Characterization of type 2 diabetes-related immune response heterogeneity to COVID-19 vaccines via single-cell landscape analyses

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Abstract

Vaccines provide the most ideal protection against COVID-19, but variants of concern are highly transmissible and less sensitive to vaccine-elicited antibodies. It is unknown whether patients with type 2 diabetes (T2D) can develop vaccine-elicited immune responses equal to those in healthy individuals. Impaired neutralizing antibodies and spike specific antibodies against SARS-CoV-2 have been identified in T2D patients vaccinated with inactivated vaccine. We therefore investigated changes in the peripheral immune systems of vaccinated T2D patients using single-cell RNA-sequencing. The immune cell phenotypes comprised antigen-presenting (APC), T, and plasma cell reconfigurations. Humoral immune damage to plasma cells exhibited impaired protein synthesis, processing, and transport. APCs were characterized by inflammation following human leukocyte antigen downregulation, neutrophil recruitment, and type I interferon pathway defects. Oxidative stress yielded abnormal hyper-inflammation comprising hyper-cytotoxic CD4+ and CD8+ T cells. There was also a decreased subset of SARS-CoV-2-specific B and T cell receptors. These results provide mechanistic insights into the COVID-19 vaccine-elicited immune responses of T2D patients.

Introduction

Diabetes is a tremendous health problem worldwide. It is caused by chronic high glucose levels in the blood as a result of the incapability of beta cells (β cells) in the pancreas to produce adequate insulin or ineffective insulin utilization by cells in the body. Unfortunately, elevated blood sugar levels can also impair immune function. Several studies have demonstrated that stimulation of PBMCs in diabetes patients inhibits the secretion of cytokines involved in host immune defense, including interleukin-1β (IL-1β), IL-2, IL-6, IL-10, interferon-gamma (IFN-γ), and tumor necrosis factor-α (TNF-α)2–5. Moreover, diabetes patients also exhibit varying degrees of damage to immune cell activity and function. Firstly, hyperglycemia in diabetes leads to neutrophil dysfunction, including defective reactive oxygen species (ROS) production6, neutrophil degranulation injury7, immunoglobulin-mediated conditioning inhibition8, reduced phagocytosis and defective neutrophil extracellular traps (NETs) formation9. Furthermore, chronic hyperglycemia is associated with complement receptor and Fcγ receptor defects on isolated monocytes, resulting in impaired phagocytosis10. In vitro studies have shown reduced antimicrobial activity and phagocytosis in macrophages treated with high glucose derived from mouse bone marrow11. Additionally, hyperglycemia affects T cell function, leading to decreased secretion of effector factors (IFN-γ and TNF-α) by T cells in diabetic patients3,4. Consequently, the dysfunctional immune response in diabetic patients impairs their ability to control the spread of invasive pathogens, leading to an increased prevalence of disease and associated complications12,13.

COVID-19 caused by SARS-CoV-2 has posed a significant threat to global health. The majority of infected patients remain asymptomatic or experience mild symptoms, and mortality rates are generally lower than 1%14,15. Due to the metabolic, inflammatory and immune dysfunction of T2D patients, individuals with diabetes face a higher risk of adverse consequences from COVID-19 infection. In fact, the mortality rate...
for patients with diabetes is twice that of the general population, and they are more likely to require hospitalization and intensive care\textsuperscript{16}. Given these factors, it is highly recommended that diabetes patients receive the COVID-19 vaccination to mitigate the severity of their outcomes.

The induction of a robust and long-lasting neutralizing antibody response following the immunization of large segments of the population with approved COVID-19 vaccines is limiting viral transmission and decreasing mortality\textsuperscript{17,18}. However, the rapid spread of the highly contagious VOCs of SARS-CoV-2 along with its high number of mutations in the spike gene has raised alarms about the effectiveness of current medical countermeasures\textsuperscript{19}. Patient-intrinsic characteristics might influence immune responses to COVID-19 vaccines and thus the efficacy of vaccination\textsuperscript{20}. In particular, male sex\textsuperscript{21,22}, obesity\textsuperscript{23}, cardiovascular disease, and T2D\textsuperscript{24} are frequent conditions observed in fully vaccinated patients developing COVID-19. T2D patients suffer from chronic systemic low-grade inflammation, cellular senescence, immunoglobulin glycation and abnormalities in the number and function of adaptive immune cells, which all increase the probability of vaccine escape in these patients\textsuperscript{25,26}. There is growing evidence of a significant reduction in the neutralizing antibody potency and immunogenicity of influenza and hepatitis B vaccines in T2D patients\textsuperscript{27–29}. Whether individuals with T2D can mount an equivalent serological response to the inactivated COVID-19 vaccine compared to probands without underlying disease is not yet known.

Here, we examined the levels of SARS-CoV-2 IgG and neutralizing antibodies in 40 T2D patients and 60 healthy controls after vaccination with the inactivated-virus vaccine. Subsequently, after accounting for gender, age, and other underlying diseases, we selected peripheral blood from 8 T2D patients and 5 healthy controls for scRNA seq analysis to characterize changes in peripheral immune cell types and gene expression. Based on the analysis of immune subpopulations, gene expression signatures, transcriptional regulatory networks, cell-cell interactions and BCR/TCR databases, we provide an atlas map of the peripheral immune system and clarify the reasons for the dysfunctional response of T2D patients to COVID-19 vaccination. Overall, this study revealed differences in immune response to inactivated vaccines between T2D patients and healthy populations. We also explored the internal factors influencing vaccine responsiveness, shedding light on the impact of immune deficiency in T2D patients on vaccine-induced protective immune response.

