

AGE-ASSOCIATED ADAPTIVE IMMUNE TRANSCRIPTOMIC REMODELING FOLLOWING INACTIVATED SARS-COV-2 VACCINATION REVEALS DISTINCT CELLULAR AND FUNCTIONAL IMMUNE SIGNATURES

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Abstract

The adaptive immune response to vaccination can be substantially influenced by aging, although the underlying transcriptional mechanisms across immune-cell populations remain incompletely understood. This study investigated age-associated adaptive immune transcriptomic remodeling following inactivated SARS-CoV-2 vaccination, with emphasis on CD4 T cells, CD8 T cells, and B cells. A retrospective transcriptomic analysis was performed using 36 RNA-sequencing samples obtained from young and older individuals before and after vaccination. Following preprocessing and quality assessment, differential gene expression analysis was conducted separately for each immune-cell population. Protein-coding immune-related genes were subsequently identified and subjected to hierarchical clustering and Gene Ontology biological process enrichment analyses. Older individuals demonstrated broader transcriptomic perturbation across all immune-cell populations compared with younger individuals. CD4 T cells exhibited the greatest degree of differential expression, with 3,885 significant genes identified in older individuals. Immune-focused analysis further revealed extensive dysregulation of immune-associated genes, particularly in CD4 T cells and B cells. Functional enrichment analysis identified pathways associated with B-cell proliferation, B-cell activation, immunoglobulin-mediated immune response, kinase-related signaling, inflammatory response, MAPK/ERK signaling, and dendritic-cell migration. Distinct clustering patterns additionally demonstrated clear transcriptomic segregation according to age group and vaccination status. These findings indicate that aging alters both the magnitude and functional direction of vaccine-induced adaptive immune responses at the transcriptomic level. The results support the value of immune-cell-specific transcriptomic profiling for understanding vaccination responses in aging populations and may contribute to future development of age-sensitive immunization strategies.

Keywords: Immunosenescence, SARS-CoV-2 vaccination, transcriptomics, adaptive immunity, immune remodeling

1. Introduction

The entire COVID-19 pandemic (coronavirus disease 2019) had a devastating impact on health, social, and economic outcomes globally. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused a disruption to healthcare systems, elevated death tolls, and caused considerable financial instability in countries (Naseer et al., 2023). To minimize the spread of the virus, public health measures like lockdowns, social distancing, and travel bans were instituted worldwide, but these measures also had long-term socioeconomic and psychological consequences (Onyeaka et al., 2021). The pandemic caused disruption in the healthcare sector, as well as in employment systems, education and public services globally, highlighting the global impacts of COVID-19 on today's society (Pawar, 2020). These challenges underscored the need for effective prophylactic and therapeutic measures that could manage the severity of the disease and mitigate mortality. The fastest developing large-scale intervention identified to address COVID-19 was vaccination. Hospitalizations, severe infections, and virus-related deaths were significantly lower in the pandemic period in the context of global immunization programs (Watson et al., 2022). The efficacy of the vaccine was also shown clinically by having very low risks of severe progression and mortality for those who have been vaccinated, while the risks for unvaccinated populations are very high (Huang & Kuan, 2022). In addition to personal protection, mass vaccination helped to re-establish healthcare stability and to help the social and economic recovery at the global level (Dye, 2022). Several vaccine platforms were thus resorted to globally to create protective immunity against SARS-CoV-2 infection, such as mRNA, viral vector, protein subunit, and inactivated vaccines.

Most of the protective action of vaccines is believed to be primarily due to the coordinated activation of adaptive immune system cells, namely, T lymphocytes and B lymphocytes. CD4 T cells coordinate immune responses and interact with cells to coordinate cytokine signaling; CD8 T cells participate in killing infected cells. B-cells function in producing antibodies and in maintaining long-term humoral immunity (Primorac et al., 2022). Integrated cellular and humoral immune activation that can establish long-term immunological memory is thus necessary for successful vaccination. Respiratory viral vaccination also triggers mucosal and systemic immune responses, which aid in viral clearance and protection from reinfection (Mettelman et al., 2022). Both innate and adaptive immune responses are highly engaged in vaccine-induced immune activation, such as inflammatory regulation, interferon signaling, and antigen-specific lymphocyte responses, which have been reported to investigate SARS-CoV-2 vaccines (Li et al., 2022).

Although COVID-19 vaccines have proven to be effective, some age groups have been found to have higher levels of immune response than others. As people age, their immune function decreases progressively, which is known as immunosenescence. This is manifested through decreased diversity of lymphocytes, impaired activation of immune cells, decreased vaccine response and dysregulated inflammatory signaling (Crooke et al., 2019). The strength and longevity of vaccine protection may be affected in the older adults. Changes in B-cell and T-cell responses are reported in the older adults and this may lead to reduced adaptive immune responses and diminished immunologic memory after vaccination (Booth & Toapanta, 2021). Thus, elucidation of the molecular mechanisms behind these age-associated immune changes is key to enhancing vaccine strategies in the older adults.

