Compartment Specific Immune Cells Expansion in Multiple Sclerosis

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1.Introduction

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (1) characterized by dysregulated immune responses and intrathecal antibody production. The presence of oligoclonal IgG bands in cerebrospinal fluid (CSF) highlights the importance of B-lineage cells, particularly antibody-secreting plasmablasts, in MS disease biology. (2-3)

However, understanding which immune cells expand, where they expand, and how their function changes across compartments is essential for understanding the mechanisms underlying disease persistence. A cell population may be present in both blood and CSF, yet acquire distinct functional states within the CNS microenvironment. (4)

Therefore I asked whether the immune alterations associated with MS represent a systemic immune response or a compartment-specific CNS process, with a particular focus on plasmablasts.

2.Methods

Raw 10x Genomics single-cell RNA-seq data from GSE138266 were analysed, including 22 samples collected from cerebrospinal fluid (CSF) and peripheral blood mononuclear cells (PBMCs) of 6 patients with multiple sclerosis (MS) and 6 individuals with idiopathic intracranial hypertension (IIH) as controls.

Quality control was performed separately for each sample using an adaptive median absolute deviation (MAD)-based approach to remove low-quality cells. The filtered data were normalised using SCTransform v2 and integrated using a CCA-based integration approach in Seurat v5.

Cell types were identified using established canonical marker genes and independently annotated using SingleR with the Monaco Immune Cell reference. Cell annotations were examined at both broad and fine levels of cell-type resolution.

To identify differences in cell abundance between MS and control samples, neighbourhood-level differential abundance analysis was performed using Milo, with spatial false discovery rate (FDR) correction. Differences in cell-type proportions were also assessed at the donor level using Wilcoxon tests.

Plasmablast-Specific Analysis and Independent Validation

Plasmablasts were identified in the single-cell RNA-seq dataset, with the population detected specifically in the CSF of patients with MS. Because plasmablasts were not detected in the control or blood compartments, a direct differential expression analysis of plasmablasts between MS and control samples was not statistically appropriate.

To investigate the transcriptional profile of MS-associated plasmablasts, an independent bulk RNA-seq dataset (GSE133028) containing flow-sorted plasmablasts from CSF and peripheral blood was analysed. Differential gene expression analysis was performed using DESeq2, using the paired study design and patient identity as a blocking factor.

The significantly upregulated genes identified in the CSF plasmablasts were subsequently used for functional characterisation. Gene Ontology (GO), KEGG, and Reactome pathway enrichment analyses were performed to identify biological processes and molecular pathways associated with the CSF plasmablast transcriptional signature.

To further investigate the cellular origin of the bulk RNA-seq signature, CIBERSORTx was used to generate a reference signature matrix from the single-cell RNA-seq data. This signature matrix was used to assess the contribution of individual cell populations to the transcriptional profiles observed in the bulk samples.

Together, this approach allowed the plasmablast-associated transcriptional program to be characterised using an independent bulk dataset while avoiding an invalid cell-level comparison in the single-cell dataset.

3. Results

3.1 MS-associated compositional changes are specific to the CSF

Milo differential abundance analysis identified significant changes in 169 of 2,514 neighbourhoods in the MS CSF samples (spatial FDR < 0.1). These changes were characterised by depletion of monocytes and dendritic cells and expansion of B-lineage cells, including plasmablasts. In contrast, the same analysis of paired PBMC samples identified no significant neighbourhoods (0/2,729), indicating that the observed compositional changes were specific to the CSF compartment.

This result was further supported by donor-level analysis of cell-type proportions, with no significant differences detected across PBMC cell populations (minimum nominal p = 0.22). TFH cells, which are known to support B-cell and plasmablast differentiation, were significantly expanded in MS CSF (Wilcoxon rank-sum test, p = 0.015), consistent with the main finding reported in the original study.



Figure 1: UMAP of cell clustering across CSF and blood samples; Milo differential abundance reveals plasmablasts and B cells enriched in MS CSF compared to control.

