Journal of Microbiology, Immunology and Infection 58 (2025) 294–303

Contents lists available at ScienceDirect

Journal of Microbiology, Immunology and Infection

journal homepage: www.e-jmii.com

BNT162b2 mRNA vaccine elicits robust virus-specific antibodies but poor cross-protective CD8^{+} memory T cell responses in adolescents with type 1 diabetes

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ARTICLE INFO

Keywords:
Adolescents
BNT162b2 vaccine
Innate immune response
Type 1 diabetes
T cell responses

ABSTRACT

Background: COVID-19 mRNA vaccines have demonstrated 95 % efficacy in the general population. However, their immunogenicity in adolescents with Type 1 Diabetes (T1D), who exhibit weaken immune responses, remains insufficiently explored.
Methods: Longitudinal analysis of innate immune responses following PRR-agonists and BNT162b2 vaccine stimulations, along with S-specific antibody responses, memory T cell recall responses, and RNA-sequencing were assessed in eight T1D adolescents and 16 healthy controls at six different timepoints.
Results: After BNT162b2 vaccination, T1D adolescents produced SARS-CoV-2-specific binding and neutralizing antibodies (Nabs) comparable to healthy controls. Lower pre-vaccination blood HbA1c level correlated with higher antibody responses among T1D adolescents. However, they exhibited impaired TLR9-induced B cells and the first vaccine-induced monocyte activation. These differences were supported by transcriptomic analysis, which revealed the impairment in innate immune-related signatures both before and after vaccination. One year post-second vaccination, T1D adolescents demonstrated compromised cross-protection of T cell against BA.1 compared to healthy controls, which correlated with impaired innate immune responses identified in this study.
Conclusion: This study reveals that while T1D adolescents vaccinated with the BNT162b2 vaccine develop robust S-specific antibodies, their cross-protective T cell responses are suboptimal.

1. Introduction

Diabetes, alongside other chronic medical conditions, stands as a pivotal risk factor for severe coronavirus disease 2019 (COVID-19) and subsequent mortality.[1] Beyond impaired glucose homeostasis, individuals with diabetes exhibit a spectrum of physiological dysfunctions, including chronic inflammation and altered immune status.[2,3] Hyperglycemia, resulting from ineffective blood sugar control in diabetes patients, exerts a multifaceted impact on immune function, encompassing impaired cytokine production, suppressed leukocyte recruitment, compromised pathogen recognition, and diminished functionality of neutrophils, macrophages, and NK cells, alongside inhibition of antibody and complement effects.[4–7] It was recently shown that diabetes induces a hyperglycemia-driven metabolic-immune axis and

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https://doi.org/10.1016/j.jmii.2024.12.009
Received 23 July 2024; Received in revised form 27 November 2024; Accepted 31 December 2024
Available online 3 January 2025

drives impaired antigen presentation and antiviral immunity.[8] Collectively, diabetes and hyperglycemia undermine both innate and adaptive immune responses, thereby diminish the resilience of diabetic patients to pathogen invasion relative to individuals without diabetes.

Emerging evidence underscores the elevated risk of infection and complications among T1D patients, which is chiefly observed in adolescents or children.[9,10] Unlike type 2 diabetes, Type 1 diabetes (T1D) is an autoimmune disease that destroys insulin-producing pancreatic β cells through T cell-mediated mechanisms triggered by environmental factors like infections and diet, as well as genetic factors.[11,12] Patients demonstrate increased proinflammatory activity in monocytes/macrophages and experience hyperglycemia, which further impairs immune function, raising infection risk and lowering T and B cell responses.[13–16] Studies have shown that impaired T cell proliferation and function are also present in newly diagnosed T1D children and insulin-dependent patients.[17] Consequently, children and adolescents with T1D have an increased susceptibility to a range of infections, including respiratory (tuberculosis, pneumococcus, influenza and more severe COVID-19 disease), urinary tract, gastrointestinal, and dermatological infections (Candida and *Staphylococcus aureus* infection).[18] Therefore, clinical guidelines strongly advocate for routine and non-routine vaccination among children and adolescents with T1D to mitigate the risk of infectious diseases. However, immune dysregulation in diabetic individuals not only predisposes them to infections and associated complications but may attenuate the immunogenicity following vaccination. A limited number of studies have examined the extent of immunodeficiency and vaccine efficacy in children and adolescents with T1D, further research is warranted to address vaccine-related concerns within this population more comprehensively.[19,20]

Our prior investigation revealed a correlation between innate immune responses following initial vaccination and subsequent Nabs production in healthcare workers receiving ChAdOx1 nCoV-19 (AZD1222) vaccine. Notably, older individuals exhibited impaired innate immune responses to Toll-like receptor (TLR) stimulation and a subdued or delayed innate immune activation profile post-vaccination compared to their younger counterparts, indicative of immunosenescence.[21] Nevertheless, the mechanisms by which early innate immune reactivation influences overall vaccination immunogenicity remain poorly understood, particularly in children, adolescents, or vulnerable populations such as diabetes patients. To address this gap, we employed a systems vaccinology approach to comprehensively characterize the innate and adaptive immune responses of adolescents with T1D and their healthy controls following administration of BNT162b2 vaccine in the present study.