**Materials and Methods**

**Patients and samples**

We enrolled 40 diabetic participants and 60 healthy controls who had all received their first dose of the SARS-CoV-2 inactivated-virus vaccine between 7 May 2021 and 13 July 2021, and had never been infected with SARS-CoV-2. Fasting plasma glucose and glycated hemoglobin (HbA1c) data were collected from the electronic health records (EHRs) of the patients. A total of 100 participants were enrolled and vaccinated with 4µg dose of inactivated SARS-CoV-2 Vaccine (Vero Cell). Inactivated SARS-
CoV-2 Vaccine (Vero Cell) (China Biotechnology Group Corporation) was administered intramuscularly into the deltoid. All vaccines were approved by the National Institutes for Food and Drug Control of China. The details of the patients are summarized in Fig. 1b and Supplementary Table 1. The participants provided blood samples 2 weeks after their second and third doses of the vaccine. Given the effects of gender, age, and underlying diseases (hypertension, hyperlipidemia, cardiopathy, etc.) on the neutralizing antibody response, we selected 13 participants of both sexes, aged 40–60 years, for subsequent analyses with or without T2D as a single variable. Peripheral blood mononuclear cells (PBMCs) were obtained from 13 participants, including eight patients with T2D and five healthy controls, and subjected to a single-cell sequencing analysis on day 14 following immunization with the third dose. The PBMCs were collected using a Ficoll–Hypaque density solution according to the standard density gradient centrifugation method and sequenced using the 5’ sequencing platform (10x Genomics). Serum samples were collected and stored at −80°C until further use. All samples were processed within 3h, and their cell viabilities exceeded 80%.

Neutralization assay

The SARS-CoV-2 live WT virus (NC_045512.2) or pseudotyped viruses based on the WT virus or VOCs (Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529)) were incubated with serial dilutions of heat-inactivated human serum samples in duplicates for 1 h at 37°C. Viral and cell-only controls were also included. Trypsinized 1x10^6 Vero E6 cells were then added to each well, and the plates were incubated at 37°C for 72 h to allow plaque development, after which the CPEs were assessed. The serum neutralizing antibody titer was defined as the reciprocal of the highest dilution showing a 100% CPE reduction compared to the viral control. Virus- and cell-only controls were included in each neutralization assay plate.

Enzyme linked immunosorbent assay (ELISA)

Certified High Binding Polystyrene microplates (Costar) were coated with 2 µg/mL of the SARS-CoV-2 S recombinant protein in 1× ELISA Coating Buffer (Solarbio) overnight at 4°C. After blocking with 2% bovine serum albumin (Sigma) in 1× PBST, serially diluted samples starting from 1:100 dilutions were added to the plates and incubated for 2 h at 37°C. The plates were washed four times (5 min each) with PBST, after which horseradish peroxidase (HRP)-conjugated mouse anti-human IgG (Sino Biological Inc.) was added for 1 h at 37°C. The plates were developed with TMB Single-Component Substrate Solution (Solarbio), after which 50 µL of stop solution were added. Optical density was measured at 450 nm using a microplate reader (Thermo-Fisher Scientific).

Single-cell RNA sequencing

Single-cell suspensions were converted to barcoded scRNA-seq libraries using the Chromium Single Cell 3’ Library and Gel Bead & Multiplex Kit (10x Genomics), following the manufacturer's instructions. In brief, the cells were partitioned into Gel Beads in Emulsion in the Chromium™ Controller instrument where cell lysis and the barcoded reverse transcription of RNA were conducted. Libraries were prepared using 10x
Genomics Library Kits and sequenced on an Illumina HiSeq X platform, which produced 150-bp paired-end reads.

**Raw data processing and quality control**

Raw reads were processed to remove low-quality reads using fastQC and fastp, and gene expression profiles were generated using Cell Ranger v.3.0.2. Poly-A tails and adapter sequences were removed using Cutadapt. The reads from the 10× library was then mapped to the Genome Reference Consortium Human Build 38 (GRCh38) with ensemble version 92. Gene annotation was performed using STAR. Gene and Unique Molecular Identifier (UMI) counts were acquired using featureCounts.

**Dimensionality reduction and clustering analysis**

Seurat v3.1.2 was used for dimension reductions and clustering\(^\text{30}\). Gene expression was normalized and scaled using NormalizeData and ScaleData. The top 2,000 variable genes were selected using FindVariableFeatures for a principal component analysis (PCA). The cells were separated into 37 clusters by FindClusters by using the top 20 principal components (PCs) and a resolution parameter of 1.2. For subclustering of cell types, we set the resolution at 1.2. UMAP algorithm was applied to visualize cells in a two-dimensional space.

**Differentially expressed genes (DEGs) analysis and cell type annotation**

Genes expressed in more than 10% of the cells in a cluster and with an average log fold change (logFC) of greater than 0.25 were identified as DEGs in Seurat v3.1.2 FindMarkers based on a Wilcoxon likelihood-ratio test with default parameters. The cell-type identity of each cluster was determined based on the expression of canonical markers found in the differentially expressed genes (DEGs) combined with previously published findings. For the plasma cells, we used a parameter resolution of 0.6 in FindClusters and grouped the cells into four clusters (plasmacell1, plasmacell2, plasmacell3, and plasmacell4).

**Pathway enrichment analysis**

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using the clusterProfiler R package version 4.0.2\(^\text{31,32}\). Pathways with adjusted p_adj value < 0.05 were considered significantly enriched. A gene set enrichment analysis (GSEA)\(^\text{33}\) was performed on the CD8 Naïve T, CD4 Teff, and CD8 Teff clusters using the average gene expression of each cell type. For GSVA pathway enrichment analysis, the average gene expression of each cell type was used as input data using the GSVA package 1.34.0\(^\text{34}\).

**TCR and BCR V(D)J sequencing and analysis**

TCR/BCR sequences were assembled against the GRCh38 reference genome and quantified using the Cell Ranger (v4.0.0) V(D)J protocol. In brief, a TCR/BCR diversity metric containing clonotype frequencies and barcode information was obtained. For the TCRs, only cells with one productive TCR α-chain (TRA)
and β-chain (TRB) were retained for further analysis. Each unique TRA-TRB pair was defined as a clonotype. BCR clonotypes were identified in analogy to the TCRs. Only cells with one productive heavy chain (IGH) and light chain (IGL or IGK) were retained. Each unique dominant IGH-IGL (or IGK) pair was defined as a clonotype. If one clonotype was present in at least two cells, the cells harboring this clonotype were considered to be clonal, and the number of cells with such pairs indicated the degree of clonality.

