Single-Cell RNA Sequencing of Peripheral Blood Reveals Immune Cell Signatures in Alzheimer’s Disease

Hui Xu¹ | Jianping Jia²

¹Department of Neurology, Innovation Center for Neurological Disorders, Xuanwu Hospital, Capital Medical University, Beijing, China, Beijing, China
²Innovation Center for Neurological Disorders and Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing, China

Correspondence
Hui Xu, Department of Neurology, Innovation Center for Neurological Disorders, Xuanwu Hospital, Capital Medical University, Beijing, China. Email: xuhui1517@126.com

Abstract

Background: Alzheimer’s disease (AD) pathogenesis involves various immune-related phenomena both in central nervous system and peripheral blood. However, the characteristics of peripheral immune cells is poorly defined in AD at a single cell level.

Method: We profile 36,849 peripheral blood mononuclear cells (PBMC) from AD patients with amyloid positive (Aβ+) and normal controls with amyloid negative (Aβ-) by 5’ single-cell transcriptome and immune repertoire sequencing with cell ranger standard analysis procedure. Based on the gene expression profile, immune cells were clustered and visualized by t-distributed stochastic neighbour embedding (tSNE). In addition, we described the features of T cell receptor (TCR) and B cell receptor (BCR) repertoire from clonotypes, V gene and J gene skewed usage, amino acid length, and diversity of complementarity determining region 3 (CDR3).

Result: We revealed five immune cell subsets, CD4+ T cells, CD8+ T cells, B cells, natural killer cells, and monocytes-macrophages cells, disentangled the characteristic alterations of cell subset proportion and gene expression patterns in AD. A total of 31 cell-type-specific key genes, comprising abundant human leucocyte antigen genes, and multiple immune related pathways were identified by protein-protein interaction network and pathway enrichment analysis. We also found High-frequency amplification clonotypes in T cells and B cells and the decreased diversity in T cells were detected in AD.

Conclusion: We found abnormal immune infiltration and complex TCR and BCR alterations were presented in AD peripheral blood. As clone amplification suggested the activation of adaptive immune response against specific antigens, we speculated peripheral adaptive immune response, especially mediated by T cell, may have a role in the pathogenesis of AD. This finding may also contribute to further research regarding disease mechanism and development of immune related biomarkers or therapy.
**Figure 1: Flow Cytometry and Single Cell RNA-seq Analysis**

**Panel a:** Schematic diagram showing the process of sample collection, followed by Chromium (10x genomics) and Flow cytometry. Single cell 5' RNA-seq and VDJ sequencing are also depicted.

**Panel b:** Heatmap illustrating the distribution of cell types, with N=36,849 samples. The subtypes include CD3D (T cells), CD4 (CD4+ T cells), CD8A (CD8+ T cells), NKG7 (NK cells), CD79A (B cells), and CD68 (Monocyte macrophage cells).

**Panel c:** Scatter plots showing the expression levels of CD79A, CD79B, and CD19 in different cell types.

**Panel d:** Heatmap depicting the expression levels of AD1, AD2, and AD3 in NC1 and NC2.

**Panel e:** Heatmap showing the expression levels of various genes including CD3D, CD3E, CD3G, CD4, CD6A, CD8B, CD68, CD14, NKG7, GZMB, GNLY, and NCR1.

**Panel f:** Pie charts comparing the distribution of cell types in AD and NC groups, including CD8+ T cell, NK cell, B cell, hemoglobin contamination, and Monocyte-macrophages.
