Single-cell DN2 B Cell Atlas Reveals Novel Subtypes and Demographic Trends

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Motivation

- Double-negative (DN2) B cells are a novel type of B cells that **lack IgD and CD27**
- DN2 B cells are found abundantly within patients with **autoimmune diseases, elderly populations, and recently, in COVID-19 patients with severe symptoms**
- There is a lack of large-scale genomic research being done on DN2 B cells; establishing a cross-population atlas to further understand this novel immune cell is of high interest

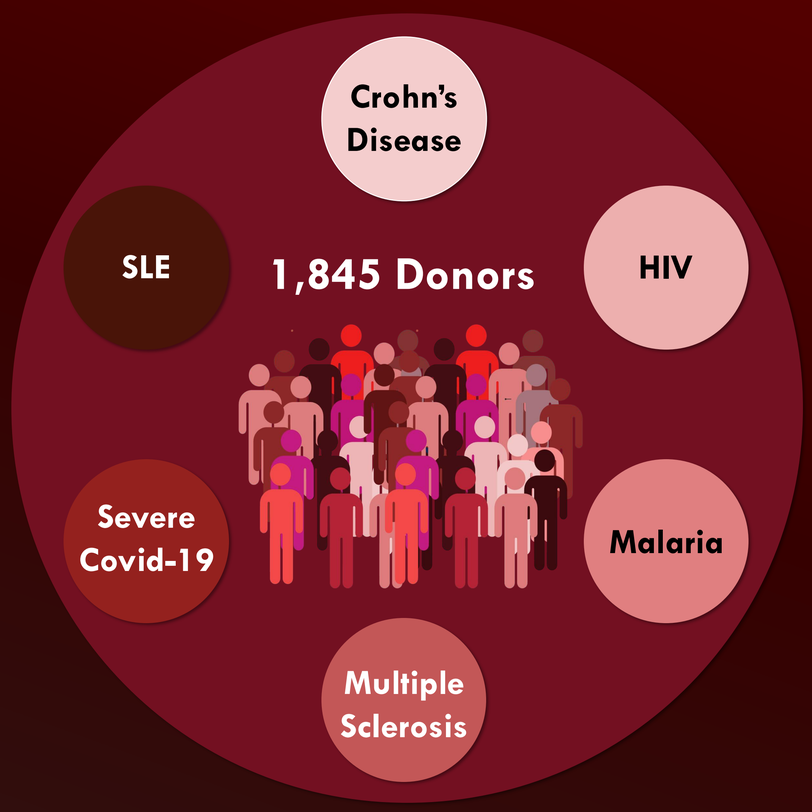
Aims:

- **Establish** a healthy baseline for DN2 B cells by creating a DN2 atlas from 4 healthy PBMC datasets
- **Incorporate** PBMC datasets from immune-related diseases into the atlas
- **Assess** demographic variations and **Compare** DN2 characteristics in healthy and disease populations

METHODS

The Healthy and Disease DN2 cell Atlas, a harmonized, metadata-rich, cross-disease, single-cell omics resource, spanning over **30K DN2 B cells.**

Data acquisition:
Publicly available scRNA-seq data (Gene Expression Omnibus)



