings suggest that the adaptive immune system is broadly altered in PD, with possible consequences for antigen presentation and autoantibody generation.

Flow Cytometry of Dura Mater

Flow cytometry confirmed the presence of $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells in the dura mater of α-synuclein–injected mice. CD4+ T cells, also known as "helper T cells," coordinate the immune response, while CD8+ T cells carry out direct killing functions. These findings suggests that immune alterations in PD extend beyond the blood and into CNS-adjacent structures. Since the dura mater has recently been recognized as an immunologically active tissue, the presence of cytotoxic T cells in this compartment raises the possibility that meningeal immunity may directly influence brain pathology.



Flow cytometric identification of $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells in the dura mater of α-synuclein-injected mice.

Immunofluorescence of Spleen

Immunofluorescence analysis revealed structural disruptions in the spleens of PD mice (A35T aSyn-AVV) than in controls (Empty vector (EV)). Normally, the spleen contains organized follicles where B cells and T cells interact to generate effective immune responses. In PD, this organization was altered, with $B220^+$ displaying a more diffuse pattern. Quantification revealed sex-dependent changes in follicle architecture; female PD spleens had slightly smaller average follicle areas (8,300 μm^2) compared to controls (11,700 μm^2), whereas male PD spleens exhibited markedly enlarged follicles (19,888 μm^2 vs. 10,889 μm^2 in controls). Follicle densities were relatively stable across groups, approximately 6-7/mm^2 in females and 7-8/mm^2 in males. Additionally, $CD138^+$ plasma cells appeared more abundant in PD spleens, consistent with enhanced antibody production. Collectively, these observations indicate that the adaptive immune system in PD is altered both structurally and quantitatively, with male mice showing pronounced follicle enlargement and females showing subtle changes in follicle size accompanied by modest increases in fluorescence intensity.

Immunofluorescence of Gut

Analysis of intestinal tissue demonstrated altered immune cell distribution in both the small and large intestines of PD mice. $CD8^+$ T cells were more abundant and widely distributed, while $B220^+$ B cells appeared to have altered localization compared to controls. Quantification highlighted sex- and region-specific changes: IgA^+ cell density increased markedly in female PD mice in both the LI (from 116 to 141 cells/mm^2) and SI (from 160 to 360 cells/mm^2), whereas male PD mice exhibited a smaller increase in LI (95 to 116 cells/mm^2) and a decrease in SI (273 to 173 cells/mm^2). $CD8^+$ cell density followed a similar pattern, with female PD mice showing pronounced increases in LI (171 to 447 cells/mm^2) and SI (175 to 261 cells/mm^2), while male PD mice showed modest increases in SI (150 to 242 cells/mm^2) and slight decreases in LI (318 to 272 cells/mm^2). These results demonstrate sex- and region-specific immune alterations in the gut, highlighting the female PD intestine as a site of substantial IgA^+ and $CD8^+$ cell expansion. The redistribution and increased number of lymphocytes further underscore systemic immune dysregulation in PD and support the emerging role of the gut in PD pathogenesis via the gut-brain axis.

Discussion and Conclusion

This study provides compelling evidence that Parkinson's Disease (PD) is associated with widespread peripheral immune dysregulation, as demonstrated through both human transcriptomic analyses and preclinical mouse models. Quantitative analysis of immunofluorescence (IF) images from spleen tissue revealed significant alterations in immune cell populations. In female PD mice, there was a notable increase in the number of CD8+ T cells per unit area, with a mean of 272.02 cells/mm², compared to 318.28 cells/mm² in male PD mice. This expansion was accompanied by elevated expression levels of activation markers such as GZMA and GZMH, suggesting a heightened cytotoxic profile of these cells. Additionally, the presence of FGFBP2, a marker associated with terminally differentiated effector CD8+ T cells, further supports the notion of chronic activation in these cells (Tansey et al., 2022).

In the gut, both small and large intestines exhibited increased densities of CD8+ T cells in PD mice. Quantitative data indicated that female PD mice had 447.44 cells/mm² in the large intestine and 261.28 cells/mm² in the small intestine, while male PD mice had 272.02 cells/mm² and 241.68 cells/mm², respectively. These findings align with previous studies linking CD8+ T cell expansion to neuroinflammatory conditions (Xu et al., 2023).

Furthermore, immunofluorescence analysis of the spleen revealed disrupted organization, with a decrease in the average follicle area in PD mice (8,293.28 μm²) compared to controls (11,699.57 μm²). This structural alteration was accompanied by an increase in plasma cell populations, as indicated by CD138+ and IgA+ staining, suggesting an adaptive immune response potentially contributing to systemic inflammation.

