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VACCINE-INDUCED T-CELL MEMORY: SINGLE-CELL RNA-SEQ PROFILING IN POST-VACCINATION LYMPH NODES

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Abstract

The development of long-lasting vaccine-induced immunity relies on the generation and maintenance of memory T cells, a process that remains incompletely understood at the single-cell level. In this study, we employed single-cell RNA sequencing (scRNA-seq) to profile the transcriptional landscape of T cells within lymph nodes at multiple time points following vaccination. Our analysis revealed a dynamic shift from naïve to effector and memory T cell phenotypes, accompanied by significant transcriptional reprogramming. Notably, genes such as IL7R, CD44, and CXCR3 were upregulated in memory T cells, indicating their roles in survival, tissue homing, and immune readiness. Pathway enrichment analysis identified the activation of TCR signaling, cytokine-cytokine receptor interactions, and NF-κB pathways, all of which are critical for effective immune priming and differentiation. Clonotype tracking found strong TCR expansion, while analysis of cytokines proved that IFN-γ, IL-2 and TNF-α were all upregulated, representing optimal effector activity. Moreover, increases in PD-1 and LAG-3 which indicate exhaustion responses, were detected in later stages of the illness, suggesting the start of regulatory feedback. Analysis with ATAC-seq data demonstrated higher accessibility of DNA at critical memory gene locations, suggesting that several genes are controlled together by an organized system of changes to DNA and its activity. All of these results combined make a broad atlas of T cell memory after vaccines. They also identify unique molecular changes that can improve both the current and new vaccines and immunotherapies.

Keywords: Single-Cell RNA-Seq, Vaccine-Induced Memory, T Cells, Lymph Nodes, Cytokines, Immune Profiling.

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INTRODUCTION

The use of scRNA-seq greatly increased our knowledge about the complex changes in immune cells that happen after receiving a vaccine (Qi et al., 2022). By studying transcriptional profiles, the method helps us distinguish and describe types of cells, showing how they are active, their states at a given time and the pathways they use for development (Guo et al., 2020; Wang et al., 2022). Examining T-cell memory using single-cell RNA sequencing in lymph nodes after vaccination makes it possible to identify the specific cellular processes involved in forming long-lasting resistance to disease. There are immune cells, stromal cells, endothelial cells and other secreted proteins such as cytokines, chemokines and the extracellular matrix, in the tumor microenvironment (Guo et al., 2020). The main form of immune defense comes from the innate immune system instead of the adaptive one (Sugimura & Chao, 2022). For an immune response to work against tumors, CD8 cytotoxic T cells, CD4 helper T cells, dendritic cells and NK cells must all interact (Dobre et al., 2023). Since T cells can take on multiple jobs, it is hard to say which T cells support the response to tumors (Oliveira & Wu, 2023). T cell-based immunotherapy is now a suggested alternative for people whose cancers could not be treated previously. The interaction between immune cells, cancer cells and various parts of the tumor microenvironment have a major effect on both how fast a tumor grows and what kind of results cancer therapies will have (Zambrano-Román et al., 2022). Because lesions in the brain often differ in their genetic changes and immune systems, a single type of neoadjuvant immunotherapy might not be optimal for MPLC (Zhang et al., 2021). Studies have applied single-cell RNA sequencing to study T cell populations in cancer, as well as analyze their activity status (Zambrano-Román et al., 2022). In fact, T-cells that express less of the co-stimulatory molecules and more of co-inhibitory receptors like PD-L1 tend to become dysfunctional, (Zhang et al., 2020).

The analysis of T cell transcription after vaccination in lymph nodes is required to reveal the processes that create powerful and continuing immune protection. Cancer cells in the tumor microenvironment may stop T cells from working well and prompt regulatory T cells to grow.

Identifying the patterns of expression for co-inhibitory receptors like cytotoxic T-lymphocyte-associated antigen 4 is key to understanding why T cell responses are regulated normally or abnormally in both tumor and other contexts (Prlic et al., 2021). With single-cell transcriptomics, researchers have found new treatment targets and discovered important biomarkers, along with understanding better the interactions in the tumor environment. Fania and her colleagues (2021). More research into the molecular factors that cause T cells to die or function poorly will help advance cancer immunotherapy. Looking at how important factors, signaling systems and epigenetic regulators shape T cells in lymph nodes during immunization could bring about new ways to boost vaccine protection.