The rapid development of transcriptomic technology has opened up novel opportunities to study vaccine-induced immune responses on a molecular level. RNA-sequencing technologies allow for the global analysis of changes in gene expression that occur during immune activation, regulation of inflammation, signaling by cytokines, and differentiation of lymphocytes. Transcriptomic profiling can therefore provide immune signatures and cellular pathways that might not be completely captured by classical serological or immunological markers. Comprehensive transcriptomic comparisons are especially useful to investigate the differential responses of immune cell subsets to vaccination under different physiological contexts.

Previous studies of SARS-CoV-2 vaccination have primarily studied antibody levels, antibody neutralization, or systemic immune activation. While some studies have reported transcriptomic changes following COVID-19 vaccination, few studies have thoroughly explored adaptive immune-cell populations in the aging landscape. In particular, there are limited transcriptomic studies that have directly compared the transcriptomes of CD4 T cells, CD8 T cells, and B cells between young and older individuals after inactivated SARS-CoV-2 vaccination. Immune remodeling following vaccination is still not fully understood and studied at the molecular level for age-dependent changes.

This study aimed to explore the transcriptomic remodeling of adaptive immunity as a result of the inactivated SARS-CoV-2 vaccine in individuals of different ages. Differential gene expression patterns and functional immune pathways were identified by comparative RNA-sequencing analyses of CD4 T cells, CD8 T cells and B cells from young and older people before and after vaccination. The study also set out to define immune signatures of vaccination-associated immune responses and immune pathways that are enriched in specific age groups. The study also attempted to characterize immune signatures of vaccine-associated immune responses and immune pathways enriched in the aged.

2. Methodology

2.1 Research Design

In the current study, the approach adopted was that of transcriptomic analysis retrospectively, whereby the aim was to assess the effects of an inactivated virus vaccine against SARS-CoV-2 on adaptive immunity based on age. This was achieved through comparing the results of RNA sequencing done using immune cells collected from young and old participants before and after vaccination.

2.2 Data Source

The RNA-seq data set used in this study was obtained through the experiment carried out by Xiao et al. (2023) to determine the impact of the inactivated SARS-CoV-2 vaccine on adaptive immunity in young and old participants. This data set comprised RNA-seq data for CD4 T cells, CD8 T cells, and B cells before and after immunization (Xiao et al., 2023).

Thirty-six RNA-seq samples obtained from human immune cell types were analyzed in this study. Both young and old subjects participated in this experiment before and after receiving a vaccine. Sequencing was done using the Illumina NovaSeq 6000 platform using the high-throughput paired-end method.

2.3 Data Preprocessing and Quality Control

The count matrix and sample metadata went through preprocessing and quality control steps. Genes that expressed very little were filtered out to minimize noise and enhance downstream statistical analysis accuracy. All remaining genes expressed across several samples were included in subsequent analyses.

Sequencing uniformity, biological clusters, and outliers in the data were assessed through library size distribution plots, principal components analysis, and sample correlation. The principal components analysis was carried out to identify differences in gene expression between immune cells, different ages, and vaccination statuses.

2.4 Differential Gene Expression Analysis

Gene differential expression analysis was independently carried out for the CD4, CD8, and B cell subtypes. The comparison was done between post-vaccination and pre-vaccination states in both young and old age classes. Prior to performing any statistical analysis, the gene expressions were normalized to account for sequencing depth variations between samples.

Differentially expressed genes were those whose adjusted p-value was less than 0.05 while their absolute value log₂ fold change was greater than or equal to 1. Genes that were up- and down-regulated for both subtypes were identified in both age categories. Volcano plots were plotted to visualize differentially expressed genes.

2.5 Immune-Related Gene Identification and Clustering Analysis

For the investigation of immune-specific transcriptional patterns, immune-related biological processes were selected, and genes involved in those processes with differential expression were obtained for subsequent analyses. Immune-related protein-coding genes were obtained and compared between different immune cell types and age groups.

Clustering and heatmap analyses were performed based on the normalized expression level of dysregulated immune genes. The clustering was done to determine the similarity of transcriptomes and obtain immune expression patterns in response to immunization and aging.

2.6 Functional Enrichment Analysis

Analysis of functional enrichment was performed to determine the biological functions of genes involved in the induction of immunity by vaccines. Genes that were up-regulated in the group of older adults were analyzed using Gene Ontology biological process enrichment analysis. The pathways that were enriched were ranked based on their adjusted p-value and enrichment score. Pathways linked with immune activation, inflammation signaling, cytokine activity, and signaling pathways inside the cell were considered.

3. Results

3.1 Quality assessment and transcriptomic profiling of immune-cell populations

Thirty-six RNA-sequencing samples comprised B cells, CD4 T cells, and CD8 T cells from young subjects and elderly before and after immunization were analyzed in this study. There were three biological replicates in each experimental group. After preprocessing and low-expression filtering, 19,482 genes were selected for further analysis.