2. Methods

2.1. Patients and samples collection

Twenty-four adolescents including eight T1D patients and 16 age-matched healthy controls were prospectively recruited. All subjects received two doses of Pfizer–BioNTech mRNA vaccine (BNT162b2) with a minimal interval of 12 weeks. Some of the individuals were infected with SARS-CoV-2 one year post-second vaccination, which were identified based on SARS-CoV-2 antigen-detection rapid diagnostic tests and Elecsys® anti-N assay (Supplementary Table 1). Peripheral blood samples were collected at 6 time points (TP): pre-vaccination (TP1), three days post-first vaccination (TP2), three days the post-second vaccination (TP3), one month post-second vaccination (TP4), five months post-second vaccination (TP5), and one year post-second vaccination (TP6) (Fig. 1A). The protocol of this study was approved by the Institutional

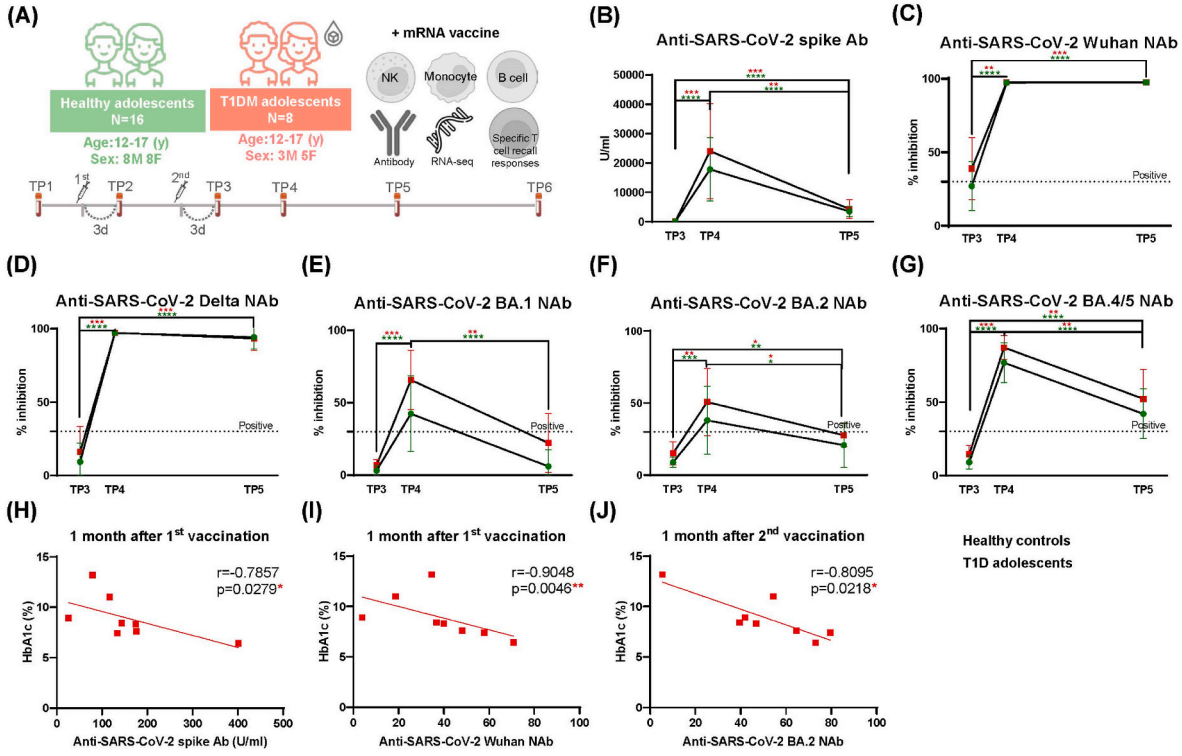

Fig. 1. Antibody responses elicited by BNT162b2 mRNA vaccine in healthy and T1D adolescents. (A) Recruited individuals and study design. (B) Anti-S antibody titers were examined by Elecsys® Anti-SARS-CoV-2 S assay at TP3, TP4, and TP5. The inhibition rate of Nabs against Wuhan (C), Delta (D), BA.1 (E), BA.2 (F), BA.4/5 (G) variants in three time points were analyzed by cPass. The correlation of HbA1c and Nab responses including anti-S antibody titer at TP3 (H), Nab against Wuhan strain at TP3 (I), and Nab against BA.2 variant at TP4 (J) were performed in T1D adolescents. Statistics within one group were calculated using the Wilcoxon test, while differences between the two groups were evaluated using the Mann-Whitney *U* test. The correlation between the two factors was measured using the Spearman rank correlation. Error bars depict ±SD. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

Review Board of National Cheng Kung University Hospital (No. A-BR-110-051).

2.2. Innate immune response after PRR-agonists stimulation and vaccination

Pattern recognition receptor (PRR) responses and vaccination responses of innate immune cell subpopulations were analyzed using flow cytometry, which were modified from the previous study.[21] We analyzed different expressions of cytokines and markers across different subpopulations of immune cells, including $CD56^{dim}$ NK cells, $CD56^{bright}$ NK cell, classical monocytes (CM), intermediate monocytes (IM), non-classical monocytes (NCM), naïve B cells, unswitched memory B cells (USM), and switch memory B cells (SwMe). The modified procedure was described in Supplementary Methods. All reagents are listed in Supplementary Table 2. The gating strategies are demonstrated in Supplementary Figs. 1A–C.

2.3. Anti-SARS-CoV-2 S antibody measurement and neutralizing antibody detection