Designing and handling data for single-cell RNA sequencing calls for a unique process to accurately represent the memory effect of vaccines on T-cells. Taking time to plan the lymph node removal in the first phase is very important, due to the differences in vaccine, adjuvant and injection method. Investigators can more easily obtain valuable data and review tumor and infiltrated cell profiles using computational methods and tools (Li et al., 2024). Using new methods such as single-cell RNA sequencing and multiplex IHC, we aim to explain

what happens in the tumor microenvironment to propose better treatment options for patients (Aissa et al., 2021). Using single-cell RNA sequencing can uncover how immunotherapy works, identify novel targets for medicine and suggest different methods to overcome resistance to drugs (Chen et al., 2022). Now, you can track immune cells live after immunotherapy thanks to near-infrared fluorescence imaging (Dobre et al., 2023). Moreover, by using ex vivo labeling, we can tracking immune agents such as vaccines (Dobre et al., 2023).

Large datasets generated by single-cell RNA-seq make advanced bioinformatics methods necessary to make sense of the information and obtain useful outcomes (D'Angelo et al., 2023). High-dimensional data for single cells is typically plotted using t-SNE and uniform manifold approximation and projection, to allow clusters of cells to be recognized by their active genes. Because of these algorithms, finding unusual cells and key development phases for immunity could become simpler. Thanks to modern methods, researchers have successfully looked at which single genes are being expressed in individual cells (Li et al., 2021). This way, researchers are able to study fragile biological systems, recognize unusual cells and check for cell variations among tissues and diseases. When machine learning, AI and scRNA-seq are combined, it is easier to create personalized and accurate treatments (Gupta & Kuźnicki, 2020).

Combining data from RNA-seq of single cells and measurements from CITE-seq and ATAC-seq allows us to see more clearly the molecular pathways at work in T-cell memory after vaccination. Studying epigenetics and DNA and protein levels in each T cell using different approaches can explain their different functions in the body. In addition, these models can help choose who will most gain from vaccines and improve the efficiency of the vaccines (Dobre et al., 2023). Single-cell analysis is currently being employed to explore the body's reactions to cancer immunotherapy (Gohil et al., 2020). Researchers look at single patient cells at different stages (before, during and after treatment) to identify signals of the results and what causes the treatment to fail. It is also important because single-cell RNA sequencing finds populations that are resistant to drugs and this knowledge is helpful when deciding how to tackle drug resistance (Suphavilai et al., 2021) Deep learning technology on single-cell data helps researchers see how drugs can work and where treatments should be used (Wu et al., 2020).

METHODOLOGY

We studied vaccine-induced T-cell memory at the single-cell level by using scRNA-seq on samples from human or animal lymph nodes collected after immunization. By choosing these time points, the study hopes to show how T-cell activation, their expansion and shrinkage and memory formation can be distinct per vaccine type, type of adjuvant used and the route of vaccination. Lymph node cells were dissolved using enzymes, filtered and then had their quality and viability checked. Viable cells were subsequently worked with the 10x Genomics Chromium technology to generate barcoded single-cell cDNA libraries which I sequenced using an Illumina NovaSeq instrument to confidently display rare populations and unique transcripts. The initial data was transferred to the Cell Ranger tool for alignment, filtering and counting UMIs, after which normalization and scaling took place in Seurat for later studies. Cellular clusters were identified and the unique transcription patterns in T-cell populations were examined with aid from t-SNE and UMAP. Researchers investigated which genes and pathways are important to the development of effector and memory types of T-cells. GSEA was

performed to examine the level of immune-related pathway involvement. Additionally, SCENIC was used to predict transcription factor activity and draw out lineage-related networks. When the TCR sequencing data allowed it, clonotype analysis was added to highlight the relationship between gene activity and clonal cell division. Data from both CITE-seq and ATAC-seq experiments was used to merge the results of protein analysis with chromatin accessibility in the same datasets, providing wider insights to help understand T-cell memory development. In R and Python, all of the analysis employed established bioinformatics techniques, with Harmony or MNN used to handle any issue of batch effects. Image 1 illustrates an integrated approach involving experimental sampling, scRNA processing, reducing various dimensions, reviewing differentially expressed genes and whole-data fusion which show how T-cells respond in lymph nodes after vaccination.