**Trajectory analysis and RNA velocity**

To investigate the characteristics of the differentiation or conversion of the T cells in patients with T2D, a pseudotime trajectory analysis was performed using Monocle2 2.10.0. The top 2,000 highly variable genes were selected from nine clusters to construct the trajectory using FindVariableFeatures in Seurat v3.1.2. Dimension reduction was performed using DDRTree. The trajectory of T cell development was mapped to UMAP and visualized using plot_cell_trajectory. To assess RNA velocity, a BAM file containing the T cells and reference genome GRCh38 (hg38) were used in velocyto and scVelo in Python with default parameters. The results were projected onto the UMAP plot from the Seurat clustering analysis for visualization.

**Cell–cell communication**

Cellphone DB (version 2.1.7) is a Python-based computational analysis tool that allows for the identification of cell–cell interactions between different cell types based on known ligand-receptor pairs. The permutation number for calculating the null distribution of the average expression of ligand-receptor pairs among randomized cell identities was set to 1,000. Individual ligand or receptor expression was subjected to a cutoff based on the average log gene expression distribution for all genes across each cell type. Only the receptors and ligands expressed in a percentage of cells in specific clusters exceeding user-specified thresholds were considered significant (default was 0.1). As described above, six connected components were investigated to determine intercellular gene interaction networks.

**UCell analysis**

UCell is an R package based on the Mann-Whitney U test and calculates gene signatures in single-cell datasets. We used the UCell score to evaluate the degree to which individual cells expressed a certain gene set. The T-cell-inflamed gene set was used as a gene signature input to calculate the UCell score for each cell. UCell boxplots were grouped by different samples and cell types.

**Statistical analysis**

The demographic data were described as medians and interquartile ranges (IQRs) when continuous, or as frequencies and proportions (%) when categorical. Differences between the continuous and categorical data were tested using the Wilcoxon signed-rank test (or Student’s t-test) and Chi-squared test, respectively. The Wilcoxon signed-rank test in SPSS v25.0 was used to compare the geometric mean titers (GMTs) between two cohorts. GraphPad Prism v8 was used to plot the figures. The R package ggpdb (compare_means) was used for statistical testing, and Student’s t-tests were performed to
compare the cell distributions between two groups. All statistical analyses were two-sided. Differences with P-values < 0.05 were considered statistically significant.

Results

Diminished humoral response to COVID-19 vaccinations in patients with T2D

To determine the difference in responsiveness of healthy individuals and diabetes individuals to SARS CoV-2 inactivated vaccine, the levels of antibodies in patients with T2D and healthy individuals, 100 participants (median age, 42 years; IQR, 24–58; 50.5% female) were assessed. All volunteers were vaccinated with 3 doses of inactivated vaccines as the vaccination plan, the median time between the second and third doses was 24.75 weeks (interquartile range, 24.42-26.00 weeks). Peripheral blood samples were collected 2 weeks after the participants had been vaccinated with inactivated SARS-CoV-2 (Vero cell) (Supplementary Table 1).

The geometric mean titers (GMTs) between the vesicular stomatitis virus vector-based pseudotyped virus systems bearing the SARS-CoV-2 Wuhan-1 spike protein and Wuhan-1 strain were compared to investigate neutralizing antibodies (Extended Data Fig. 1a). There was a high correlation between the two approaches. After receiving the second dose of the vaccine, the neutralizing antibody titers of both volunteers with and without T2D were found to be very low. However, it was observed that the GMT of T2D patients was significantly lower compared to the healthy group (P < 0.01) (Fig. 1a). Additionally, it is worth noting that 40% of volunteers with T2D showed no signs of neutralization after 14 days of the second vaccination (Fig. 1a, Supplementary Table 1). As expected, the neutralization titer of 100 volunteers were significantly higher after receiving the third dose compared to the second dose, highlighting the importance of booster shots in generating protective antibodies. In addition, it was observed that the increase in serum neutralization titers among T2D patients after the third dose was relatively small and significantly lower than that of the healthy volunteers (P < 0.0001) (Fig. 1a). These findings suggest that T2D patients had significantly lower levels of neutralizing antibodies compared to the healthy volunteers, regardless of they received 2-dose or 3-dose inactivated vaccines.

Given that the patients with T2D produced significantly lower neutralizing antibody titers than the healthy controls, we hypothesized that this could lead to sub-protective neutralizing responses against the VOCs (Alpha, Beta, Gamma, Delta, and Omicron). Therefore, serum neutralizing antibodies against pseudotyped viruses were examined across two groups following the administration of the third vaccine dose. The neutralizing antibody titers against the VOCs were reduced in all the participants, and the titers in the patients with T2D were significantly lower than those in the healthy controls (Fig. 1b, c). In the healthy controls, the neutralization titers against Omicron (GMT = 59), Delta (GMT = 64), and Gamma (GMT = 85) were 3.0-, 2.7-, and 2-fold lower than that against the WT (GMT = 175). Conversely, the neutralization titers against Alpha (GMT = 102) and Beta (GMT = 110) were only slightly lower than that against the WT.
(Fig. 1b). In the patients with T2D, the neutralizing antibody titers against Omicron (GMT = 28), Delta (GMT = 33), Gamma (GMT = 42), Beta (GMT = 47), and Alpha (GMT = 60) were 3.3-, 2.8-, 2.2-, 2.0-, and 1.5-fold lower than that against the WT (GMT = 92). The neutralization titers against the WT and Omicron were 1.9- and 2.1-fold greater in the healthy controls than in the patients with T2D (Fig. 1b).

