B Cell Compartmental Features and Molecular Basis for Therapy in Neuromyelitis Optica Spectrum Disorder

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Introduction

- Neuromyelitis optica spectrum disorder (NMOSD) is a devastating autoimmune disease of the central nervous system (CNS), predominantly leading to relapsing optic neuritis and longitudinal extensive transverse myelitis.
- B cells play important roles in pathogenesis of NMOSD and have been proposed to contribute to astrocytic damage and demyelination in NMOSD. But the triggers for pathogenic B cell activation remain elusive.
- A critical assessment of the B cell landscape across different organ systems would advance the understanding in immune mechanisms of diverse B cells that drive NMOSD relapses.
- We performed high-dimensional single-cell sequencing by use of 10×Genomics to comprehensively characterize the signatures of B cells in CSF, peripheral blood, and bone marrow from patients with NMOSD.

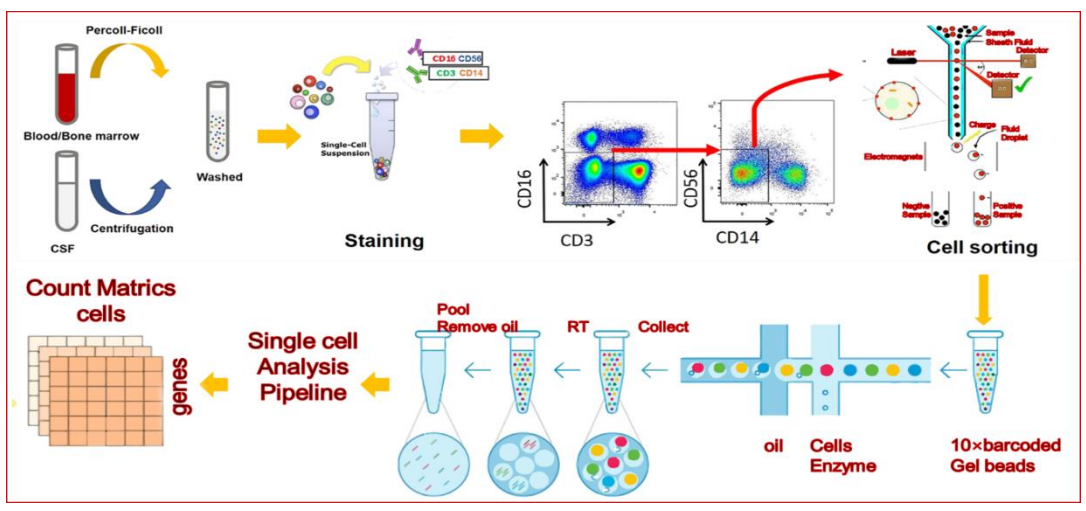
Objective

- We aim to characterize B cell programming towards autoimmunity across different compartments in patients with NMOSD.

Methods

- Eleven NMOSD patients with positive AQP4-IgG were enrolled for single cell sequencing.
- Library preparation and RNA sequencing of B cells from the CSF, peripheral blood, and bone marrow samples.
- Single cell RNA-seq data processing.
- Gene Set Variation Analysis (GSVA) and Calculation of gene set-based scores.
- B cell subsets analysis by flow cytometry and specific expression of type I interferon related genes.
- AQP4-IgGs measurement and Assessment of peripheral blood antibody secreting cells (ASCs).