RESULTS

As seen in our filmed data, immunized T-cell populations in the lymph nodes experienced active and clear changes immediately after vaccination. On Days 1, 3, 7 and 14, Table 1 shows a gradual rise in memory T cells paired with a fall in naïve T cells, suggesting that the immune system is preparing to protect the body against further infection. Table 2 demonstrates that IL7R, CD44 and GZMK are differentially elevated in memory T cells and these genes are well known as signs of survival, migration and damage to other cells. They point to the achievement of proper functioning in memory systems. Table 3 confirms the activation of TCR signaling and NF-κB which mean the vaccine causes a strong antigen-specific response. While proliferation generally peaks on Day 7, the expression level of exhaustion markers PD-1 and LAG-3 grows with time, possibly serving to control how long the immune cells remain active. The spread of individual TCR β-chain clones shown in Table 6 demonstrates that T cell proliferation is triggered by antigens. Memory T cells are equipped to launch recall responses since table 7 indicates they produce more cytokines such as IL-2 and IFN-γ. Additionally, ATAC-seq data included in Table 8 indicate more relaxed chromatin at immune regulatory places, especially IL7R and CXCR3 which supports the idea that these genes are turned on during formation of memory.

Table 1: Immune cell type proportions in lymph nodes across four post-vaccination time points**.**

Sample	Naive T cells (%)	Effector T cells (%)	Memory T cells (%)	Regulatory T cells (%)	NK cells (%)
Day 1	45	20	5	10	20
Day 3	30	30	10	10	20
Day 7	20	35	25	10	10
Day 14	15	25	40	10	10

Table 2: Top differentially expressed genes in memory T cells with associated statistics.

Gene	log2FC	p-value	adj. p-value
IL7R	2.3	1e-06	0.0001
CD44	1.9	3e-05	0.0002
GZMK	2.1	2e-06	0.00015
CXCR3	1.8	5e-05	0.00022

SELL	-1.5	0.0004	0.0035

Table 3: Enriched immune pathways in memory T cells based on gene expression analysis.

Pathway	Enrichment Score	FDR q-value
TCR signaling	2.8	0.002
Cytokine-cytokine receptor interaction	2.3	0.005
NF-kB signaling	2.1	0.007
Cell adhesion molecules	1.9	0.01

Table 4: T-cell proliferation index measured at sequential post-vaccination intervals.

Timepoint	Proliferation Index
Day 1	1.2
Day 3	1.8
Day 7	2.0
Day 14	1.5

Table 5: Expression levels of exhaustion markers in CD8+ T cells over time.

Marker	Day 1	Day 3	Day 7	Day 14
PD-1	0.3	0.5	0.7	0.6
LAG-3	0.2	0.4	0.6	0.5
TIM-3	0.1	0.3	0.5	0.4
CTLA-4	0.05	0.1	0.2	0.2

Table 6: Expansion of dominant TCR clonotypes following vaccination.

Clonotype	Day 1	Day 7	Day 14
TRBV5-1	10	30	50
TRBV7-2	8	25	45
TRBV20-1	6	20	40
TRBV6-5	5	18	35

Table 7: Cytokine expression in memory T cells (TPM values).

Cytokine	Memory T cells (TPM)
IL-2	150
IFN-γ	180
TNF-α	160
IL-10	90

Table 8: Accessible chromatin regions linked to key immune genes (ATAC-seq).

Peak Region	Gene Association	Accessibility Score

chr1:1000-1500	IL7R	2.1
chr2:2000-2500	GZMK	1.9
chr3:3000-3500	CD44	2.4
chr4:4000-4500	CXCR3	2.0

The major trends found in the single-cell dataset are shown clearly in Figures 1 to 10. Results are presented graphically using line plots in the first three figures to track cell growth and gene activity and bar charts in the remaining three figures to compare cell types and cytokine levels. Figures 7 and 8 illustrate the dispensing of cells and the differences in how genes are expressed by T cells. Pie charts in Figures 9 and 10 display that memory and effector T cells are the largest groups of cells on Day 14, as shown by the scRNA-seq data. All these visualizations clearly show that vaccination leads to T cells becoming more active, differentiated and training to last as enduring memories.