The Roche Elecsys® Anti-SARS-CoV-2 S assay (Roche Diagnostics, Switzerland) is for quantifying total antibodies against receptor binding domain (RBD) of spike protein (S) in plasma. All procedure were conducted according to the manufacturer's protocol. A concentration of the analyte <0.80 U/mL was regarded as negative, and ≥0.80 U/mL was considered positive. The Nabs against the Wuhan, Delta (B.1.617.2), Omicron sublineages (B.1.1.529/BA.1 and BA.2) strains were detected by the cPass SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript, USA). Nabs against Omicron sublineages BA.4/5 were detected by Anti-SARS-CoV-2, B.1.1.529, Neutralizing Antibody Titer Serologic Assay Kit (AcroBiosystem, USA). The assays were performed according to the manufacturer's instructions. If the percent signal inhibition was ≥30 %, which served as the cutoff value, the results were considered positive.

2.4. RNA-sequencing and data analysis

Total RNA extraction from whole blood preserved in PAXgene® Blood RNA Tube (BD Biosciences, San Diego) were performed using QIAamp RNA Blood Mini Kit (Qiagen) following the manufacturer's protocol. In brief, library preparation were conducted with KAPA mRNA HyperPrep Kit (KAPA Biosystems, Roche, Basel, Switzerland) according to manufacturer's guidelines. The quality of RNA was assessed through Qsep 100 DNA/RNA Analyzer (BiOptic Inc., Taiwan). NGS sequencing was conducted on the Illumina NovaSeq6000 sequencer, producing 150 base pair paired-end reads. The detailed versions of software and analysis are provided in the Supplementary Methods.

2.5. SARS-CoV-2–specific memory T cell recall responses

SARS-CoV-2 specific memory T cell recall responses were examined by activation-induced marker (AIM) assay and bromodeoxyuridine (BrdU) cell proliferation assay after stimulation with SARS-CoV-2 peptide pools. The AIM assay were modified from a previous study,[22] and all reagents are listed in Supplementary Table 3. In brief, PBMCs were stimulated with peptide pools (PepTivator®SARS-CoV-2 Prot_S B.1.1.529/BA.1 WT Reference Pool and Mutation Pool) and stained with activation markers and anti-BrdU-AF488 antibody. Data were acquired using BD FACSCanto II flow cytometry. The details of modified procedure were described in Supplementary Methods. The gating strategy for activated T cells is demonstrated in Supplementary Figs. 1D and E.

2.6. Statistical analysis

Statistical analyses were performed in GraphPad 9. The immune responses after PRR-agonists stimulation within each group were tested using Kruskal-Wallis test followed by Dunn's multiple comparisons test. The different dose of vaccine responses within each group were tested using the Wilcoxon matched-pairs signed rank test. Differences between the two groups were evaluated using the Mann-Whitney *U* test. The correlation of two factors was testing using the Spearman r. A *p*-value <0.05 was considered statistical significance: *p-value <0.05; ** p-value <0.01; *** p-value <0.001; **** p-value <0.0001. Values represent the mean ± standard deviation (SD).