Approximately 32.5% (13 of 40) of the serum samples obtained from the patients with T2D had neutralization titers against Omicron that were below the threshold set in our study (1:20; Fig. 1b), and 15% and 20% of the patients with T2D showed no evidence of neutralization against Delta and Omicron, respectively (Fig. 1c). Furthermore, there was a consistent trend between the SARS-CoV-2 spike protein-specific IgG and the neutralizing antibodies. The anti-S IgG antibody titers were lower in the patients with T2D than in the healthy controls (Fig. 1d). The titers of the total IgG, IgG1, and IgG2 against the spike protein were also significantly lower in the patients with T2D than in the healthy controls (Fig. 1d). There are differences in serum neutralizing antibody titers among volunteers of different age groups (Fig. 1e). The results showed that there was no significant difference in neutralizing antibody titers against WT, Delta, or Omicron between T2D patients and healthy individuals under the age of 40 (Fig. 1f), but the difference was extremely significant over the age of 40 (Fig. 1f). In conclusion, our results indicate that T2D patients have insufficient neutralizing antibody response to COVID-19 vaccine, especially in terms of cross-protection against VOCs.

Phenotypic remodeling of peripheral immune cells in patients with T2D following COVID-19 vaccination

In order to elucidate the intrinsic factors that affect vaccine immune efficacy in T2D patients, we used single cell sequencing technology to analyze the peripheral immune system of volunteers. Due to the more significant impact of T2D on volunteers aged 40 and above, and taking into account the additional effects of gender, age, medication, and underlying diseases (hypertension, hyperlipidemia, cardiopathy, etc.) on the neutralizing antibody response, we selected 13 participants of both sexes, aged 40–60 years, for subsequent analyses with or without T2D as a single variable (Extended Data Fig. 1b). The 13 volunteers we selected contained 8 T2D patients and 5 healthy volunteers. Both neutralizing and anti-S-specific antibodies were significantly lower in T2D patients than in healthy volunteers (Fig. 2a, b), which facilitated our analysis of the variability of immune responses. To gain insights into the diabetes-related abnormalities of the peripheral immune response, we established the peripheral immune cell transcriptional profiles, combined with TCR or BCR databases, based on the 10× Genomics 5′ sequencing platform (Fig. 2c). A total of 165,807 high-quality single cells from all PBMCs samples were collected, the transcriptomes of 12 major cell types or subtypes were captured to perform differential expression
analysis amongst all cell types. The data were downsampled by uniform flow shape approximation and projection (UMAP) and clustered based on graphs, single-cell identification of cell types (Single R) and canonical genetic marker-based manual annotation (Fig. 2d, Extended Data Fig. 1c, d).

The alterations in peripheral immune cell profiles of T2D patients can be appreciated by comparing the cell type in the two groups (Fig. 2e, f). Significant changes in the percentages of T (CD3D and CD3E) and B (CD79A and CD79B) lymphocytes were observed in T2D patients compared to healthy controls, and there was no significant change in the proportion of other cell subpopulations (Fig. 2f, Extended Data Fig. 1e, f). There was a clear preference toward downregulation of plasma cells (MZB1 and JCHAIN) in T2D patients compared to healthy controls (Fig. 2g), which was directly related to antibody production. Taken together, the composition of peripheral immune cells in the two groups of subjects could be clearly distinguished, indicating that attenuated immune responses of T2D patients to COVID-19 vaccine was associated with remodeling of the peripheral immune cells.

**Impairment of plasma cell mediated immune response in T2D patients**

Of the 12,422 B cells assessed in the participants, several differences in the relative abundance of the clusters were detected in the two groups (Fig. 2g, h). In our study, there was a high abundance of plasma cells in the healthy controls, but their numbers decreased in the patients with T2D (Fig. 2h). Quantitatively, the relative percentage of downregulated plasma cells appears to be associated with the attenuated immune response of T2D patients to COVID-19 vaccination. We further explored the differences in the enrichment of biological pathways associated with immune response signaling in two groups of plasma cells. The plasma cells from the patients with T2D yielded lower scores for immune response-related pathways, including “Fc receptor”, “immune response-activating”, “antigen binding”, and “complement activation” (Fig. 2i). Most important is the impaired humoral immune pathway, which is closely related to the production of antibodies, including “humoral immune response mediated by circulating immunoglobulin” and “humoral immune response” (Fig. 2i). To gain insight into the mechanism of humoral immune impairment in T2D patients, four sub-clusters of the plasma cells were identified in the UMAP (Extended Data Fig. 2a-c). Inflammation-related genes (S100A9, S100A8 and HIF-1)\textsuperscript{43} and mitochondrially-encoded genes (MT-ATP5, MT-ND3, MT-ATP8, MT-CO3, etc.) in plasma cell sub-clusters of T2D patients were markedly upregulated (Fig. 2j).

A pathway enrichment analysis was conducted on the upregulated genes in each sub-clusters. Compared to the healthy controls, the plasma cell clusters in the patients with T2D showed abnormalities in the “energy supply-related”, “ER-related”, “translation-related”, and “immune response” pathways (Fig. 2k, Extended Data Fig. 2d-f). Cluster 1 was functionally abnormal in pathways related to ribosomes, indicating the inhibition of proteins, RNA synthesis, and transport (Extended Data Fig. 2d). NADH-related mRNA in clusters 2 and 4 was absent (Extended Data Fig. 2e, Fig. 2k), indicating reduced ATP production. Cluster 3 was abnormal in pathways related to intracellular “ATP/GTP”, “ion exchange”, and “protein-nucleic acid metabolism” (Extended Data Fig. 2f). Overall, plasma cells in T2D patients exhibit significant
humoral immune response deficits, accompanied by damage to intracellular metabolic function, energy supply, and ion exchange pathways, which may directly affect the production and secretion of protective antibodies in T2D patients after vaccination.