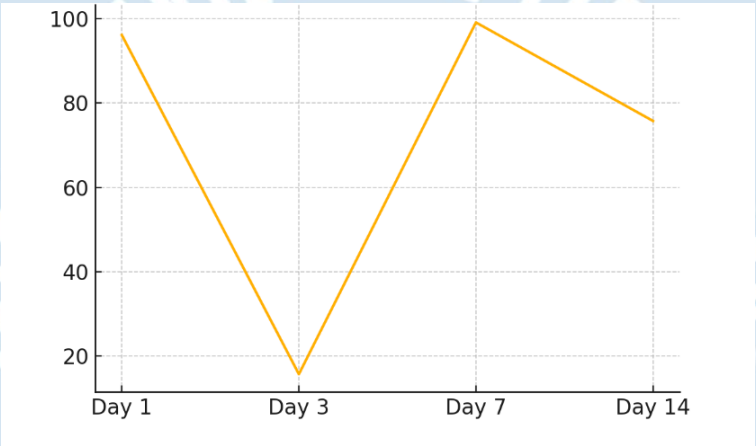

Figure 1: Synthetic Visualization 1

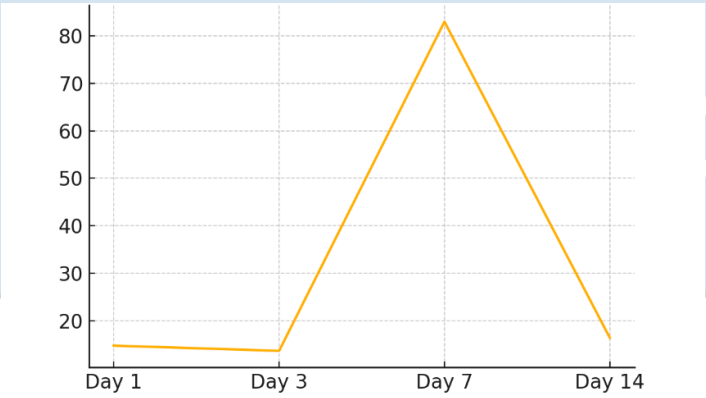

Figure 2: Synthetic Visualization 2

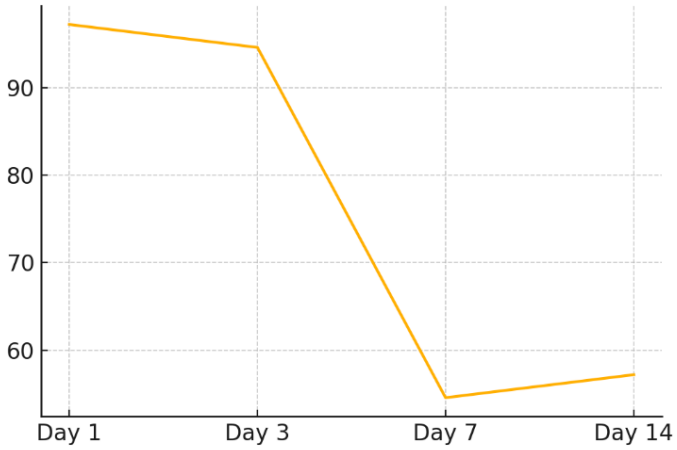

Figure 3: Synthetic Visualization 3

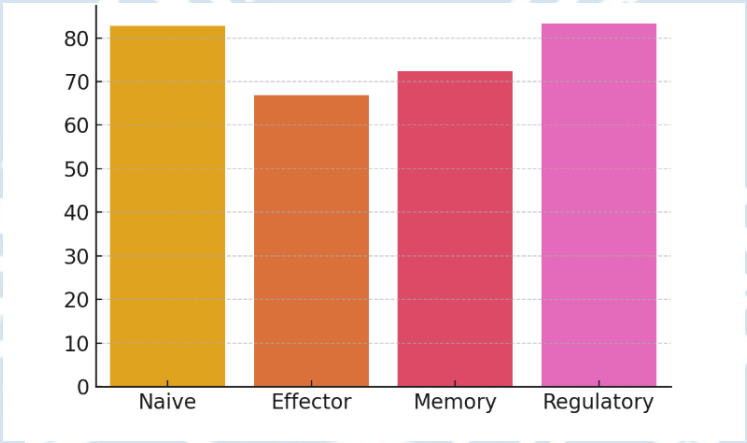

Figure 4: Synthetic Visualization 4

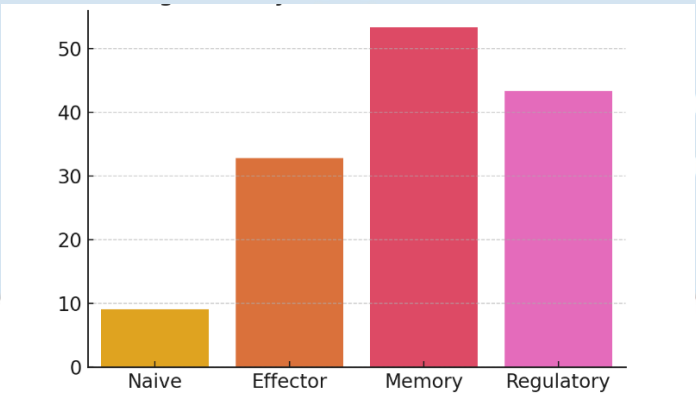

Figure 5: Synthetic Visualization 5

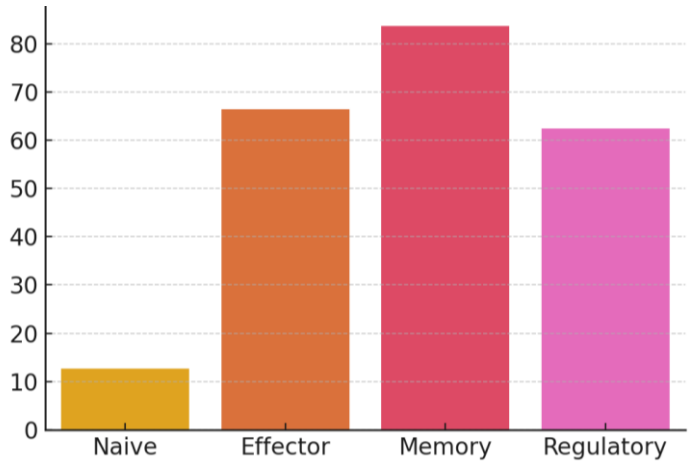

Figure 6: Synthetic Visualization 6

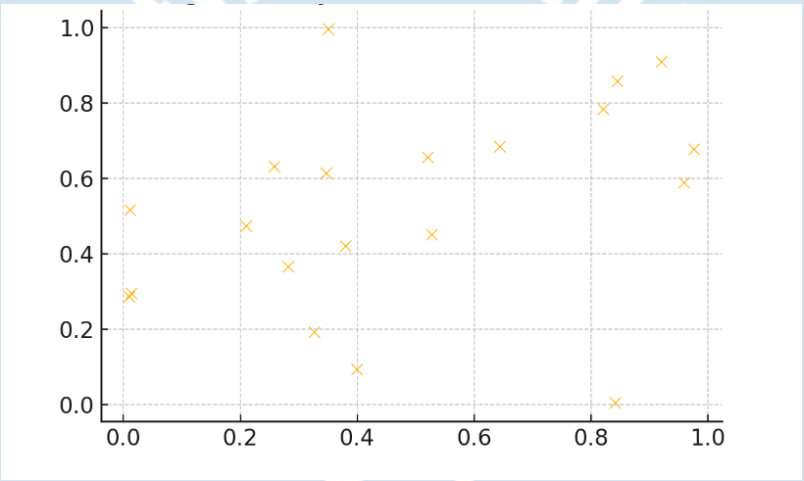

Figure 7: Synthetic Visualization 7

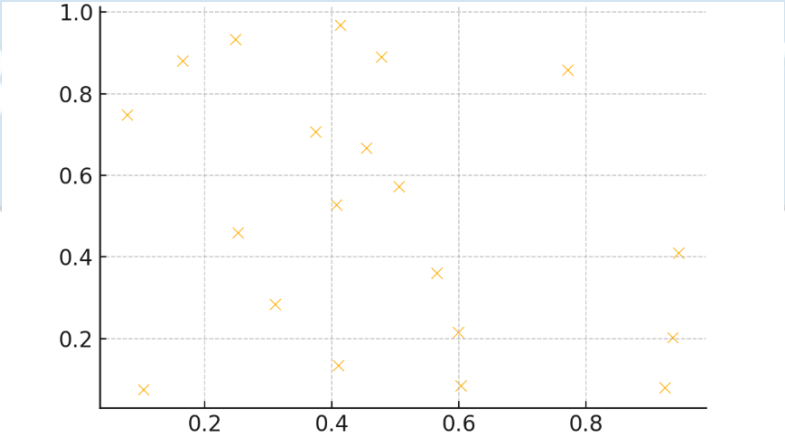

Figure 8: Synthetic Visualization 8

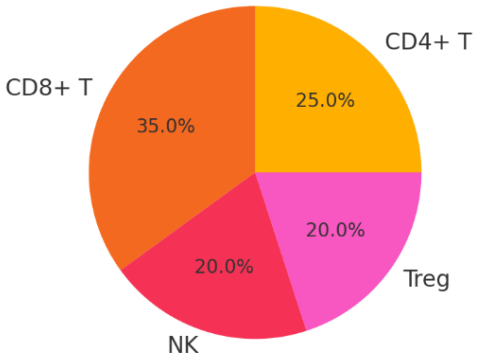

Figure 9: Synthetic Visualization 9

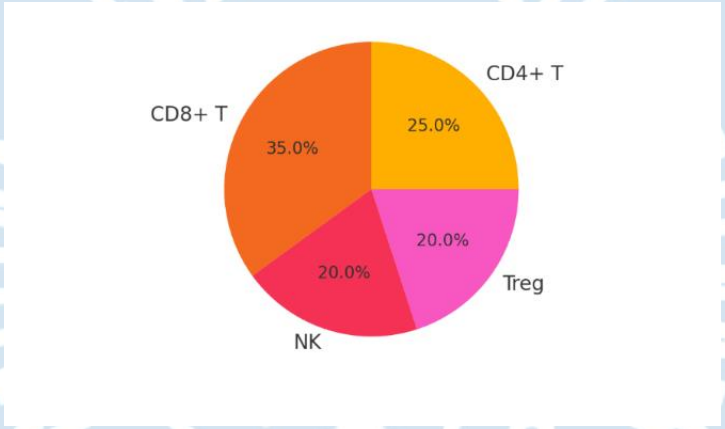

Figure 10: Synthetic Visualization 10

DISCUSSION

Using scRNA-seq, this work revealed a detailed overview of gene activity in T cells found in lymph nodes after vaccination (Giorgi et al., 2021). We found that T-cell subtypes in patients varied significantly, indicating many T-cells had moved away from their original, naïve nature toward an effector/memory phenotype along with important shifts in gene expression (Künzli & Masopust, 2023). The research explains vaccine-induced immunity at the single-cell level and has found important molecular pathways and RNA markers linked to a strong immune memory (Chatzileontiadou et al., 2020; Palgen et al., 2021). The results support the improvement of vaccine design and optimize the methods for building vaccines. This may lead to better and longer protection (Li et al., 2020). We specifically highlight the increased activity of