3. Results

3.1. Robust antibody responses in T1D adolescents after BNT162b2 mRNA vaccination

Fig. 1 illustrates the levels of anti-S antibody and Nab against Wuhan and variants of concern (VOCs) at TP3, TP4, and TP5. Anti-S antibody titers significantly increased by approximately 183-fold ($p < 0.0001$) and 154-fold ($p = 0.0078$) at TP4 compared to TP3 in healthy and T1D adolescents, respectively. However, they declined by about 5.08-fold ($p < 0.0001$) and 5.49-fold ($p = 0.0027$) at TP5 compared to TP4 (Fig. 1B). The second vaccination significantly boosted Nab inhibition rates against Wuhan and Delta strains, sustaining above 97 % for five months post-second vaccination in both groups (Fig. 1C and D). While the Nab activity against BA.1, BA.2, and BA.4/5 increased following the second dose, the response proved to be suboptimal and short-lived (Fig. 1E–G). Overall, there are no significant differences in anti-S titer and Nab levels against Wuhan and VOCs between healthy and T1D adolescents across different time points.

In T1D adolescents, their glycated hemoglobin (HbA1c) levels before vaccination were negatively correlated with anti-S antibody titers ($p = 0.0279$) and Nabs against the Wuhan strain ($p = 0.0046$) post-first vaccination (Fig. 1H and I). This correlation extended to Nab responses against BA.2 variants ($p = 0.0218$, Fig. 1J). These results underscore that well-managed blood sugar control prior to vaccination correlates with enhanced antibody responses. Meanwhile, the most common adverse events after vaccination in this group were mild and transient (malaise, muscle soreness, fever), resolving within 48 h. No deterioration in glycemic control was noted afterward.

3.2. Impaired TLR9-mediated IFN-α production of B cell subsets in T1D adolescents before vaccination

To unveil innate immune responses affecting BNT162b2 vaccination efficacy in T1D adolescents, we analyzed immune cell responses to PRR agonists before vaccination. B cell subset percentages showed no significant differences between groups (Fig. 2A). Both groups displayed increased expression of CD86 across all B cell subsets post-stimulation with PRR agonists (Fig. 2B). Despite similar frequencies and CD86 expression in B cell subsets, T1D adolescents had significantly reduced CpG-induced IFN-α expression in naïve ($p = 0.029$) and USM B cells ($p = 0.037$) compared to healthy controls (Fig. 2C). In NK cells, there were no significant differences in either the percentage of subsets or the expression of IFN-γ between the two groups (Supplementary Fig. 2). In monocytes, the expression of CD86, IL-6, and IFN-α were significantly increased in healthy controls after poly(I:C), LPS and CpG stimulation. However, no significant differences were observed between the two groups (Supplementary Fig. 3). Collectively, T1D adolescents have aberrant TLR9-mediated IFN-α production in naïve and USM B cells when compared with healthy controls.

3.3. Suboptimal BNT162b2 mRNA vaccine-induced cytokine production of monocytes subsets in T1D adolescents after the vaccination

We next analyzed the matched innate immune responses three days post-first and the second vaccination and compare them with baseline