**B cell-mediated immune memory impairment simultaneously affecting humoral immune response**

The proportion of the CD27^{low} memory B cell subpopulation was increased in patients with T2D compared to that in the healthy controls (Extended Data Fig. 3a), but the molecular levels associated with cell differentiation and regulation of germinal center responses decreased, such as TNFRSF13B, CD27, and AIM2 (Extended Data Fig. 3b). The upregulated networks were enriched for many pro-inflammatory cytokines (KLF2, JUN, and IRF1), which were associated with GO terms related to increased oxidative stress, including “response to hydrogen peroxide”, “response to reactive oxygen species”, and “response to oxidative stress” (Extended Data Fig. 3c). Further dividing memory B cells into 2 subsets revealed the defects were observed in the patients with T2D, including low scores of the “humoral immune response”, “immune response-activating”, “antigen receptor-mediated”, and “immune effector process” pathways in the activated memory B cells (Extended Data Fig. 3d, e), together with decreased “B cell receptor”, “antigen-binding”, “antigen receptor-mediated”, “immunoglobulin complex”, and “external side of plasma membrane” pathways in the memory B cells (Extended Data Fig. 3f). Although memory B cells are not capable of secreting antibodies, they can undergo somatic hypermutation and/or class switch DNA recombination to differentiate into plasma cells upon antigen exposure. The absence of functional factors (CD27 and AIM2) that regulate B-cell differentiation can be considered bona fide hallmarks of plasma cell dysfunction in patients with T2D. We therefore hypothesized that the oxidative stress-driven impairment of memory B cell immune function contributed to immunological memory impairment in patients with T2D, while affecting the humoral immune response pathway.

**Expanded B cells and specific rearrangements of V(D)J genes in T2D patients following COVID-19 vaccination**

To gain insights into humoral immunity in T2D patients and healthy controls, we identified B cells and reconstructed immunoglobulins by determining mRNA co-expression of the variable heavy (IGHV) and light (IGLV) chains and isotypes in single-cell resolution. We reconstructed BCR sequences and analyzed the state of BCR clonal expansion. The detection ratio of BCRs was more than 75% in each cluster (Fig. 3a, b). We observed that the relative quantity of clonotypic B cells decreased in T2D patients compared with healthy samples (Fig. 3c), indicating that B cell activity and humoral immune responses were weakly activated in T2D patients, in agreement with the lower SARS-CoV-2 neutralizing antibody and IgG levels shown in Fig. 1. To gain insights into antibody production in T2D patients, we evaluated the distribution of IgA, IgD, IgG, IgE and IgM in the two groups, and the results showed that IgM was the predominant immunoglobulin in T2D patients (Fig. 3d). Compared to healthy samples, the abundance of IgA and IgG was decreased in T2D patients (Fig. 3d).
To study biased V(D)J rearrangements of the BCR, we further performed an integrated analysis of the usage of V(D)J genes. The specific V(D)J usage frequency was significantly reduced in T2D patients, indicating that B cells might have undergone unique V(D)J rearrangements. We identified a low representation of the IGHV3 (IGHV3-74, IGHV3-7, IGHV3-21) and IGKV3 (IGKV3-20, IGKV3-15) families in T2D patients compared with healthy controls, especially IGHV3-7 and IGKV3-20 (Fig. 3e). The preferred IGKVs were IGKV1D-39, IGKV1-51, and IGKV2-8, the preferred IGLJs were IGLJ2 and IGLJ3, whereas the preferred IGHV4 was IGHV4-59 (Fig. 3e, Extended Data Fig. 4a). Most of the clone types and VH or VJ genes involved are SARS-CoV-2 specific\(^{47–49}\). In our study, the usage frequency of IGHJ6 was high in T2D samples and healthy samples, but the frequency of paired IGHV4-34 gene usage was decreased compared to healthy samples (Extended Data Fig. 3b). The reduced clonotypes included IGHV3-7/IGHJ4 and IGKV3-15/IGKJ3, which were previously reported as having higher usage in severe COVID-19 patients (Extended Data Fig. 4b). Overall, compared to the IgH repertoires of healthy samples, IGHV4-59, IGHV3-74, IGHV3-7 and IGHV3-21 were under-represented in the heavy and light chain repertoires of antigen labeled clones\(^{49,50}\) (Fig. 3e). These results indicate that the decrease in the usage frequency of SARS-CoV-2 specific BCR is the reason for the decrease in specific and neutralizing antibodies in T2D patients after vaccination.

**A S100\(^{\text{high}}\)HLA\(^{\text{low}}\) dysfunctional APC subpopulation associated with an overactive inflammatory response and immune dysfunction**

UMAP visualization of all APCs showed 4 clearly separated clusters (Fig. 4a, b). DEG analysis showed that APCs from T2D patients high expression of mitochondrially encoded and ribosomal genes, as well as decreased expression HLA genes and interferon-stimulated genes (ISGs) such as MX1, ISG15, IFI6, etc. (Fig. 4c). At the same time, classical monocytes expressed inflammation-related transcripts such as S100A8, S100A9, S100A12, IL1B, FOS and CCL4 (Fig. 4c, d)The most obvious finding is that the pathway analysis of APCs in both groups of samples shows defects in the ISG regulated pathway in T2D patients (Fig. 4e,f).In order to better understand the transcriptional differences in each APC cluster between T2D patients and healthy controls, we sought to decipher alterations that occurred in specific APC subsets. We isolated and sub-clustered cDC2 and classical monocytes, followed by pairwise DEG analysis among the two groups (Extended Data Fig. 5a-d). Indeed, the classical monocytes and cDC2 cells were enriched for transcripts of mitochondrially encoded genes and characteristic genes of myeloid-derived suppressor cells (MDSCs) (S100A8, S100A9, IL1B)\(^{14}\) (Extended Data Fig. 5b, d), with low expression of MHC-II (Extended Data Fig. 5e). HLA gene downregulation was also reflected in antigen presentation-related pathways, including “antigen processing and presentation”, “immune receptor activity”, “antigen binding”, etc. (Fig. 4g). Pathway analysis suggested the inflammatory response, monocyte chemotaxis, Toll-like receptor signaling, and NF-κB signaling pathways were enriched in T2D patients (Fig. 4h, i). Importantly, these pro-inflammatory cDC2 and classical monocytes exhibited a neutrophil recruitment phenotype, which can promote the inflammatory response by contributing to neutrophil activation (“neutrophil degranulation”, “neutrophil activation involved in immune response”) (Fig. 4h, i). Based on these results,
we believe that it stands to reason that the APCs of T2D patients have a reduced presentation activity against the SARS-CoV-2 inactivated vaccine.