Collectively, these results underscore the hypothesis that peripheral immune activation contributes to PD pathogenesis. The expansion and activation of cytotoxic CD8+ T cells, along with alterations in B cell and plasma cell populations, suggest a complex interplay between innate and adaptive immune responses, involving antibody production or antigen presentation. The observed immune changes in both the spleen and gut highlight the systemic nature of immune dysregulation in PD and point to the importance of peripheral immune compartments in disease progression. These findings have significant implications for understanding the role of peripheral immunity in neurodegenerative diseases and may inform future therapeutic strategies targeting immune pathways to mitigate disease progression. Additionally, the presence of immune changes in the dura mater underscores the importance of meningeal immunity as a

frontier for understanding how peripheral immune cells may interact with the CNS in neurodegenerative disease (Alves de Lima et al., 2020).

Future Directions

Future studies will aim to validate the murine findings in human peripheral organs, such as the spleen, gut, and dura, thereby strengthening translational relevance. Increasing the sample size in both human and preclinical cohorts will be critical to further evaluate the potential sex differences observed in the current quantifications, enabling more definitive conclusions regarding sex-specific immune alterations in PD. Functional characterization of $CD8^+$ T cell subsets is also planned, with a particular focus on determining whether these cells exhibit an IL-7Rα^low phenotype, which has been associated with highly activated but short-lived effector cells (Young et al., 2023). Gain- and loss-of-function approaches using preclinical PD models will be employed to delineate the contributions of $CD8^+$ T cells and B/plasma cells to PD progression. Finally, immune dysregulation in the meningeal niche will be further investigated to clarify how dura mater immunity influences neurodegeneration.

Acknowledgments

This research was supported by the Laidlaw Foundation. I extend my sincere gratitude to my research advisor, Dr. Olga Rojas, for her invaluable guidance and mentorship throughout the course of this project. I also acknowledge the University of Toronto Laidlaw Scholars Programme for its support and for providing the resources and framework that made this research possible.

Figure 1. Differential Gene Expression in Human PBMCs of PD Patients

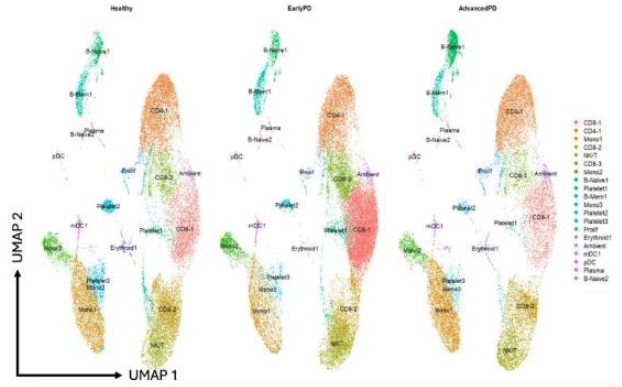


Figure 1a. UMAP of PBMC Clusters by Disease Stage.

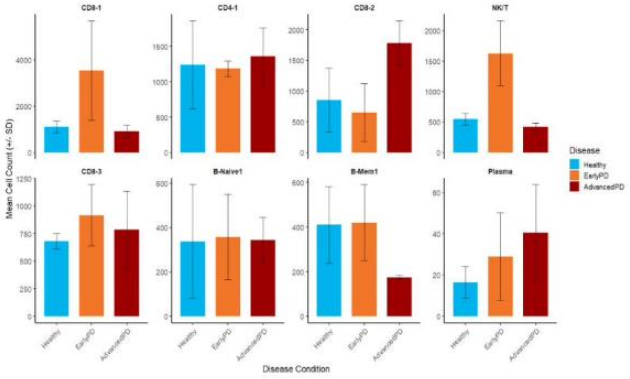


Figure 1b. Lymphoid Cell Cluster Abundance Across Disease States.