Principal component analysis revealed good clustering by immune cell lineage, which suggested excellent transcriptome reproducibility and clear biological distinction between different cell types. CD4 T cells, CD8 T cells, and B cells exhibited good clustering, while age-based clustering was also apparent within each lineage. There was no obvious outlier, thus ensuring the quality and reproducibility of the RNA-seq data. Figure 1 illustrates the transcriptome distribution of all RNA-sequencing samples. The distribution of samples among different age groups, immunization conditions, and immune cell lineages was presented in Table 1.

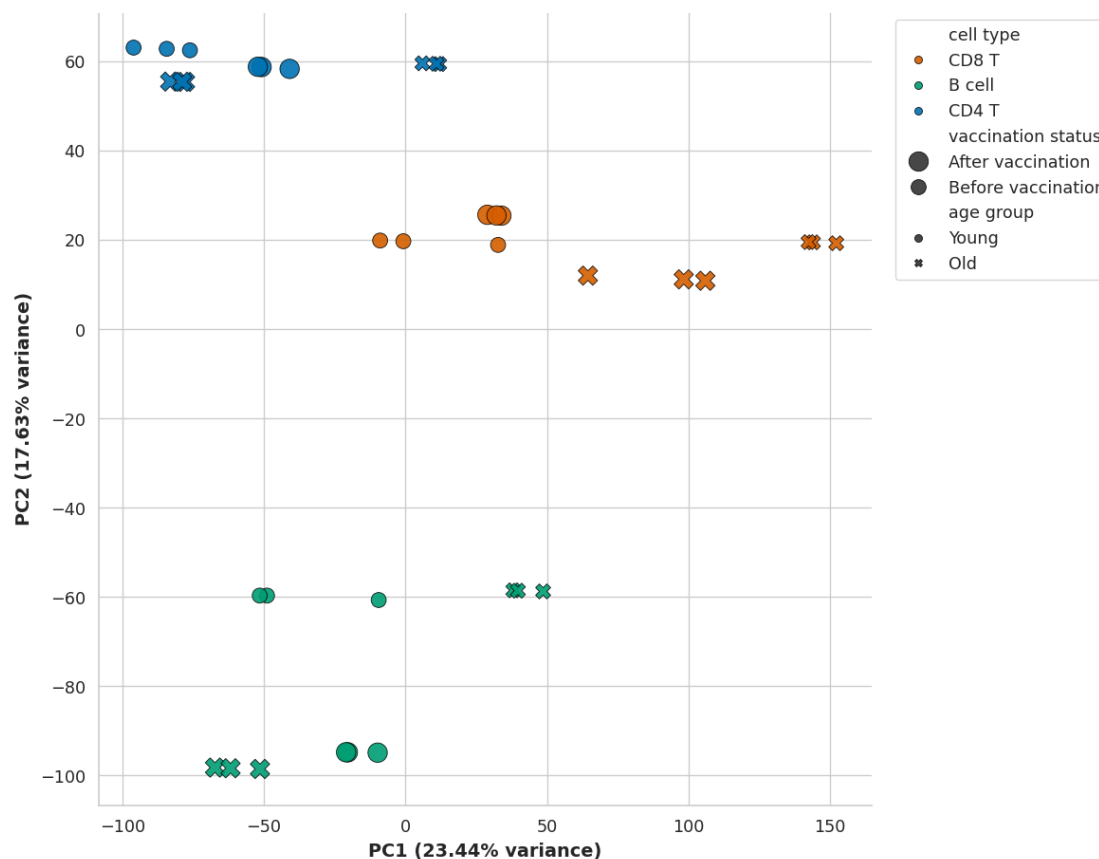


Figure 1. Principal component analysis of RNA-sequencing samples.

Table 1. Dataset sample distribution according to age group, vaccination status, and immune-cell type

Age group	Vaccination status	Cell type	Number of samples
Old	After vaccination	B cell	3
Old	Before vaccination	B cell	3
Young	After vaccination	B cell	3
Young	Before vaccination	B cell	3
Old	After vaccination	CD4 T	3
Old	Before vaccination	CD4 T	3
Young	After vaccination	CD4 T	3
Young	Before vaccination	CD4 T	3
Old	After vaccination	CD8 T	3
Old	Before vaccination	CD8 T	3
Young	After vaccination	CD8 T	3
Young	Before vaccination	CD8 T	3

3.2 Differential gene expression following vaccination

Transcriptional remodeling following vaccination was highly prevalent among all immune cell populations, according to the differential expression analysis. It was observed that older patients had more DEGs than their younger counterparts. The greatest changes were observed in CD4 T cells, with 3,885 and 2,719 DEGs noted for older and younger individuals, respectively. Likewise, there were 3,389 DEGs in older B cells but only 2,442 DEGs in young B cells. On the other hand, CD8 T cells experienced fewer transcriptional changes than CD4 T cells, despite older patients having more DEGs than younger ones. Figure 2 shows the overall frequency of gene expression differences due to vaccinations among all immune cell types regardless of age. The quantitative frequency of DEGs, including those that are upregulated and downregulated, is shown in Table 2.