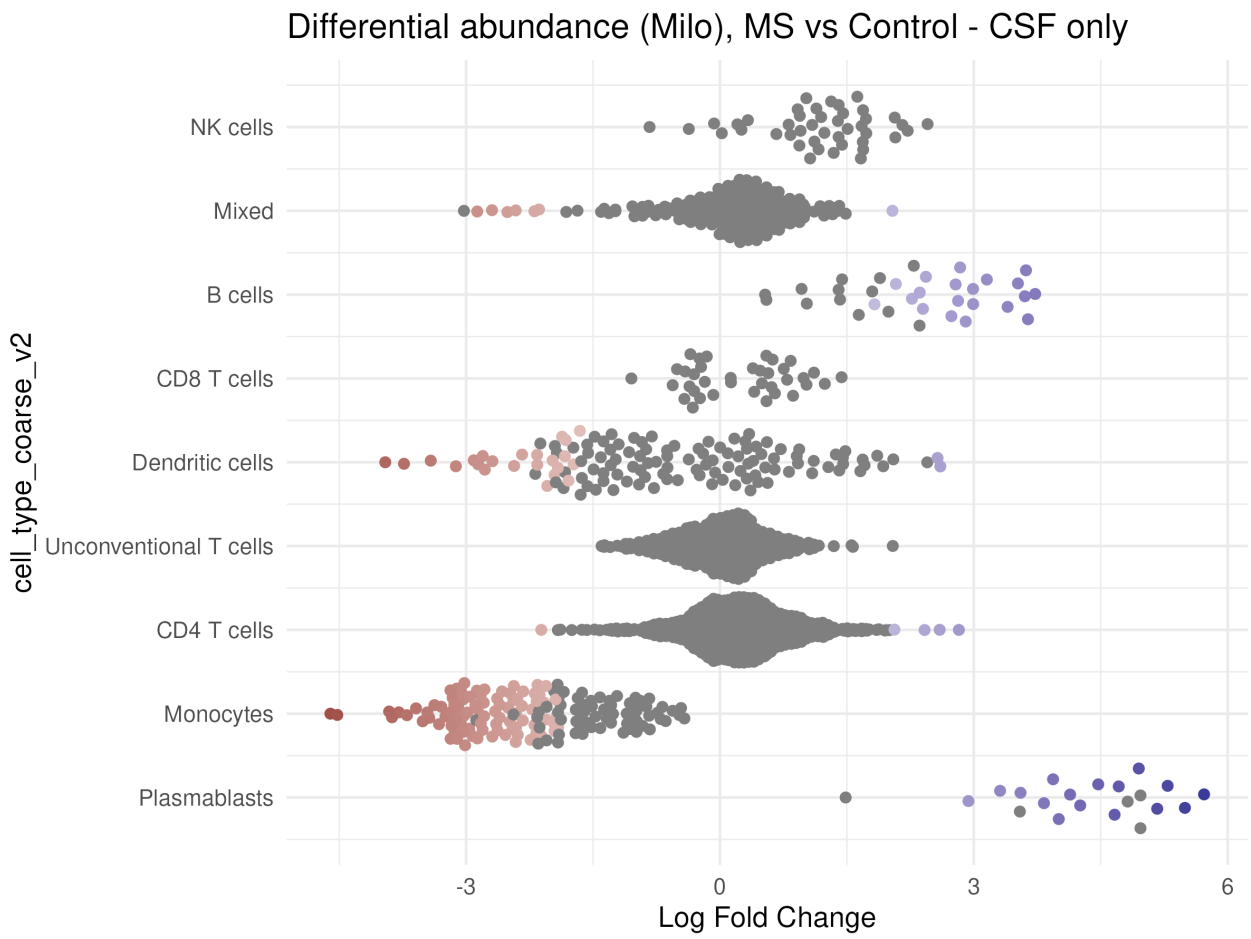


Figure 2: Milo analysis show a differential abundance of plasmablast and B-cell in MS CSF compared with the control group.