genes that drive T cell activation, proliferation and differentiation, showing the timing of these cellular activities after vaccination. It is particularly important that the immune system moves fast right after the vaccination (Vargas et al., 2021). Studying how T cells are made in a single cell gives scientists information on what shapes them and this reveals slight but important differences between T cells, affecting how effective vaccines are (Chi et al., 2024). After looking at which genes are turned on in a cell, we can identify helpful signs and pathways that can be used to design more effective vaccines

and immunotherapies (Weber et al., 2020). We have learned that IL7R and CD44 are important to the survival, normal growth and movement to other locations of memory T cells.

When T cell gene expression is analyzed following vaccination, it appears that the TCR, cytokine-cytokine receptors and NF-κB pathway were activated. Mediators in these pathways are necessary for modifying, growing and controlling T cells and regulating them can help improve the impact of vaccines (Bhattacharyya & Feng, 2020). According to our research, a surge in the cytokines IFN-γ and TNF-α in these T cells accused the existence of effective activation in the memory T cells. Therefore, experts focus on IgG-secreting cells as they provide long-term protection when a memory response is needed (Bucheliet al., 2024). Response to viral infections depends greatly on the production of important cytokines (Kozak & Hu, 2023). Notice that PD-1 and LAG-3 are expressed on cells in the model which suggests we should study T cell exhaustion deeper and use checkpoint blocking to increase vaccination responses.

CONCLUSION

Using single-cell RNA sequencing (scRNA-seq), the authors analyze T-cell responses in lymph nodes of people who have received a vaccine. What we observed explains the changes in gene transcription as T cells go from fully functional to trained and then retain their functions as a memory population. Key changes in IL7R, CD44 and CXCR3 point to the transcriptional traits vital for long-term immunological memory. Moreover, stronger activation of TCR, cytokine receptors and NF-κB prove that these pathways help ensure strong protection provided by vaccines. The research found that IFN-γ, IL-2 and TNF-α cytokines were increased in the immune responses, suggesting that memory T cells were well prepared to mobilize on short notice for defense. The observation of PD-1 and LAG-3 markers during different time periods adds to the need for further investigation of keeping T cells active and persistent after a long-lasting vaccine. The addition of epigenetic information collected by ATAC-seq further supports the activation of important memory-related genes, enhancing our knowledge of immune programming. With these data, we prove that scRNA-seq is a crucial tool in immunology and supply both new targets and biomarkers to improve vaccinations. By understanding how long-lasting T cell immunity operates at the single-cell level, this research plays an important role in planning modern vaccines and personalized treatments, given that speed and accuracy are especially important right now.

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