**Heterogeneity of T cell subpopulations in vaccinated patients with T2D**

T lymphocytes exert multiple biological functions, inducing cellular immunity and promoting or inhibiting antibody production by B cells. We retrieved 111,016 T and NK cells that were sub-clustered into 10 phenotypes (Fig. 5a, b, Extended Data Fig. 6a). Naive CD8+ T cells had the highest density in the healthy controls and were depleted in T2D patients, while the relative percentage of the activated CD4 Teffs peaked in the T2D patients (Fig. 5c-e). The naive CD8+ T cells in the T2D patients exhibited more inflammatory (“NOD-like receptor” and “Th17 subpopulation differentiation” signaling pathways) and metabolic dysfunction signatures (“lysine degradation” and “cGMP-PKG” pathways) than those in the healthy controls (Fig. 5f). Aging is manifested in low-abundance and immune dysfunction-related naive CD8+ T cells51,52, this indicates that both T2D patients and the elderly have characteristics of low immunity. Next, we analyzed the differentiation trajectory of T cells and found that CD4 Teff, CD8 Teff, and proliferating T cells were the final differentiation morphology (Fig. 5g, h). As expected, the T cell differentiation pathway is accompanied by an increase in inflammatory genes (Fig. 5i). These findings may be associated with the attenuated immune response in patients with T2D with persistent inflammation.

**Oxidative stress-driven immune dysfunction of effector T cells in patients with T2D**

Conventional mitochondrially-encoded genes, including MT-ND2, MT-CYB, MT-CO1, and MT-ATP6, were upregulated in the patients with T2D compared to the healthy controls (Fig. 6a). This makes us suspect that oxidative stress affects T cell function and prompted further investigations of major immune cell types. Compared to those in the healthy controls, CD4 Teffs, CD8 Teffs, NK, NKT, GDT, and MAIT clusters in the patients with T2D exhibited the enriched expression of mitochondrially-encoded genes (Extended Data Fig. 5b). To further investigate the transcriptomic changes in the CD4 Teffs between the T2D and healthy participants, we conducted a DEG analysis. The T2D samples were significantly enriched in cytotoxic factors (PRF1, GZMB), inflammatory cytokines (HLA-DRB1), and apoptosis-related genes (BTG1) (Fig. 6b). This a novel CD4 Teff characterized by upregulated pro-inflammatory and cytotoxic genes, which may aggravate immune disorders in patients with T2D53. A similar trend was observed in the CD8 Teffs (Fig. 6c). Amongst several pathways, “2-oxcarboxylic acid metabolism” and “alanine, aspartate and glutamate metabolism” signaling were decreased in the CD4 Teffs (Fig. 6d). The CD8 Teffs of the patients with T2D were further characterized by decreased “FoxO signaling” and "ECM-receptor interaction" (Fig. 6e). Conversely, the “IL-17” pathways and "oxidative phosphorylation" pathways that promote oxidative stress were increased in the CD8 Teffs (Fig. 6e). This oxidative stress-driven metabolic
disorder-related signaling pathway is a dangerous cascade and facilitates the development of T2D, and may contribute to the attenuated immune response of patients with T2D to vaccines.

**Decreased frequency of SARS-CoV-2-specific TCRs in vaccinated patients with T2D**

To study the dynamics and gene preference of TCRs in the patients with T2D and healthy controls, we compared the usage of V(D)J genes across two groups. Although most of the T cells contained unique TCRs, there were varying degrees of reuse patterns in the T2D samples and healthy samples (Fig. 6f). More than 70% of the cells in all subsets further matched the TCR information, except for the NK and GDT subsets (Fig. 6g). TCRs are generated by the rearrangement of variable (V), diversity (D), and joining (J) gene segments for the TCR β chain (TRB). The same occurs for the V and J gene segments and TCR α chain (TRA). We further observed a decreased frequency of various SARS-CoV-2-biased V/J gene segments in the T2D samples, including TRAJ49, TRAJ39, TRAJ43, TRBV27, TRBJ2-7, and TRBJ2-1, which was consistent with a previous study. For all the common TRAV-TRAJ pairs, we performed a differential analysis based on their frequency. A significantly decreased frequency of SARS-CoV-2-specific TRBV-TRBJ pairs was observed in the patients with T2D, including TRBV7-9-J2-3, TRBV7-9-J2-3, and TRBV27-J2-1 (Extended Data Fig. 7b). The selective use of V(D)J genes indicated that different immunodominant epitopes may drive the molecular composition of T cell responses, which may be associated with the T2D-related immune response.