1 An Integrated B Cell Map of Health and Disease

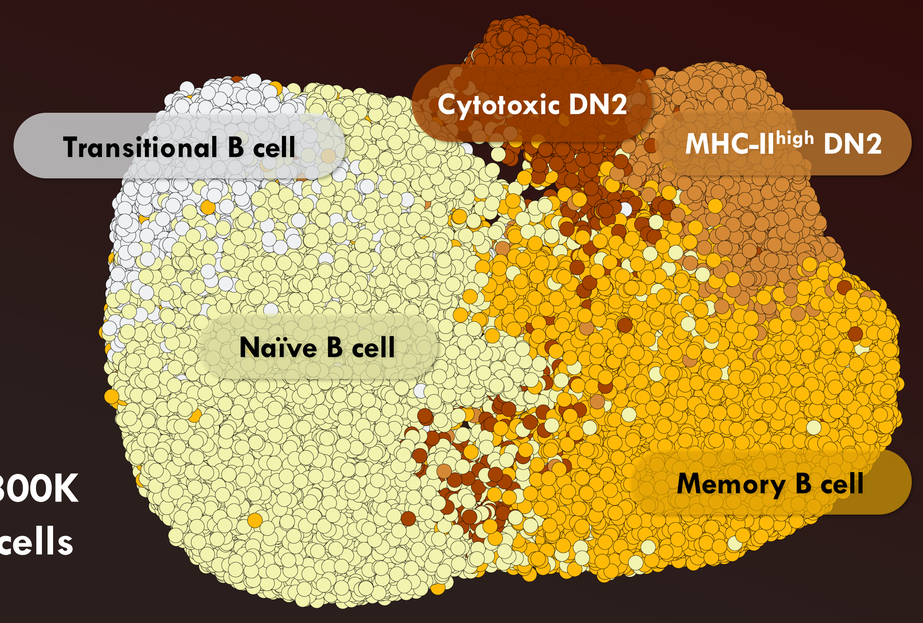


Fig. 1a| UMAP projection of healthy & diseased ~300K human peripheral blood mononuclear B cells using scRNA-seq and clustered into 5 subsets.

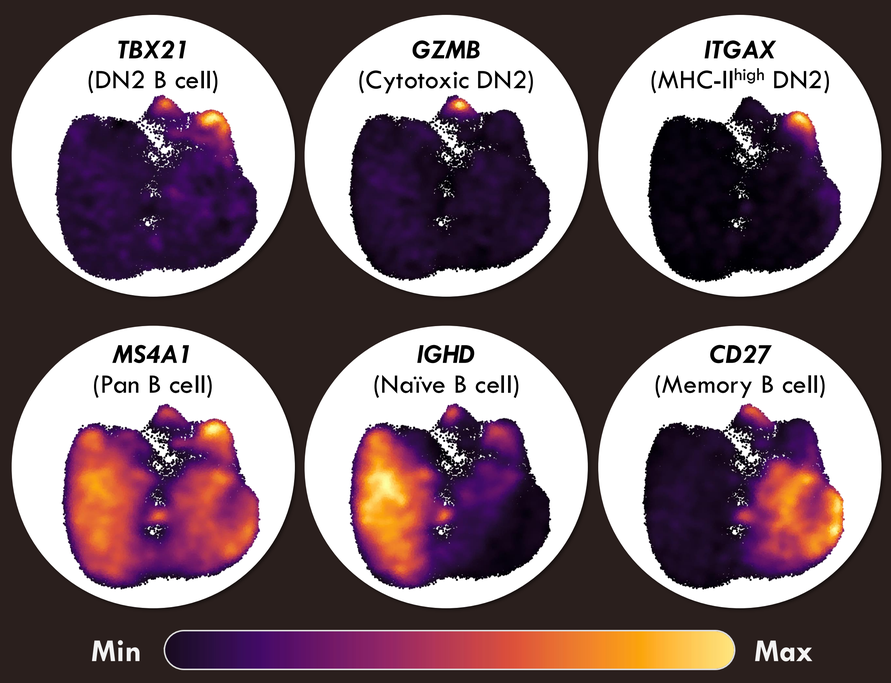


Fig. 1b| Density maps of above UMAP projection with characteristic B cell markers.

2 Proportion Analysis by Disease & Demographics

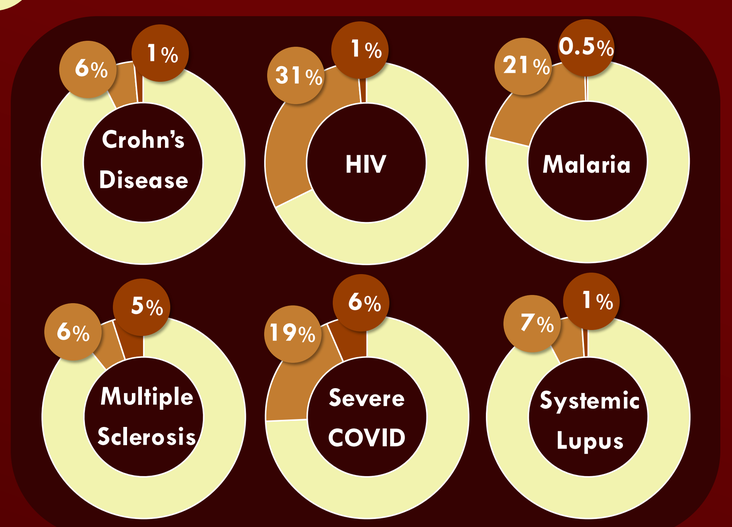


Fig. 2a | Proportions of MHC-II^{high} DN2s (orange), cytotoxic DN2s (red), and other B cells (yellow) based on 6 different immune disease types