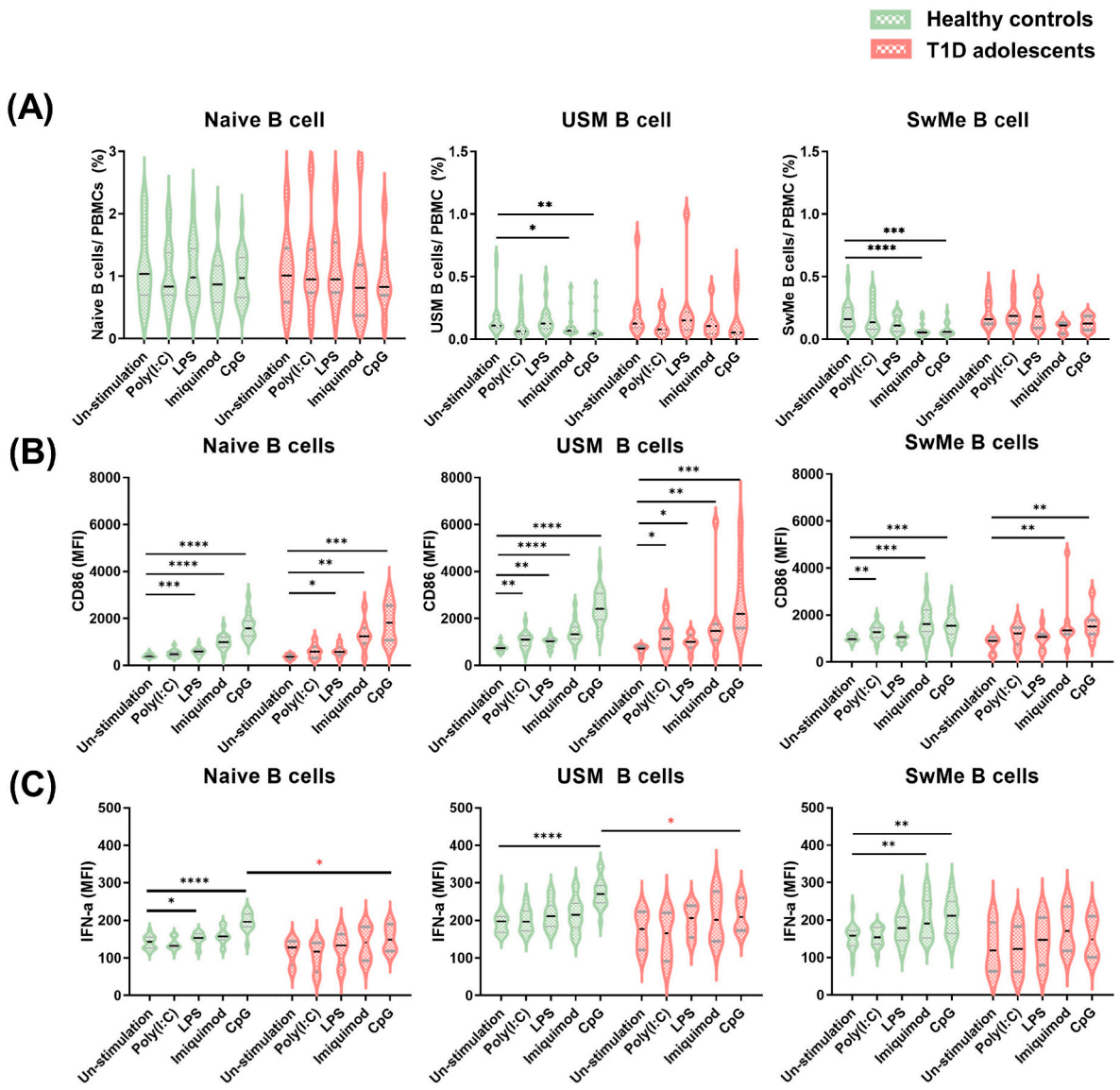

Fig. 2. B cell responses induced by PRR-agonists before vaccination in healthy and T1D adolescents. (A–C) The percentages (A), the expression of CD86 (B), and the production of IFN-α production (C) of naïve B cells, USM B cells, and SwMe B cells were identified with flow cytometry after 48 h PRR-agonists stimulation. The results were showed in violin plots, with the black line indicating the median and the gray lines representing quartile intervals. Statistics within one group were calculated using the Kruskal-Wallis test, while differences between the two groups were evaluated using the Mann-Whitney *U* test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

responses. In monocytes, elevated frequencies and CD86 expression were identified post-second vaccination (Fig. 3A and B). IL-6 expression in CM and IM, and IFN-α expression of IM, were increased post-first vaccination exclusively in healthy controls. T1D adolescents exhibited a significantly reduced IL-6 expression in CM ($P = 0.038$) and in IM ($P = 0.028$), and IFN-α expression in IM ($P = 0.019$) compared to healthy controls. Moreover, IL-6 and IFN-α expression in NCM was elevated after first vaccination, with a more pronounced increase observed post-second dose in healthy controls (Fig. 3C and D). Both groups exhibited elevated IL-10 expression in monocyte subsets post-first vaccination (Fig. 3E). The subsets frequency of $CD56^{dim}$ NK cells and IFN-γ expression of $CD56^{dim}$ and $CD56^{bright}$ NK cells were consistently increased in T1D and healthy adolescents after each dose of vaccination (Supplementary Fig. 4). In B cells, both groups had increased frequency three days post-second vaccination, while no significant elevation of CD86 and IFN-α expression (Supplementary Fig. 5). Together, the results suggest that T1D adolescents have compromised first BNT162b2 vaccine-induced IL-6 and IFN-α production in monocyte subsets compared to healthy controls.