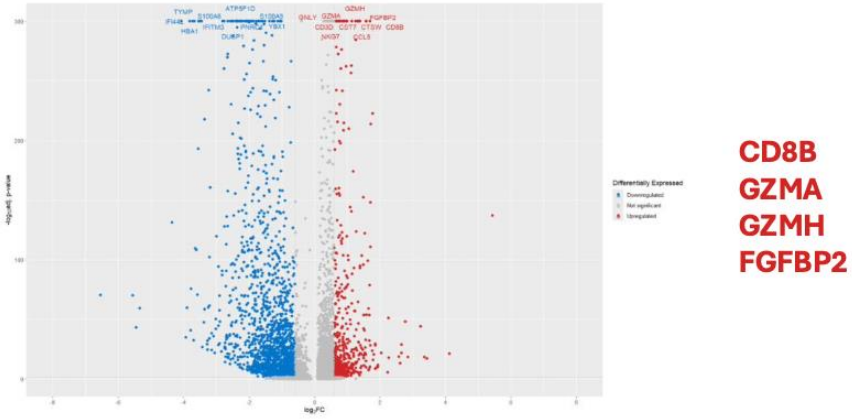


Figure 1c. Volcano plot showing differentially upregulated genes in Early PD with Advanced PD as the reference group. Notable upregulated genes include CD8B, GZMA, GZMH, and FGFBP2, consistent with expansion and activation of cytotoxic $CD8^+$ T cells in PD. FGFBP2 is a marker of highly cytotoxic, proliferative CD8 T cells and has been implicated in neuroinflammatory states. (Xiong et al., 2024)

Figure 2. Altered Splenic Follicle Architecture in α-Synuclein Mice

EV = empty vector
α-Syn = AAV A53T α-Synuclein

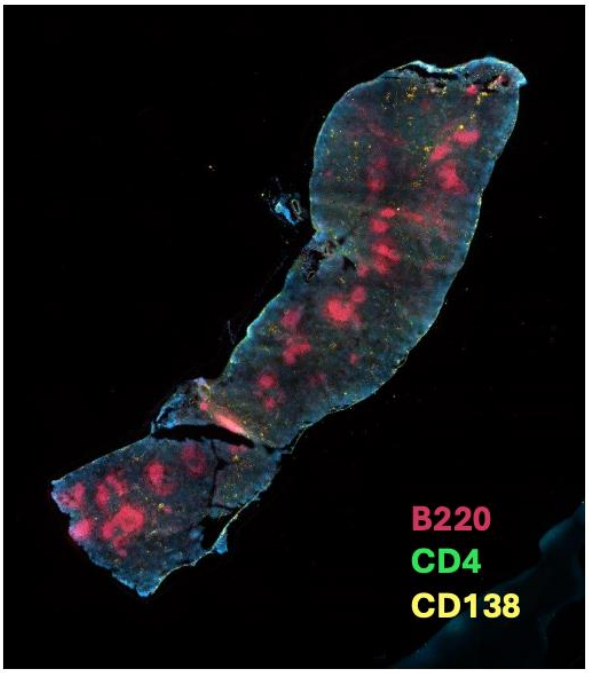


Figure 2a. Spleen 10x EV.

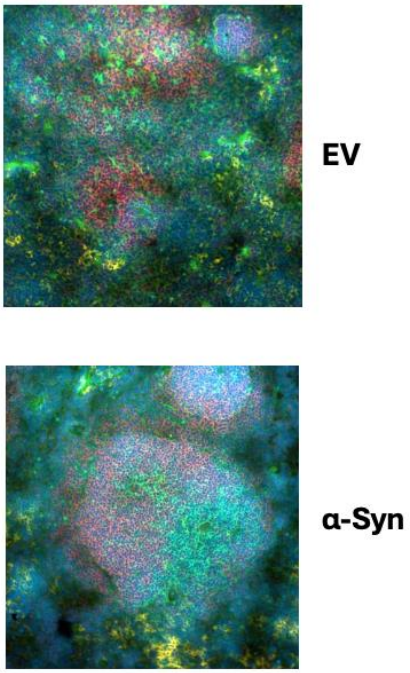


Figure 2b. Spleen 20x EV vs α-Syn.

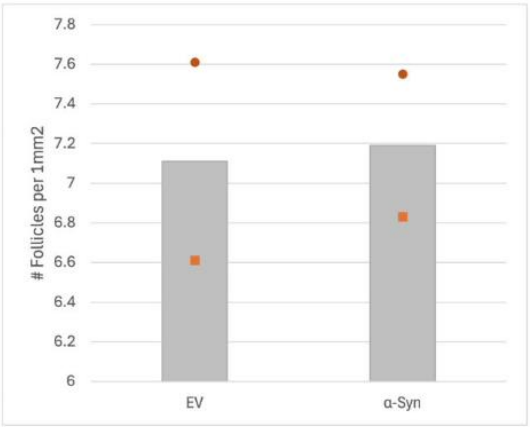


Figure 2c. Number of spleen follicles per 1mm^2. Squares depict females and circles depict males.