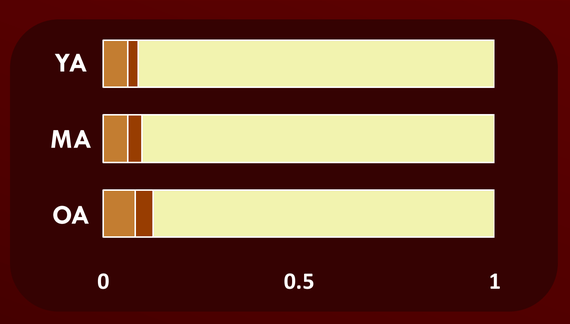


Fig. 2b | Proportions of classical DN2s (orange), cytotoxic DN2s (red), and other B cells (yellow) in healthy individuals based on age differences (Young Adult (YA) <45yrs, Middle-aged (MA) 46-65yrs, Older Adult (OA) >65 yrs)

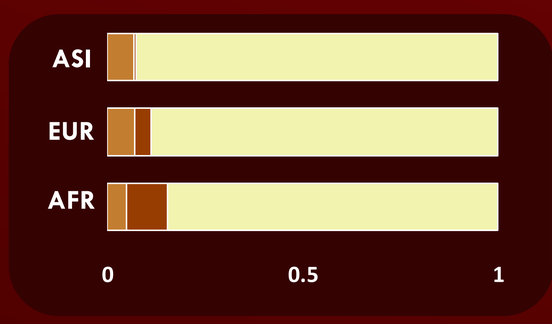


Fig. 2c| Proportions of classical DN2s (orange), cytotoxic DN2s (red), and other B cells (yellow) in healthy individuals based on ethnicity differences (ASI= Asian, EUR= European, AFR= African American)

3 Signaling Pathways Associated with Disease

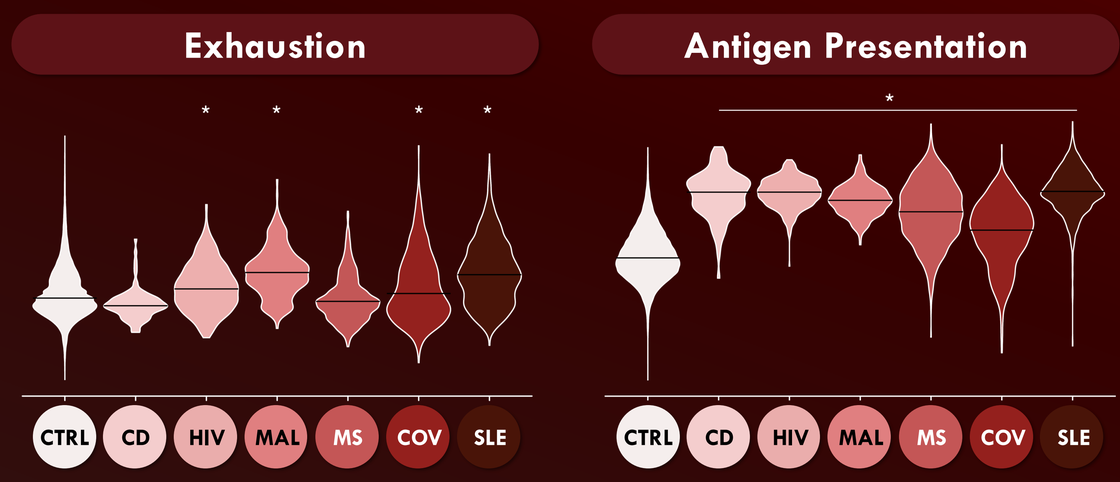


Fig. 3| Violin plots depicting average expression of exhaustion, antigen presentation, hypoxia, and type 1 interferon-stimulated gene (ISG) signatures in DN2 B cells across individuals in cases and controls. Significant expression changes between cases vs controls are highlighted *$P_{adjusted}$ < 0.05 (Wilcoxon). CTRL= Healthy; CD= Crohn's Disease; MAL= Malaria; MS= Multiple Sclerosis; COV= Severe Covid-19; SLE= Systemic Lupus Erythematosus

CONCLUSION

- **scRNA-seq of PBMC reveals distinct DN2 B cell subpopulations**
- **DN2 subpopulations are differentially expanded across chronic and autoimmune diseases**
- **Ratio of DN2s to other B cells increases with age**
- **DN2 B cell subpopulations vary across age and race**
- **HIV and malaria express a significantly high proportion of MHC-II^{high} DN2 B cells**
- **Increase of cytotoxic DN2s in Severe COVID-19 and multiple sclerosis**
- **Disease DN2s express distinct gene signatures including genes encoding hypoxia, antigen presentation, exhaustion, and type 1 IFN**

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