3.2 Plasmablast expansion is associated with MS CSF

Plasmablasts were detected in all six MS-CSF donors but were almost completely absent from control CSF, with only one plasmablast detected across all control samples. At the available sample size, the comparison reached the minimum possible p-value. To determine whether this represented a general difference in plasmablast abundance between CSF and blood, plasmablast proportions were compared between paired CSF and PBMC samples from the same MS donors. No significant difference was observed ($p = 0.31$, $n = 5$). This suggests that the major difference is associated with disease status within the CSF, rather than simply reflecting preferential localisation of plasmablasts to CSF compared with blood.

3.3 Independent bulk RNA-seq validation

Because plasmablasts were essentially absent from control CSF, a direct MS-versus-control differential expression analysis within the single-cell plasmablast population was not statistically appropriate. Therefore, an independent bulk RNA-seq dataset (GSE133028) containing flow-sorted CSF and blood plasmablasts was analysed using DESeq2 with patient identity included as a blocking factor.

All core plasma-cell identity genes examined, including MZB1, JCHAIN, XBP1, IGHG1/2/3, SEC61B, PDIA4, CD38, and PRDM1, showed substantial expression in the independently sorted plasmablast samples, supporting the biological identity of the analysed population.
Comparison of CSF and blood plasmablasts identified 441 significantly differentially expressed genes. Unsupervised PCA and hierarchical clustering based on the full transcriptome clearly separated CSF and blood plasmablast samples, with samples clustering according to their anatomical compartment.

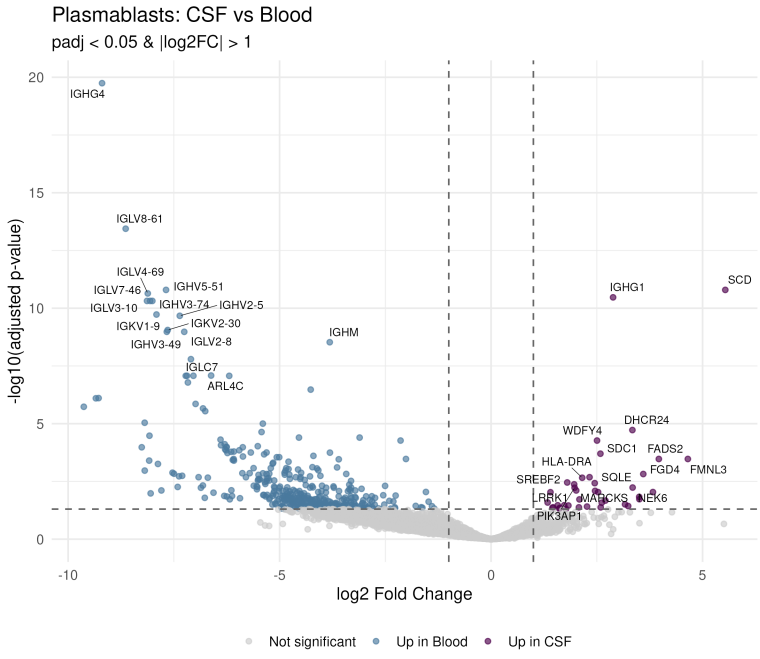


Figure3: differentially expressed genes in CSF vs blood in MS bulk RNA-sequence data

3.4 Plasmablast identity is distinct from conventional B cells

To further validate the plasmablast identity, plasmablasts were compared with B cells from the same patients in the independent bulk dataset. The paired DESeq2 analysis identified 922 significantly differentially expressed genes (FDR < 0.05).