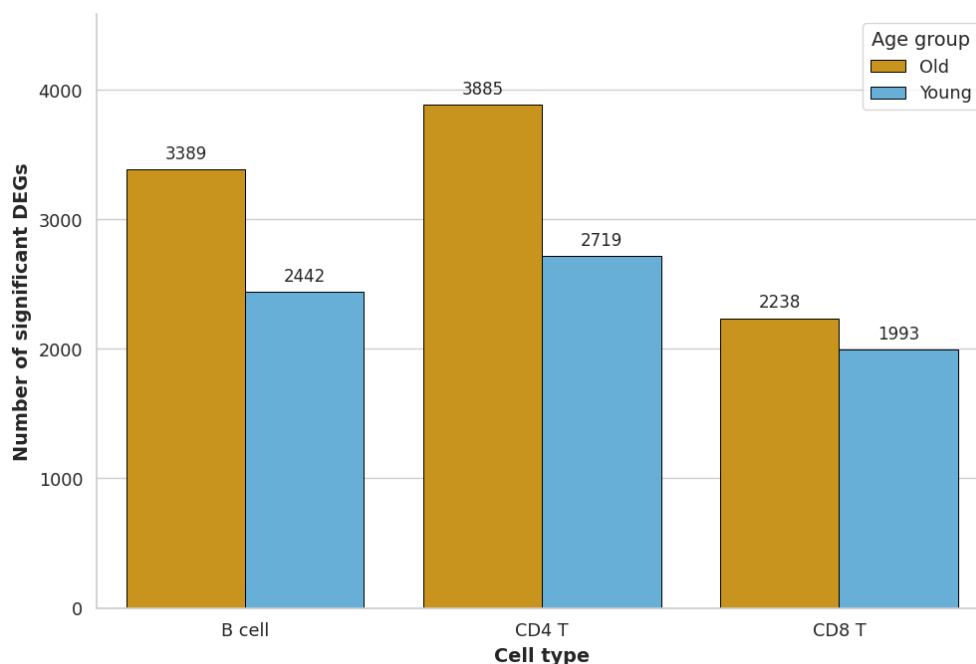


Figure 2. Differentially expressed genes after vaccination across immune-cell populations

Table 2. Summary of differential gene expression after vaccination

Cell type	Age group	Total significant DEGs	Upregulated DEGs	Downregulated DEGs
B cell	Old	3389	1520	1869
B cell	Young	2442	1002	1440
CD4 T	Old	3885	1550	2335
CD4 T	Young	2719	1459	1260
CD8 T	Old	2238	1106	1132
CD8 T	Young	1993	1077	916

3.3 Immune-related transcriptional regulation after vaccination

Differential gene expression analysis focusing on immune-related genes showed a huge number of immune-associated genes being differentially expressed due to the vaccine. Immune transcriptional changes were seen prominently in CD4 T cells of older patients, where 270 genes involved in the immune response pathway had shown significant differences. B cells of older subjects also underwent prominent changes in immune transcription, which was evident in 201 genes involved in the immune pathway. In most types of immune cells, the down-regulation of genes outnumbered the up-regulation of genes. For example, in CD4 T cells of the older population, 199 immune genes were downregulated while only 71 genes were upregulated. This indicates that there is extensive immune reprogramming taking place post-vaccination.

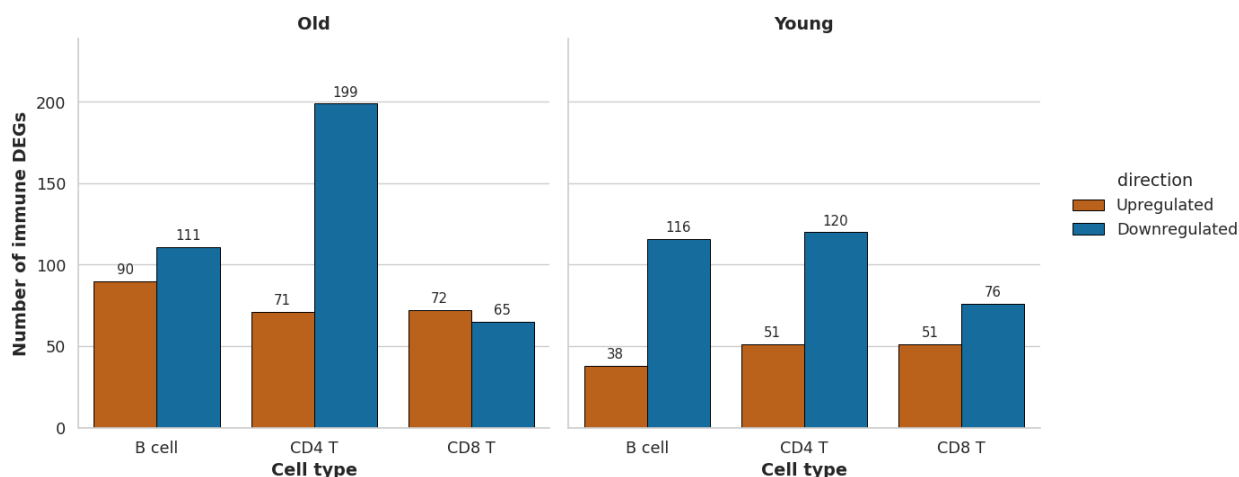


Figure 3. Direction of immune gene regulation after vaccination in young and older individuals.