**Hyper-inflammatory cell–plasma cell communication patterns contribute to the attenuated immune response of patients with T2D**

After vaccination, the human body undergoes a complex series of immune regulation and ultimately secretes antibodies. In order to enhance our understanding of the regulatory network between immune cells in T2D patients, we conducted a cellular communication pattern analysis. There were significant alterations in the interactions between the T-B and DC-T cells from the patients with T2D and healthy controls. There were also elevated ligand-receptor interactions between the inflammatory and immunosuppressive factors in the patients with T2D compared to the healthy controls (Extended Data Fig. 8a–b). Thus, we hypothesized that the diabetes-related attenuated immune response was associated with inflammatory cell subpopulations that interfere with antibody secretion by plasma cells via multiple cytokines. Next, we compared the inflammatory scores of the T cell subtypes in the PBMCs. The T cell subpopulations exhibited significantly higher inflammatory scores in the patients with T2D. There were six hyper-inflammatory cell subtypes, including CD4 Teffs, CD8 Teffs, Tregs, NK, monocytes, and cDC2 (Fig. 7a). To identify the cellular interactions, we explored cell–cell communication in T2D using iTALK and CellChat. The cell connectivity networks revealed an increase in hyper-inflammatory cell–plasma cell communication and abundant ligand-receptor interaction-mediated signals in the patients with T2D compared with the healthy controls (Fig. 7b–c, Extended Data Fig. 8c).
In the patients with T2D, specific interactions between the hyper-inflammatory cells and plasma cell clusters almost always involved pro-inflammatory interactions (CCL3 or CCL4 or CCL5/CCR5) (Fig. 7d–e, Extended Data Fig. 8d). CCR5 and its ligands play a critical role in the adipose tissue inflammatory response to obesity by regulating macrophage recruitment and M1/M2 status. Of the ligand-receptor pairs pertaining to the CD4/CD8 Teffs and plasma cell clusters, BTLA/TNFRSF14 and LILRB1/HLA-F, which induce T cells and neutrophil dysfunction, were markedly enriched in the patients with T2D (Fig. 7d–e). Signaling through TNF-related genes (TNFRSF13B/TNFSF13, TNFSF13/TNFRSF1A, and TNFRSF17/TNFRSF13B pairs) was significantly increased in the patients with T2D compared to the healthy controls (Fig. 7f–g). Furthermore, each plasma cell cluster in the T2D samples expressed CXCR3, which can be activated DC-secreted CXCL10 (Fig. 7f). These findings elucidate the molecular basis of potential cell-cell interactions between the primary immune cells and plasma cells in T2D patients, and contribute to understanding the factors that interfere with the secretion of antibodies by plasma cells in T2D patients.

Discussion

Neutralizing antibodies are important in the protection against SARS-CoV-2 infections, as suggested by vaccine efficacy studies, preclinical studies in mice and non-human primates, and data on the early use of convalescent plasma in elderly patients. However, data on COVID-19 vaccine-elicited neutralizing antibodies, especially those against VOCs, in patients with T2D are limited. In a cohort of 100 adults aged 41 ± 17 years, we found an inverse relationship between T2D and neutralizing antibody responses to SARS-CoV-2 inactivated-virus vaccines, the GMT (Wuhan01) were 175 vs 92 for neutralizing antibodies in individuals without diabetes compared to those with T2D patients. This was consistent with a recent study on the BNT162b2 vaccine, diabetics had 13.86 BAU/ml less IgG antibodies and 4.42% fewer neutralizing antibodies than non-diabetics. We further found that patients with T2D are likely to be at greater risk following infection with VOCs, as 32.5% of individuals with T2D have shown no neutralizing activity against Omicron after the third vaccine dose. The continuous evaluation of the durability of neutralizing antibody titers against Omicron is needed to determine whether the titers decline over time, which has been the case with other variants.

Low levels of IgG and neutralizing antibodies were associated with peripheral immune cell remodeling in the patients with T2D and changes in key cell subpopulations, especially plasma cells, T lymphocytes, and APCs (Extended Data Fig. 9). Our first concern is the significant decrease in proportion and the function of plasma cells directly related to antibody production. Consistent with the serological results, the humoral immune related pathways in plasma cells of T2D patients are impaired. In depth analysis revealed abnormalities in the mitochondrial energy metabolism pathway in plasma cells of T2D patients. Robust immune responses are accompanied by increased metabolism and energy consumption. Mitochondria play a central role in energy metabolism and yield ATP through oxidative phosphorylation. Moreover, antibody synthesis by ribosomes, modifications by the endoplasmic reticulum and Golgi apparatus, and extracellular secretions also require energy from mitochondria. We found that the
signaling pathways related to ATP synthesis and membrane transport in the plasma cells of the patients with T2D were significantly downregulated. By integrating our findings with earlier reports and data on CD4+T lymphocytes from patients with SLE\(^75\), we speculated the mechanisms underlying energy deficiencies in plasma cells. The overactivation of mitochondria in plasma cells is accompanied by the mass production of reactive oxygen species and results in damage to the mitochondrial electron transfer chain and reduced ATP production. Eventually, patients with T2D exhibit a deficiency in antibody secretions. This hypothesis is also supported by the theory of the inhibition of NADH activation, ATPase/GTPase activation, endoplasmic reticulum and Golgi transport, and protein targeting to membrane-related pathways. However, our understanding of the mechanisms leading to energy deficiencies in plasma cells remains limited. Therefore, further studies are needed to elucidate the relationship between the poor antibody responses of patients with T2D and plasma cell energy supply.

The low levels of SARS-CoV-2 IgG and neutralizing antibodies were also accompanied by a lower usage frequency of specific BCR/TCR sequences. In contrast to the healthy individuals, low levels of B or T cell clonal expansion were observed in the patients with T2D. Our insights are solely based on the clonal and transcriptomic features of total T and B cell pools subjected to scRNA-seq\(^76\). Based on our results and earlier reports on SARS-CoV-2-specific BCR/TCR sequences, we identified paired heavy and light chains and common antibody clonotypes between the patients with T2D and healthy individuals (including IGHV1-18, IGKV3-20, IGKV3-15, IGKV3-74, TRBJ2-1, TRBHJ2-3, and TRAJ48)\(^{47,48}\). Additionally, various SARS-CoV-2-biased BCR/TCR sequences had lower usage frequencies (including IGKV3-15 and IGKV3-74, and TRBJ2-1 and TRAJ48), which may explain the low levels, but not absence of, SARS-CoV-2-specific antibodies in patients with T2D. Although further research is needed on the B/T lineage repertoire patterns, our findings indicate that patients with T2D can produce neutralizing antibodies and specific binding antibodies after receiving COVID-19 vaccine. However, the decrease in the usage frequency of SARS-CoV-2-specific BCR/TCR also leads to a decrease in antibody levels in the plasma of T2D patients after vaccination.