Plasmablasts showed increased expression of genes involved in endoplasmic-reticulum function and protein secretion, including XBP1, HSPA5, HSP90B1, PDIA4, SEC61B/G, SEC11C, and SSR3/4, together with increased expression of immunoglobulin-secretion genes such as IGHG2/3 and MZB1. In contrast, B cells showed higher expression of genes associated with B-cell identity and antigen presentation, including MS4A1, BANK1, CD37, and HLA-DRA. These transcriptional differences support the annotation of the single-cell population as plasmablasts.

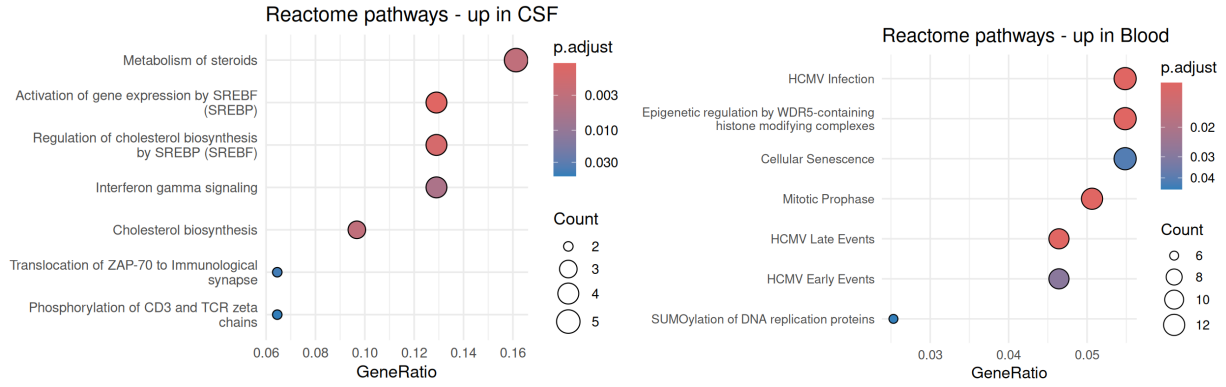


Figure4: pathway analysis of plasmablast unregulated genes in CSF and blood

3.5 CSF and blood plasmablasts show distinct transcriptional programs

Although plasmablast abundance did not differ significantly between CSF and PBMC within MS patients, their transcriptional profiles differed substantially between compartments. Functional enrichment analysis of the 441 differentially expressed genes using Gene Ontology, KEGG, and Reactome identified distinct biological programs. Genes upregulated in CSF plasmablasts were enriched for cholesterol and fatty-acid biosynthesis. Reactome analysis identified SREBF/SREBP-regulated pathways, including activation of gene expression by SREBF and regulation of cholesterol biosynthesis by SREBP (adjusted $p < 0.01$). Key genes contributing to this signature included SQLE, DHCR24, SCD, and FADS2.

CSF plasmablasts also showed increased expression of antigen-presentation genes, including HLA-DRA, HLA-DQB1, and CIITA, as well as the signalling kinase PRKCD.

In contrast, blood plasmablasts showed stronger enrichment of immunoglobulin-related and adaptive immune-response pathways, with enrichment significance reaching an adjusted p-value of approximately 1×10^{-26}.

Finally, we tested whether the CSF lipid-associated transcriptional program could be explained by increased antibody-secretory activity. The relationship between the lipid signature and immunoglobulin transcript fraction was not significant (Wilcoxon test, $p = 0.70$). Therefore, the relationship between the CSF lipid program and antibody secretion remains unresolved and requires further experimental investigation.

4. Discussion

This study shows that immune changes in MS are concentrated in the CSF, with expansion of B-lineage cells and plasmablasts and increased TFH cells. Because plasmablasts were largely absent from control CSF, their disease-associated transcriptional changes could not be directly tested by single-cell differential expression. Independent bulk RNA-seq instead confirmed that CSF and blood plasmablasts have distinct transcriptional programs.

CSF plasmablasts showed enrichment of lipid and cholesterol biosynthesis pathways, particularly SREBF/SREBP-regulated pathways, together with increased antigen-presentation genes. These findings suggest that plasmablasts may adopt a distinct functional state within the CSF rather than simply increasing in number. However, the lack of association between the lipid signature and immunoglobulin transcript fraction means that its role in antibody secretion remains unclear.