The immunological remodeling in relation to vaccination was additionally investigated using volcano plot analysis on CD4 T cells obtained from older subjects. The extensive bidirectional differential gene expression was observed, whereby a large number of significantly up- and down-regulated genes were found with a wide range of fold change. Many genes exhibited high statistical significance and a fold change higher than the cutoff point, demonstrating the profound effect of vaccination on transcription. This is evident from Figure 4 below.

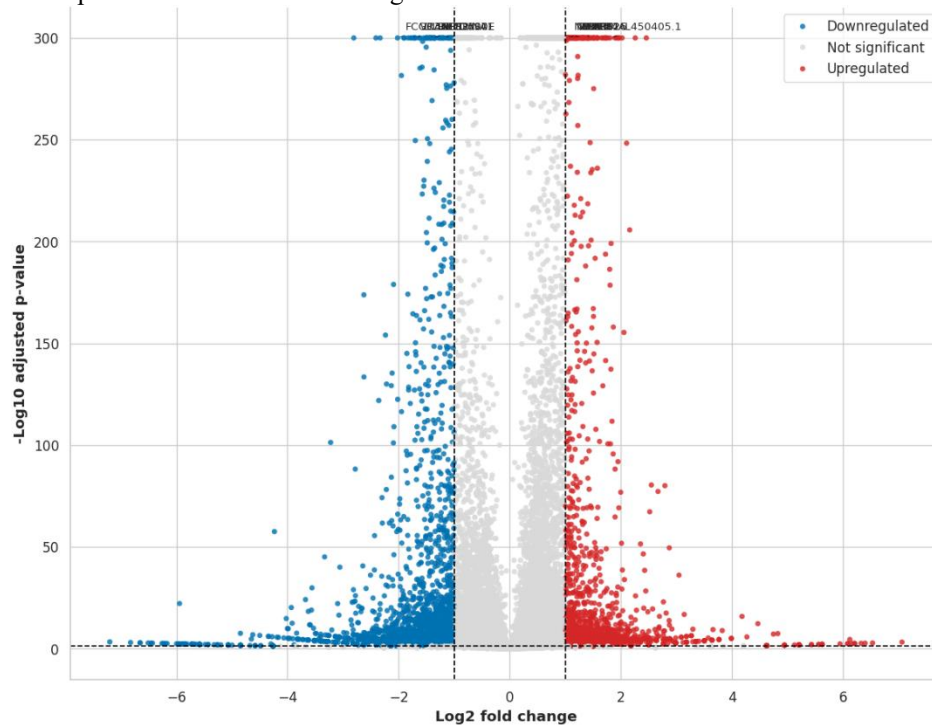


Figure 4. Volcano plot of differential gene expression in CD4 T cells from older individuals.

3.4 Immune transcriptomic signatures in CD4 T cells

Hierarchical clustering of the top immune-related DEGs indicated clear gene expression clusters based on age groups and vaccine status. Gene expressions from older adults who had been vaccinated were clearly segregated from those of younger vaccinated and unvaccinated individuals. A few immune-related genes involved in inflammation control, immune stimulation, and antigen presentation showed clear differences in expression among the groups. Some of the highly differentially expressed immune-related genes include ICOS, CCR4, NFKB1, FCGR3A, CSF1R, and HLA-DRB1. Figure 5 below illustrates the expression patterns of the top immune-related differentially expressed genes among CD4 T cells.