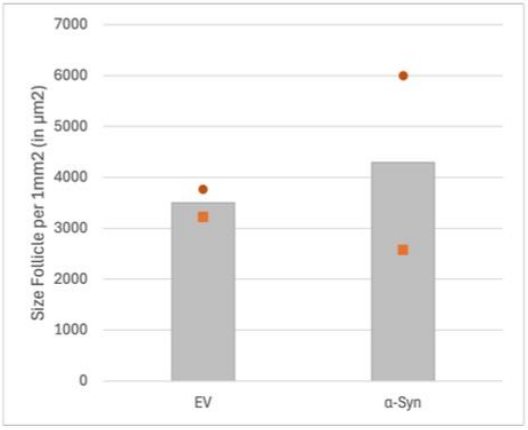


Figure 2d. Size of spleen follicles per 1mm^2, represented in µm^2. Squares depict females and circles depict males.

Figure 3. Small Intestine Immune Cell Alterations in α-Synuclein Mice

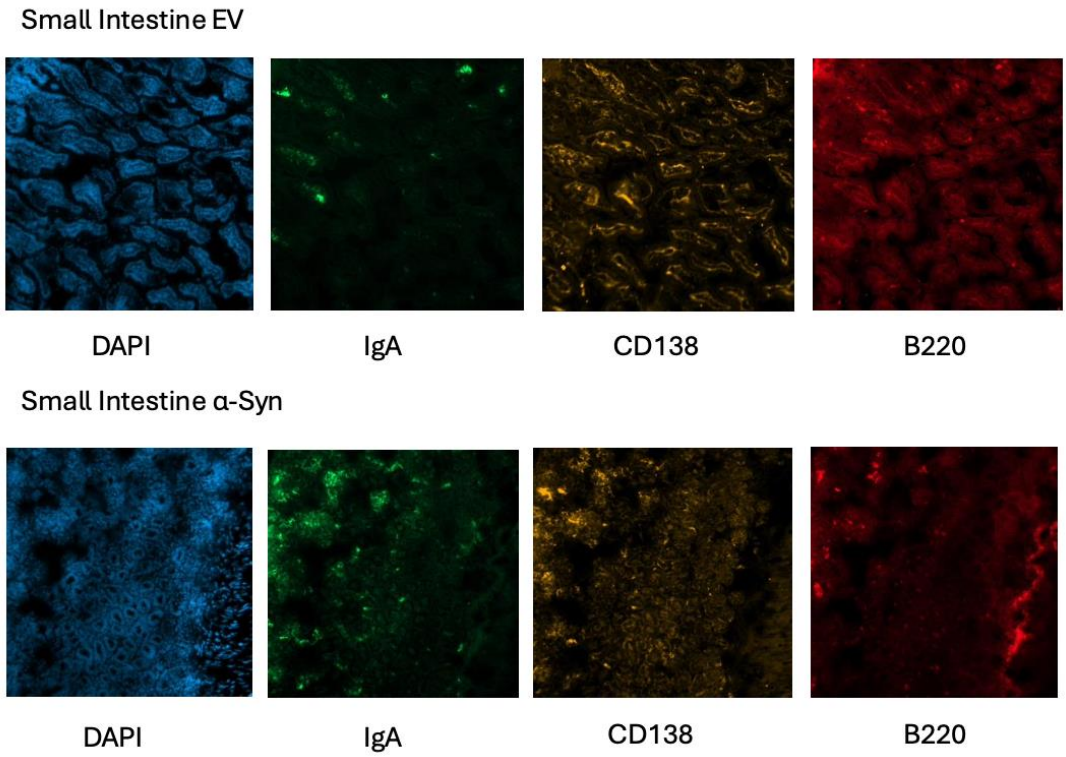


Figure 3a. Representative immunofluorescence images of IgA and plasma cells in small intestine of EV and α-Synuclein mice.

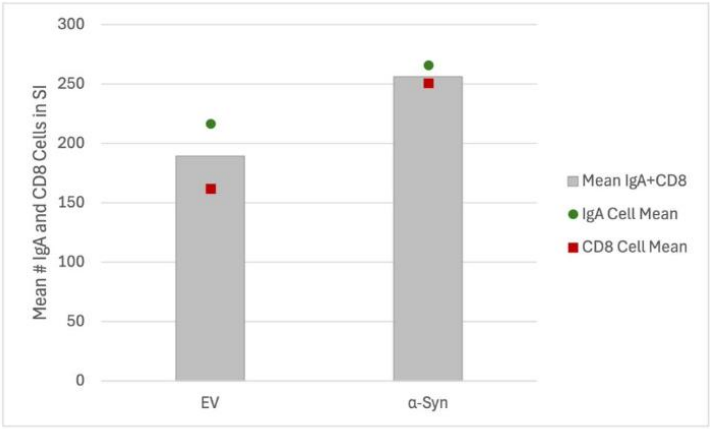


Figure 3b. Mean number of IgA and CD8 cells in EV and α-Synuclein groups of both sexes in small intestine. Mean IgA cells are depicted in green circles and mean CD8 cells are depicted in red squares.