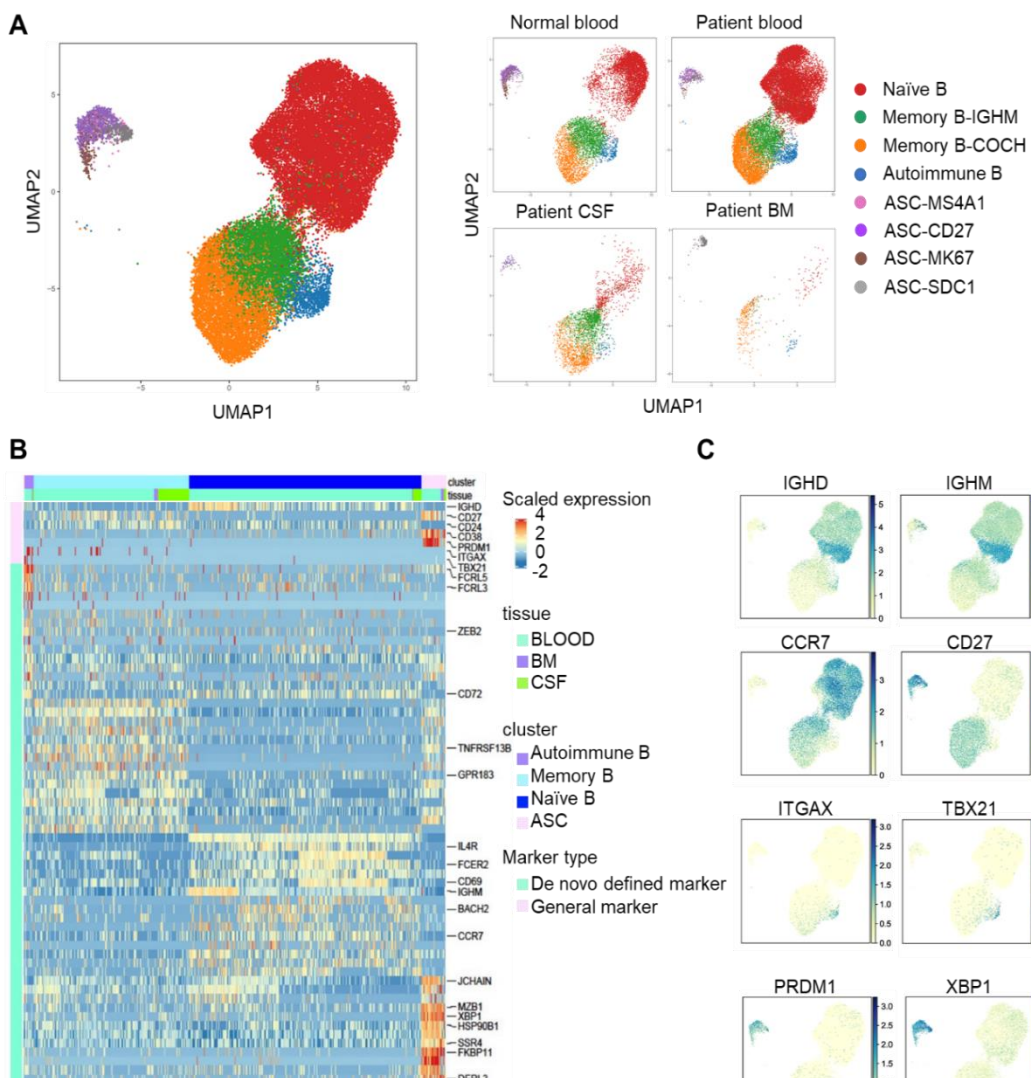
Figure 1. An overview of procedures for single cell sequencing of B cells in blood, bone marrow and CSF obtained from NMOSD patients.



Results

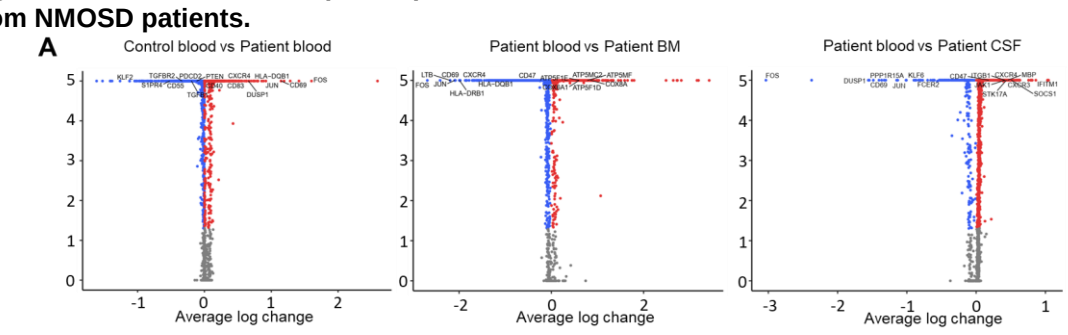
Distinctive distribution pattern of B cell subpopulations across bone marrow, blood, and CSF in NMOSD.