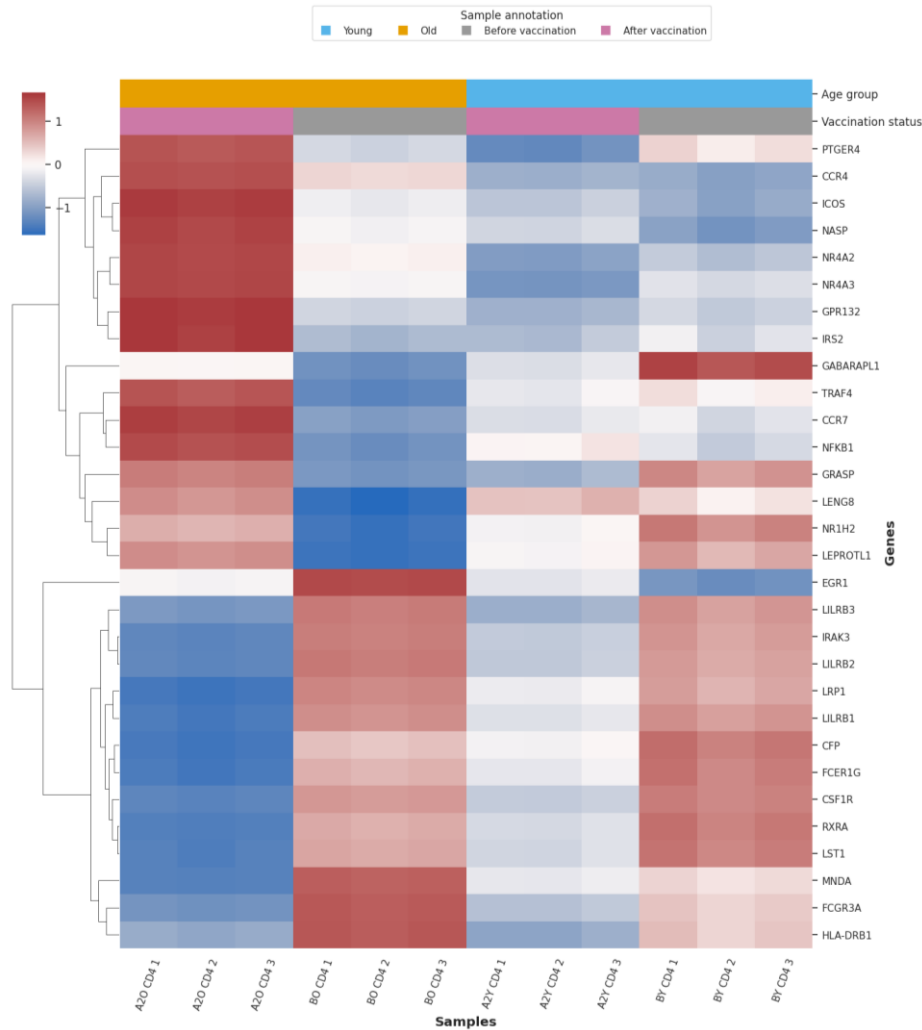


Figure 5. Heatmap of top immune differentially expressed genes in CD4 T cells.

3.5 Functional enrichment analysis of vaccine-associated immune responses

Analysis of Gene Ontology enrichment led to the identification of various biological pathways affected by vaccines in the immune system of the older adults. There was a significant enrichment of pathways associated with adaptive humoral immunity in B cells, including proliferation, activation, immunoglobulin-mediated immunity, and other signaling processes involving cytokines.

The enrichment of B cells in the biological pathways associated with adaptive humoral immunity, including proliferation, activation, and immune response mediated by immunoglobulins, is shown in Fig. 6A. CD4 T cells had their significant enrichment in intracellular signaling, inflammatory response, and kinase activity regulation-related pathways, namely regulation of kinase activity, receptor tyrosine kinase signaling, inflammatory response, and mast cell activation-related pathways. It can be seen from Fig. 6B that there was a significant enrichment in CD4 T cells in the intracellular signaling pathways and inflammatory responses in these immune cells, which indicates considerable immune signaling reprogramming due to vaccination. Significantly enriched pathways in CD8 T cells included the MAPK/ERK signaling pathway, dendritic-cell chemotaxis and migration, and phosphorylation-mediated intracellular signaling. As seen in Fig. 6C, there was a significant enrichment of CD8 T cells in MAPK/ERK cascade signaling and dendritic-cell migration, which indicates immune surveillance and trafficking in these immune cells.