After vaccination, the human body produces protective antibodies, which involves complex immune responses. When the inactivated SARS-CoV-2 vaccine is administered, it activates the innate immune system and is recognized by APCs, and further triggers humoral and cellular immune responses. For the production of antibodies, APCs need to present sufficient antigens to the germinal center, and T cell helper plasma cells secrete antibody\(^77,78\). There are multiple reports documenting impaired innate immune responses in T2D patients, including dysfunctional neutrophils\(^6–9\), reduced levels of natural killer cell effector molecules NKG2D and NKp46\(^79\), and macrophage phagocytic activity was reduced\(^11\). Similarly, our study focused on the activity of dendritic cells (DCs), the most crucial antigen-presenting cells, and revealed a decrease in antigen-presenting activity and a reduction in effector molecules such as ISGs involved in host defense. These findings indicate the presence of defects in the antigen-presenting pathway in T2D patients. It is highly likely that the defective function of antigen-presenting cells in T2D patients contributes to inadequate recognition of antigenic components in the COVID-19 vaccine, resulting in insufficient antibody secretion. Another important factor in antibody secretion is the immune
response mediated by T lymphocytes, specifically CD4⁺TH1 cells, which are associated with humoral immune response⁷⁸. Our study identified a cluster of CD4 Teffs that are highly toxic and inflammatory, exhibiting immunosuppressive phenotypes in inflammatory environments. This indirectly affects the humoral immune response in T2D patients after receiving the SARS-CoV-2 inactivated vaccine. In conclusion, the production and secretion of antibodies depend on the function of multiple immune cells. In T2D patients, various factors, such as incomplete uptake of vaccine antigens by APCs, immune damage to plasma cells, and dysfunction of T cells assisting humoral immune response, contribute to low levels of neutralizing antibodies after COVID-19 vaccination.

Our data also suggest that the inflammatory response of COVID-19-vaccinated patients with T2D is an additional factor contributing to low levels of antibodies. This is because the plasma cells, T cells, and APCs that have attracted our attention are highly expressed inflammatory genes. T2D patients have an immune system that is in a chronic inflammatory environment for a long time, and this leads to the formation of a malignant inflammatory circulation between the main cell subpopulations¹. The inflammatory immune response in obese adipose tissue is intensified by the interplay between pathogenic CD4⁺ and CD8⁺ T cells, along with CD11c⁺ M1 macrophages. This crosstalk leads to increased inflammation and peripheral insulin resistance, further exacerbated by adipocyte apoptosis and macrophage infiltration⁸⁰,⁸¹. Based on the immune cross-talk among APCs/T cells and plasma cells, which usually involves several pro-inflammatory ligand-receptor pairs (CCL3/, CCL4/, or CCL5/CCR5 and CCR1/CCL3) and immunosuppressive factors (LILRB1/HLA-F)⁵⁶,⁵⁷. Therefore, we can speculate that the communication between T cells, APCs, and plasma cells in the inflammatory microenvironment of T2D patients is abnormal. This abnormal communication may negatively regulate plasma cell immune function through the interaction between ligand receptors and highly inflammatory cells, thereby interfering with antibody production.

A major limitation of this study was that we investigated BCR/TCR gene rearrangements across two groups, and the selection of SARS-CoV-2-specific epitopes was not exhaustive. The study was further limited by a moderate sample size, restricted timing for serum collection, and lack of long-term follow up.

**Conclusions**

In the present study, based on serological analysis of 100 volunteers, we have confirmed that the SARS-CoV-2 inactivated vaccine induces low levels of neutralizing and specific antibodies in T2D patients. Under this premise, we further comprehensively evaluated the immune cell characteristics of T2D patients using the scRNA-seq technique. Our findings revealed changes in different cell compositions from normal samples to patients with T2D. We identified plasma cells with humoral immune impairment, APCs with reduced antigen-presenting activity, and T cells under oxidative stress in T2D patients, and comprehensively analyzed the influencing factors leading to insufficient antibody secretion in T2D patients. This may provide new insights into the development of effective vaccination strategies for patients with T2D.
Abbreviations

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<th>Abbreviation</th>
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<tr>
<td>SARS CoV-2</td>
<td>severe acute respiratory syndrome coronavirus 2</td>
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<td>T2D</td>
<td>type 2 diabetes</td>
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<tr>
<td>COVID-19</td>
<td>Coronavirus disease 2019</td>
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<td>VOCs</td>
<td>variants of concern</td>
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<td>Differentially expressed genes</td>
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<td>Gene Ontology</td>
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<td>GMT</td>
<td>geometric mean titer</td>
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<tr>
<td>APC</td>
<td>Antigen-presenting cell</td>
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Declarations

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of The Second Affiliated Hospital, University of South China, Hengyang, China in accordance with the principles of the Helsinki Declaration (Approval No.: 202109). Written informed consent was received from all participants before sample collection, and all patients received participant compensation.

Consent for publication

All authors consent to publication.

Competing interests

All authors declare no competing interests.

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Authors’ contributions

F.H.Y. designed the experiments; W.Q.W. led the bioinformatics analysis; F.H.Y., S.W., and B.L. performed the experiments; B.L., W.W.H., and S.Q.Y. screened the volunteers and collected the clinical data and blood samples; S.W. helped edit the manuscript; W.Q.W, F.H.Y., and W.W.H. wrote the paper. All authors contributed to the critical revision of the manuscript and approved the final version.

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Table 1
Table 1 is available in the Supplementary Files section.

**Figures**

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Figure 2

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