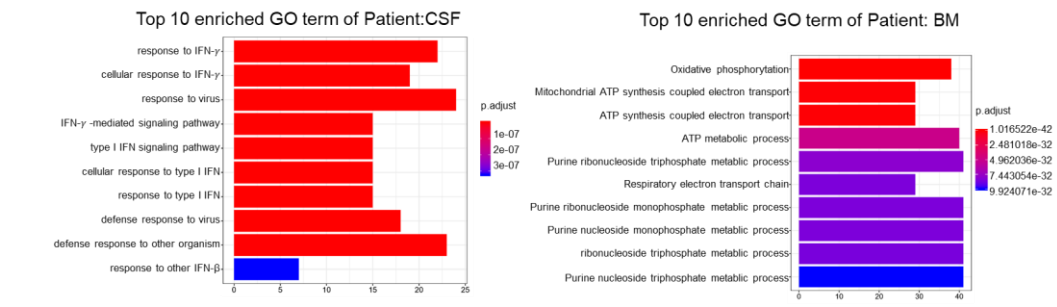
Figure 2. B cell clusters and distribution revealed by high-throughput single cell RNA sequencing in NMOSD.



A. Left: Integrated analysis reveal distinct subpopulations of B cells in NMOSD including naïve B cells, memory B cells, autoimmune B cells, and antibody secreting cells (ASCs). Right: Individual plots showing B cell subpopulations in control blood, NMOSD blood, NMOSD CSF, and NMOSD bone marrow (BM). Two clusters of memory B cells were identified: *IGHM*^{high}*COCH*^{low}*ITGB1*^{low} and *IGHM*^{low}*COCH*^{high} *ITGB1*^{high} memory B cells. Four clusters of ASCs were identified: *MS4A1*^{high}, *CD27*^{high}, *MK67*^{high} and *SDC1*^{high} ASCs. **B.** Heatmap showing unsupervised hierarchical clustering of gene expression pattern in B cells from NMOSD blood, bone marrow and CSF. Scaled expression of discriminative gene sets for each B cell subpopulation (naïve B cells, memory B cells, autoimmune B cells and ASCs) is shown. Color scheme is based on gene expression abundance from -2 (blue) to 4 (red). Top margin color bars highlight gene sets specific to the respective B cell subpopulation and tissue type (i.e. blood, bone marrow and CSF). **C.** Plots showing the expression of indicated markers in NMOSD B cell subpopulations.

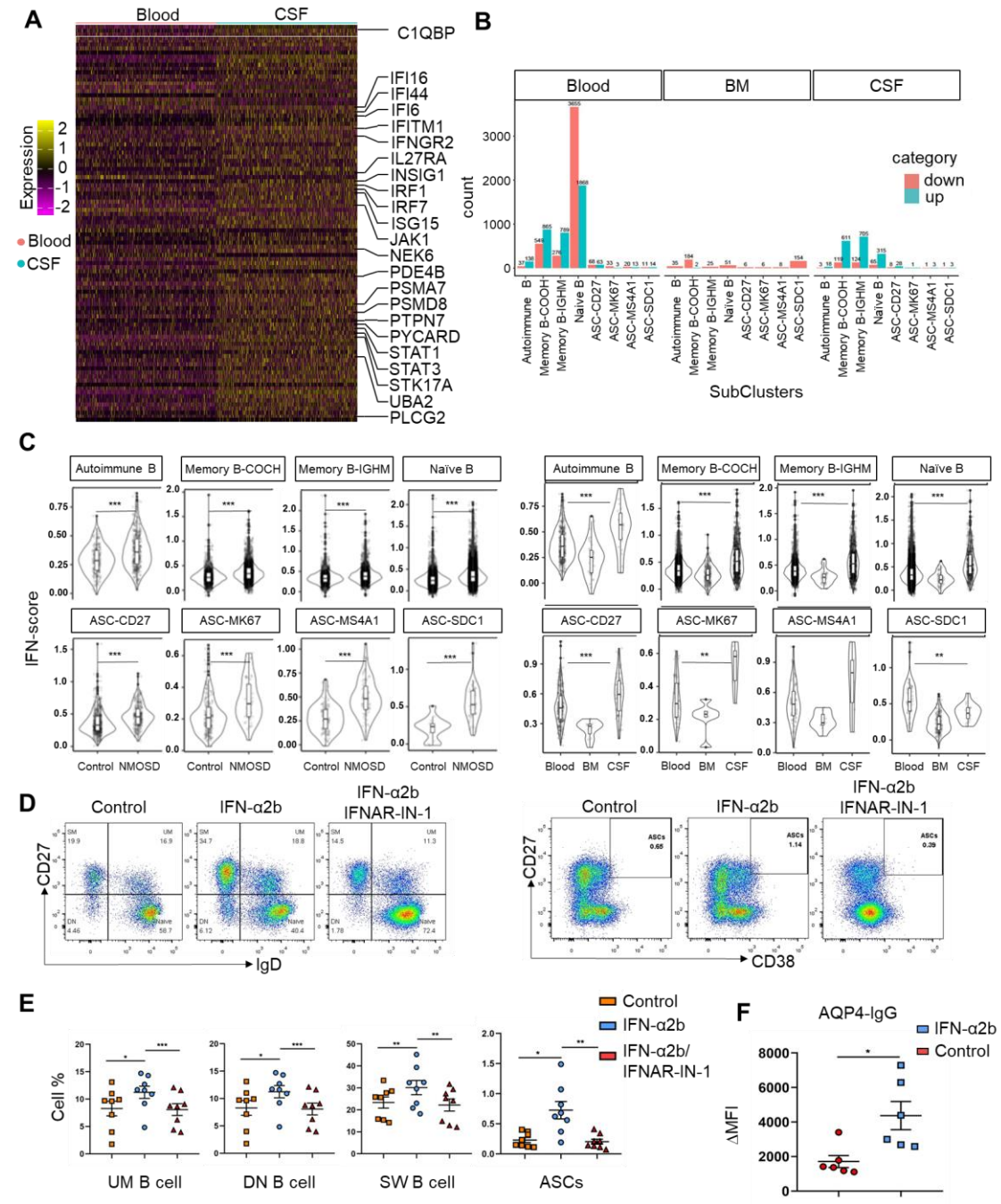
Figure 3. Distinctive transcriptome profiles of B cells across blood, bone marrow and CSF from NMOSD patients.