3.4. Transcriptomic analyses revealed lower BNT162b2 mRNA vaccine-induced innate immune activation in T1D adolescents

We performed RNA-seq on blood samples from 4 healthy and 4 T1D adolescents at three time points: pre-vaccination and three days post-first and second doses. In differential gene expressions (DEGs) analysis, 36 common up-regulated DEGs and two common down-regulated DEGs were identified between healthy and T1D adolescents (Fig. 4A). Those correlated with COVID-19 vaccine or SARS-CoV-2 infection responses were listed in the heatmap.[23–26] Most of the genes exhibited lower up-regulation in T1D adolescents, including interferon-stimulating genes (ISGs), FCGR1A, and LY6E (Fig. 4B). The volcano plots showed that both healthy and T1D adolescents exhibited up-regulated ISGs and complement-related genes three days post-second vaccination compared to baseline. Notably, T1D adolescents exhibited down-regulated DEGs including MZBI, TNFRSF17, and several

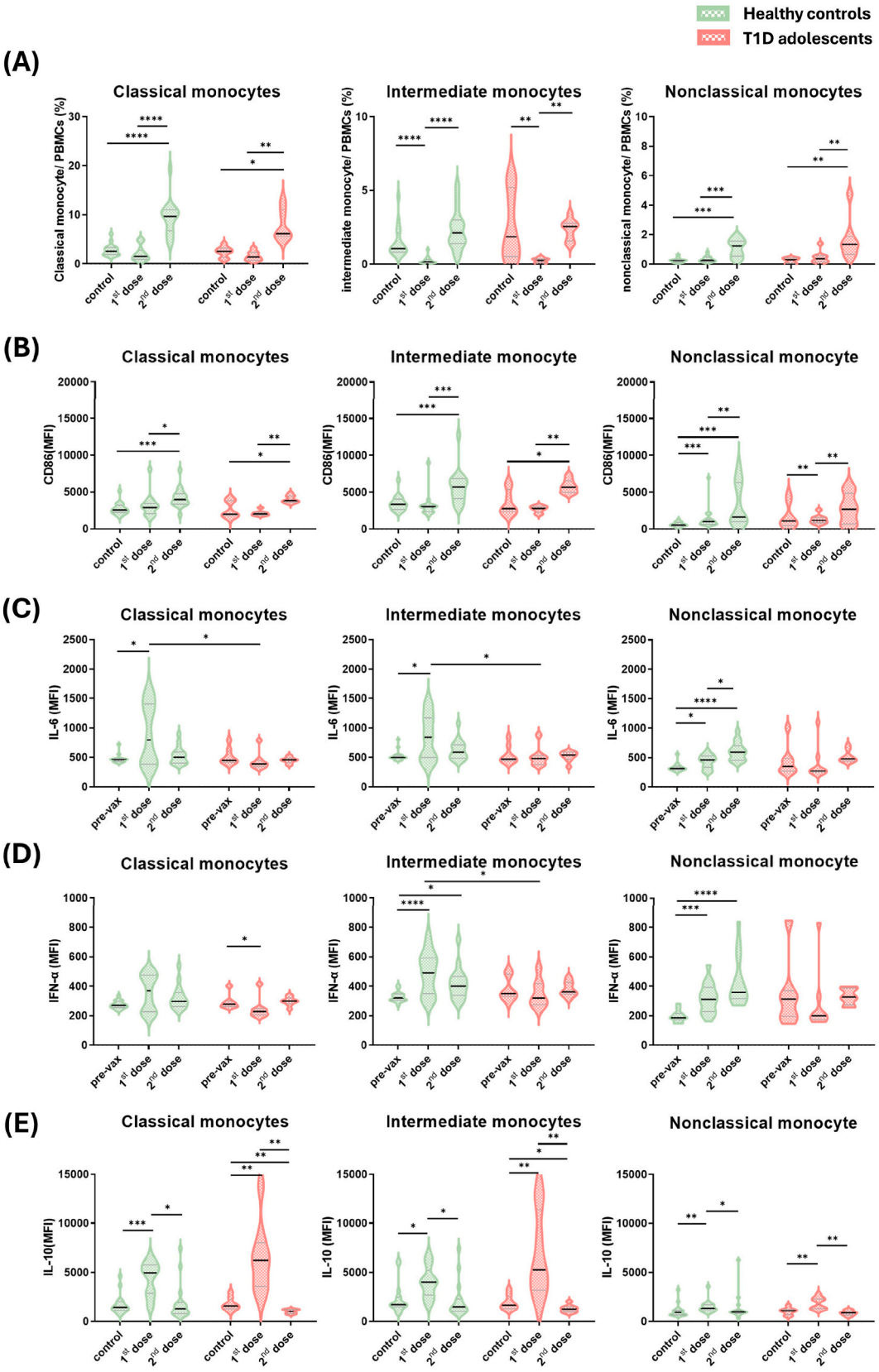

Fig. 3. Monocyte responses of healthy and T1D adolescents elicited by the first and second BNT162b2 vaccination. (A–E) The percentages (A), the expression of CD86 (B), the production of IL-6 (C), the production of IFN-α (D), and the production of IL-10 (E) in CM, IM, and NCM were examined by flow cytometry before vaccination and three days after vaccination. The median (black lines) and quartiles (gray lines) were presented in violin plots. Statistics within one group calculated using the Wilcoxon test, while differences between the two groups were evaluated using the Mann-Whitney *U* test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