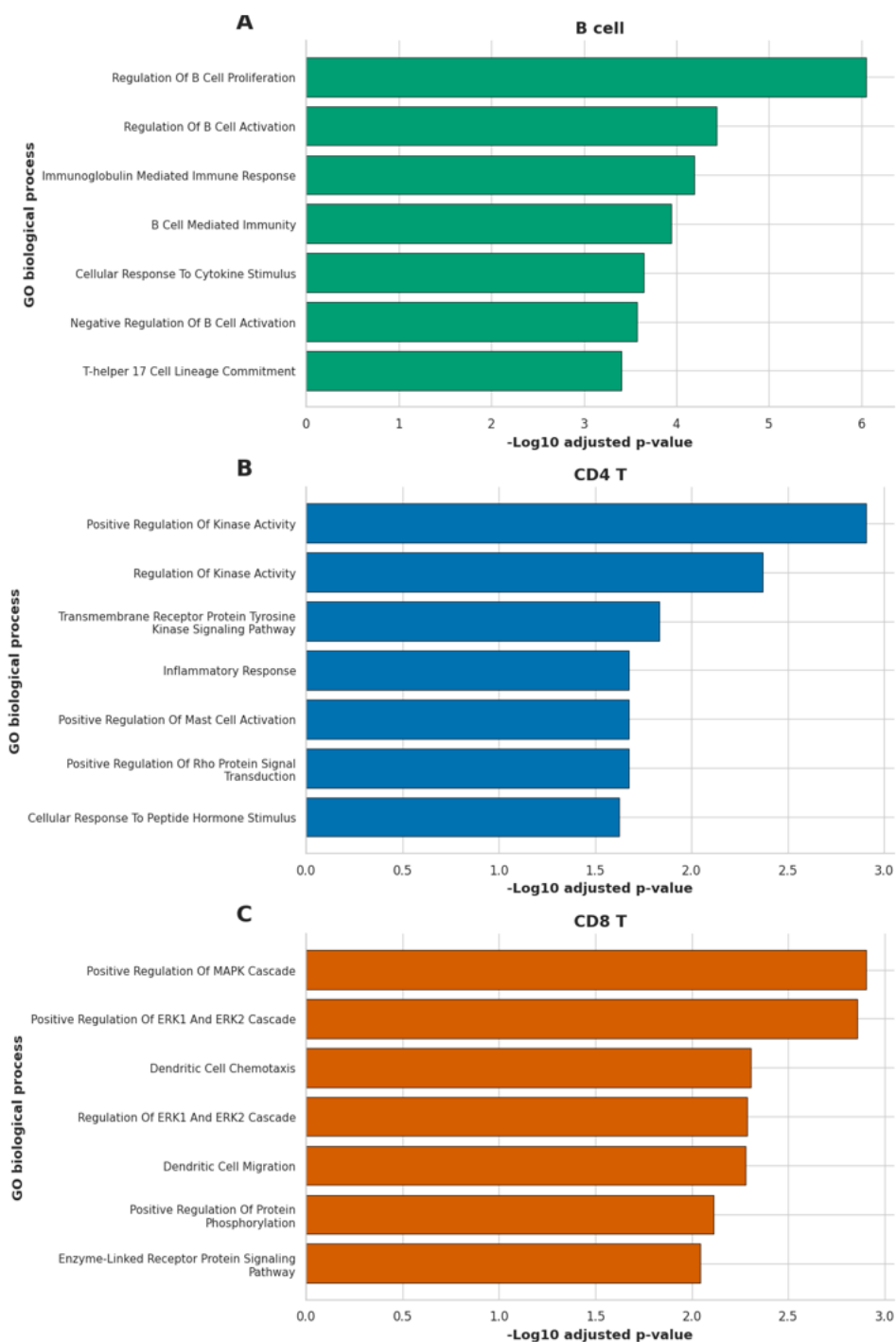


Figure 6. Top enriched GO biological processes in older individuals: (A) B-cell-associated pathways, (B) CD4 T-cell-associated pathways, and (C) CD8 T-cell-associated pathways.

Table 3. Top enriched GO biological processes in older individuals after vaccination

Cell type	GO biological process	Adjusted p-value
B cell	Regulation of B-cell proliferation	8.89×10^{-7}
B cell	Regulation of B-cell activation	3.68×10^{-5}
B cell	Immunoglobulin-mediated immune response	6.46×10^{-5}
B cell	B-cell-mediated immunity	1.13×10^{-4}
CD4 T	Positive regulation of kinase activity	0.0012
CD4 T	Regulation of kinase activity	0.0043
CD4 T	Transmembrane receptor protein tyrosine kinase signaling pathway	0.0146
CD4 T	Inflammatory response	0.0212
CD8 T	Positive regulation of MAPK cascade	0.0012

CD8 T	Positive regulation of ERK1 and ERK2 cascade	0.0014
CD8 T	Dendritic-cell chemotaxis	0.0049
CD8 T	Dendritic-cell migration	0.0053

4. Discussion

Adaptive immune transcriptional changes were observed in all immune-cell types tested after inactivated SARS-CoV-2 vaccination, in a manner that is different from that of young individuals. The aged immune system showed greater transcriptomic remodeling in response to vaccination than younger people, based on the number of differentially expressed genes observed. This trend was seen in B cells, CD4 T cells, and CD8 T cells, which showed that aging affected both humoral and cell-mediated immune compartments and not just one immune lineage. The higher DEG burden in the older adults could be due to immune system regulation changes, compensatory up-regulation of transcription, or immune-system dysregulation after vaccination. The most perturbed transcriptomes were found in CD4 T cells, especially in older people. The strong transcriptomic remodeling of CD4 T cells indicates that aging may play a significant role in shaping the function of helper T cells post vaccination, given that these cells are key regulators of adaptive immune responses via cytokine signaling, B-cell help, and cellular immunity. The activation of kinase-associated signaling, inflammatory response and receptor-associated pathways also suggests that CD4 T cells are re-programmed immunologically in older adults. Up-regulated immune genes in CD4 T cells also might indicate post-vaccination immune regulatory activity or loss of maintenance of selected helper T-cell programs.

Humoral immune remodeling was clearly evident in B cells. Pathways enriched with B-cell proliferation, B-cell activation, immunoglobulin-mediated immune response and B-cell mediated immune response suggest that vaccination activated antibody-related immune pathways in older persons. At the same time, there were also a number of genes related to the immune system that were depressed, indicating that this immune response was not consistent. In contrast, B-cell transcriptional responses may be a dynamic equilibrium of activation, differentiation and regulation following vaccination. CD8 T cells also had a relatively low degree of DEG burden compared with the other two cell types, with pathway analysis showing significantly enriched pathways related to dendritic-cell chemotaxis, dendritic-cell migration and MAPK/ERK signaling, among others, with biological relevance. These results suggest that the cellular mechanisms of immune responses and communication after vaccination to cytotoxic immune responses can be modulated. The relatively balanced expression of upregulated and downregulated immune genes observed in CD8 T cells indicates that there is a more balanced transcriptional response than in CD4 T cells.