A. Scatter dot plots showing expression of inflammation-related genes in B cells from NMOSD blood, bone marrow and CSF. Blood from healthy individuals were used as control. Red dots: upregulated genes. Blue dots: down-regulated genes. **B.** Bar graphs showing top 10 enriched gene ontology (GO) terms of indicated pathways in B cells from NMOSD blood or bone marrow GO pathway analysis shows **significant increase of type I interferon signaling pathway in CSF B cells**.

Figure 4. B cell reactivity to type I interferon in NMOSD patients.



A. Unsupervised hierarchical clustering of 145 IFN-related genes that are expressed in blood B cells versus CSF B cells obtained from NMOSD patients. Scaled expression of discriminative gene sets for B cells is shown. Color scheme is based on gene expression abundance from -2 (purple) to 2 (yellow). **B.** Counts of B cell subpopulations with upregulation or down-regulation of interferon-related genes in blood, bone marrow and CSF. Upregulation: green; down-regulation: red. **C.** Left panel: Z-scores of IFN-related genes in NMOSD patients versus controls. Right panel: Z-scores of IFN-related genes in main clusters of B cells from peripheral blood, CSF, and bone marrow in patients with NMOSD. **D-E.** Flow cytometry plots and bar graphs show B cells subpopulations from NMOSD blood in response to IFN-α2b stimulation with or without an IFN-α2b inhibitor. UM B cells: memory IgM cells (IgM+IgD+CD27+); DN B cells: double negative B cells (CD27−IgD−); SW B cells: switched memory B cells (IgD–CD27+); ASCs: antibody-secreting cells (CD27highCD38high). n = 8 per group. **F.** Effects of IFN-α2b stimulation on production of AQP4-IgG in the supernatant of cultured B cells. B cells were obtained from NMOSD blood and cultured in the presence of sCD40L (100 ng/ml), IL-2 (50 ng/ml), IL-21 (50 ng/ml), IL-6 (5 ng/ml), TNF-α (5 ng/ml) for 10 days, with or without IFN-α2b (5000 U/ml). AQP4-IgGs were quantitatively measured by flow cytometry. Data are expressed as median fluorescence intensity (ΔMFI) values. n = 6 per group.

Conclusions

- Four distinct subclusters of B cells were identified in NMOSD, including autoimmune B cells
- B cells are compartmentally fine-tuned towards autoreactivity in NMOSD.
- CSF B cells acquire hyper-reactive to type I interferon signaling pathway. Inhibition of type I interferon pathway may provide a new therapeutic avenue for NMOSD.

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Disclosures

None.

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