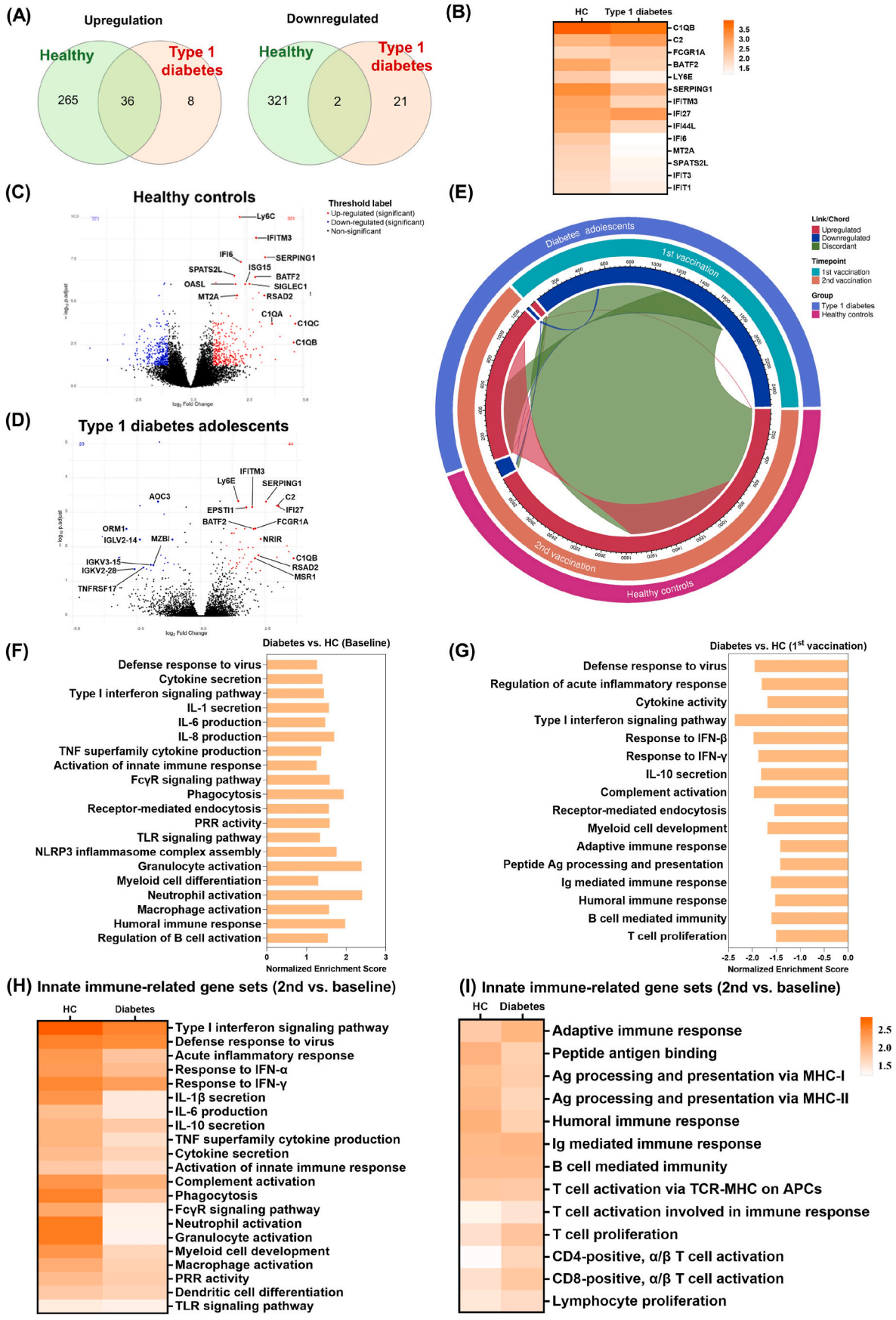

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Fig. 4. Transcriptional signatures of healthy and T1D adolescents before and after BNT162b2 mRNA vaccination. (A–D) The DEGs (adjusted *p*-value <0.05, absolute log_2-transformed fold-change (|log2FC|) ≥ 1) observed three days after the second vaccination compared to baseline in healthy and T1D adolescents were shown. (A) The Venn diagrams showing the overlaps of upregulated (left panel) and downregulated (right panel) DEGs between healthy (green) and T1D (pink) adolescents. (B) The heatmap showing the log2FC of shared DEGs between healthy and T1D adolescents. The volcano plot depicting DEGs after the second vaccination compared to baseline in healthy controls (C) and T1D adolescents (D). (E–I) GSEA (adjusted *p*-value >0.25, absolute normalized enrichments scores (|NES|)>1) identifying enrichment of modules at different time points. (E) Circos plot of the overlapping gene sets across healthy and T1D adolescents after the first and second vaccination analyzed using GSEA. Two segments of the outer circle represent the two groups. Two segments of the middle circle represent the responses after the first and second vaccination compared to baseline. The extracted modules were divided into upregulated and downregulated categories in the inner circle. Lines connect upregulated (red), downregulated (blue), and discordant (green) genes. (F) The immune-related gene sets expression of T1D adolescents compared to HC at baseline. (G) The immune-related gene sets of T1D adolescents compared to HC three days after the first vaccination. (H) The heatmap showing innate immune-related gene sets three days after the second vaccination compared to baseline in both HC and T1D adolescents. (I) The heatmap showing adaptive immune-related gene sets three days after the second vaccination compared to baseline in both HC and T1D adolescents.

COVID-19-vaccine related immunoglobulin light chain variable region genes (IGLV2-14, IGKV2-28, and IGKV3-15), indicating impairment in B cell development (Fig. 4C and D).[27,28]