The greater immune heterogeneity seen in older people is in line with previous studies showing age-related differences in SARS-CoV-2 vaccine immune responses, with older people having a different cellular and humoral immune profile than younger people (Collier et al., 2021). The present results also corroborate the notion that inflammaging and immunosenescence may affect the effectiveness of vaccine-induced immunity and the threshold for immune activation in older adults (Pereira et al., 2020). Evidence across the population of age-associated variations in antibody response to SARS-CoV-2 vaccination has been observed (Wei et al., 2021) to support the observed differences in immune response. In addition, mRNA vaccination studies in older adults have demonstrated that vaccines are immunogenic in older adults, albeit with a different magnitude and/or kinetics of immune response than younger adults (Anderson et al., 2020). These reports provide a supportive argument for the notion that vaccination continues to be immunologically active in older individuals, and that it may trigger qualitatively different immune programs.

These key changes in CD4 T cell and CD8 T cell transcription are in line with the known role of T cell immunity in the pathogenesis of SARS-CoV-2 infection and vaccination response, specifically in orchestrating antiviral immunity and promoting immune memory (Toor et al., 2021). Preliminary vaccine studies of older adults also have shown good immunogenicity and safety, but there is a need for a better understanding of immune responsiveness in older adults (Wu et al., 2021). The pathway-level results are similar to those of systems vaccinology studies that identified coordinated innate and adaptive immune gene programs that are detectable after SARS-CoV-2 vaccination using transcriptomic profiling (Arunachalam et al., 2021). Cell-type specific immune profiling (Tong et al., 2023) has also demonstrated immune cellular and molecular variability among peripheral immune cells in recipients of an inactivated SARS-CoV-2 vaccine, which underscores the importance of single-cell analysis of cells. Time-series transcriptomic studies following SARS-CoV-2 vaccination have also revealed dynamic changes in immune gene expression following vaccination, further highlighting the benefit of transcriptomics for identification of vaccine-associated molecular signatures (Watanabe et al., 2023).

The results indicate that the immune system of adaptive immunity changes its transcriptional architecture during ageing. It is important to note that the higher DEG burden in older people does not automatically reflect a greater protective response, but might signify more widespread immune remodeling with activation, regulation and inflammatory signaling. This difference is significant as the immune function can be impaired despite enhanced transcription activity in older individuals. The findings indicate that CD4 T cells are a key point of age-associated immune remodeling following vaccination. Age-related changes in CD4 T cells can potentially impact the quality of vaccine-induced immunity since they play a role in regulating B-cell maturation, antibody responses and cytotoxic T cell function. The enrichment of B-cells for humoral pathways also points to the need to consider antibody-associated molecular pathways in conjunction with T-cell responses. On a practical level, transcriptomic signatures for immune cells could be used to monitor vaccines in older adults in the future. These strategies can assist in the discovery of immunomarker genes that are linked to vaccine responsiveness, booster needs or immune persistence. Such results can also be implemented into vaccine design, such as vaccine schedule optimization or the use of adjuvants in older adults.

There are a couple of caveats to keep in mind. The data analyzed were publicly available transcriptomic data with relatively few biological replicates per condition. The data was balanced and had good sample clustering, but a larger sample size would increase the statistical power and generalizability of the data. This study also concentrated on gene-expression changes, so there were no protein-level validation or functional immune assays performed.

Key immune genes and pathways need to be validated in separate cohorts using flow cytometry, cytokine profiling, antibody assays, and functional assays of T cells in future studies. Longitudinal studies at several post-vaccination time points would also shed light on whether the observed transcriptional remodeling is early activation, delayed regulation or ongoing immune remodeling in older individuals.

5. Conclusion

Age-associated remodeling of adaptive immune-cell populations was observed following inactivated SARS-CoV-2 vaccination. CD4 T cells have the highest transcriptomic burden of differential expression, while older individuals have a wider differential expression burden than younger individuals. An immune-focused analysis also identified significant dysregulation of protein-coding immune genes, especially in CD4 T cells and B cells. Significant differences in cell programs emerged as a result of functional enrichment, such as B-cell proliferation and activation, immunoglobulin-mediated immunity, kinase-related signaling, inflammatory response, MAPK/ERK signaling and dendritic-cell migration pathways. These results provide evidence of shifts in the strength and direction of adaptive immune transcriptional changes with age following vaccination. The results underscore the importance of immune-cell-specific transcriptomic profiling as a tool to examine vaccine responsiveness in older adults, as well as to help identify molecular markers of vaccine-induced immunity, immune durability, and age-sensitive vaccination strategies in the future.

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