Overall, the findings support compartment-specific plasmablast biology in MS and highlight lipid metabolism, particularly SREBP-associated pathways, as a potential mechanism for further investigation. Larger patient cohorts and experimental studies are needed to determine whether this metabolic program contributes to plasmablast function and whether it could be therapeutically targeted.

5. Limitations and Future Work

Limitations

This study has several limitations. First, the single-cell analysis included a relatively small number of donors, which limits statistical power and requires validation in larger cohorts. Second, plasmablasts were almost absent from control CSF, preventing a direct differential expression comparison between MS and control plasmablasts. Therefore, the transcriptional analysis relied on an independent bulk RNA-seq dataset. In addition, the observed pathway associations are based on transcriptomic data and cannot establish causality.

Future Work

Future studies should validate the plasmablast and SREBP-associated signature in larger and independent MS cohorts. Experimental studies should then investigate whether the identified lipid-metabolic pathways, particularly SREBP signalling, directly influence plasmablast survival, differentiation, or antibody production. This could help determine whether the identified pathway represents a functional mechanism of MS-associated plasmablast biology and whether it could provide a potential target for therapeutic intervention.

6. Conclusion

Plasmablasts in MS show distinct compartment-specific biology. Although their relative abundance was comparable between CSF and blood, MS CSF plasmablasts were almost absent in controls and displayed a distinct transcriptional program enriched in SREBP-regulated lipid and cholesterol metabolism and antigen presentation. These findings suggest that plasmablast function, rather than abundance alone, differs between compartments, highlighting SREBP-associated metabolism as a potential mechanism for further investigation.

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References:

1. Schafflick, D., Xu, C. A., Hartlehnert, M., Cole, M., Schulte-Mecklenbeck, A., Lautwein, T., Wolbert, J., Heming, M., Meuth, S. G., Kuhlmann, T., Gross, C. C., Wiendl, H., Yosef, N., & Meyer Zu Horste, G. (2020). Integrated single cell analysis of blood and cerebrospinal fluid leukocytes in multiple sclerosis. *Nature communications*, *11*(1), 247. https://doi.org/10.1038/s41467-019-14118-w

2. Ramesh, A., Schubert, R. D., Greenfield, A. L., Dandekar, R., Loudermilk, R., Sabatino, J. J., Jr, Koelzer, M. T., Tran, E. B., Koshal, K., Kim, K., Pröbstel, A. K., Banerji, D., University of California, San Francisco MS-EPIC Team, Guo, C. Y., Green, A. J., Bove, R. M., DeRisi, J. L., Gelfand, J. M., Cree, B. A. C., Zamvil, S. S., … Wilson, M. R. (2020). A pathogenic and clonally expanded B cell transcriptome in active multiple sclerosis. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(37), 22932–22943. https://doi.org/10.1073/pnas.2008523117

3. Straeten, F., Zhu, J., Börsch, A. L., Zhang, B., Li, K., Lu, I. N., Gross, C., Heming, M., Li, X., Rubin, R., Ouyang, Z., Wiendl, H., Mingueneau, M., & Meyer Zu Hörste, G. (2022). Integrated single-cell transcriptomics of cerebrospinal fluid cells in treatment-naïve multiple sclerosis. *Journal of neuroinflammation*, *19*(1), 306. https://doi.org/10.1186/s12974-022-02667-9

4. Alvermann, S., Hennig, C., Stüve, O., Wiendl, H., & Stangel, M. (2014). Immunophenotyping of cerebrospinal fluid cells in multiple sclerosis: in search of biomarkers. *JAMA neurology*, *71*(7), 905–912. https://doi.org/10.1001/jamaneurol.2014.395

Dataset

scRNA-seq data: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE138266

Bulk RNA-seq: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE133028