Figure 4. Large Intestine Immune Cell Alterations in α-Synuclein Mice

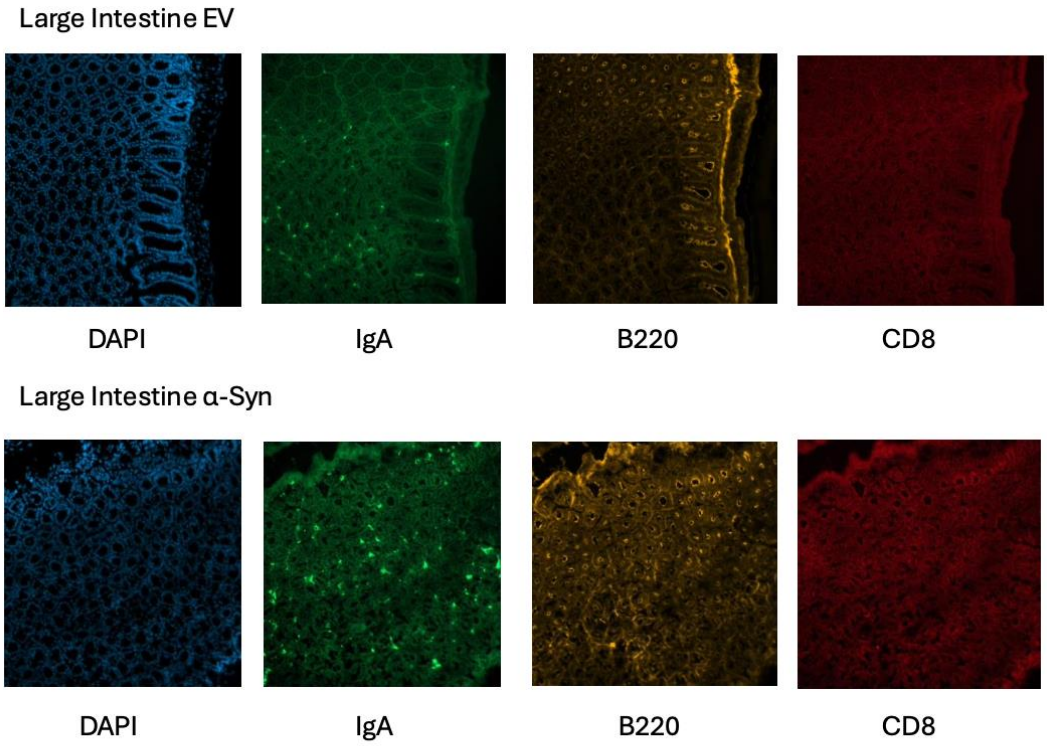


Figure 4a. Representative immunofluorescence images of IgA and CD8 T cells in large intestine of EV and α-Synuclein mice.

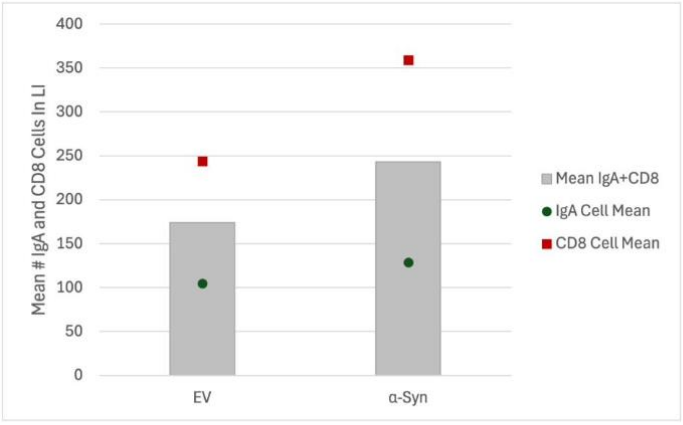


Figure 4b. Mean number of IgA and CD8 cells in EV and α-Synuclein groups of both sexes in large intestine. Mean IgA cells are depicted in green circles and mean CD8 cells are depicted in red squares.

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