Although both control and T1D groups shared upregulation of immune-related gene sets post-second vaccination, T1D adolescents showed downregulation of these gene sets post-first vaccination compared to controls (Fig. 4E). Pre-vaccination, T1D adolescents exhibited higher expression of immune-related gene sets, suggesting chronic inflammation (Fig. 4F).[29] However, T1D adolescents exhibited lower up-regulation in both innate and adaptive immune-related gene sets compared to HC post-first vaccination, including the pathways of type I interferon, humoral responses, and T cell proliferation (Fig. 4G). Moreover, lower type I interferon signaling pathway was aligned with IM responses in T1D adolescents (Fig. 3D). Three days post-second vaccination, T1D adolescents continued to demonstrate lower up-regulation in innate immune-related gene sets, including variety cytokine production and dendritic cell differentiation. Notably, T1D adolescents exhibited lower signatures of phagocytosis, consistent with previous studies indicating impaired phagocytosis in patients with hyperglycemia (Fig. 4H).[6] Unlike the response post-first vaccination, T1D adolescents demonstrated similar expression of adaptive immune-related gene sets as HC post-second vaccination (Fig. 4I). In summary, RNA transcriptomic analysis shows T1D adolescents manifest chronic inflammation and attenuated immune responses early post-first vaccination. Post-second vaccination, they still display downregulated B cell development-related DEGs and lower innate immune responses.

3.5. *Weakened CD8$^+$ T cell cross-protection against omicron strain in T1D adolescents*

Besides antibody responses, vaccine responses could also be determined by specific memory T cell responses. One year post-second vaccination, T1D adolescents exhibited comparable frequency of AIM$^+$ CD4$^+$ T cells but a slightly lower frequency of AIM$^+$ CD8$^+$ T cells after Wuhan peptide pools stimulation compared to HC (Fig. 5A and B). T1D adolescents displayed compromised T cell recall responses after BA.1 peptide pools stimulation, especially in CD8$^+$ T cells (Fig. 5C and D). In alignment with the AIM$^+$ T cell responses, T1D adolescents had lower proliferation rate after BA.1 stimulation, while exhibiting similar responses after Wuhan strain stimulation (Fig. 5E).

Our results showed that T1D adolescents had both lower cross-react S-specific CD8$^+$ T cells and impaired innate immune responses based on Figs. 2C, 3C and 3D, and 5D. We then divided the individuals into two groups (AIM^{high} and AIM^{low} groups) based on the frequency of AIM$^+$ CD8$^+$ T cells to understand whether the specific T cell responses would be affected by impaired innate immunity. We found that AIM^{low} group

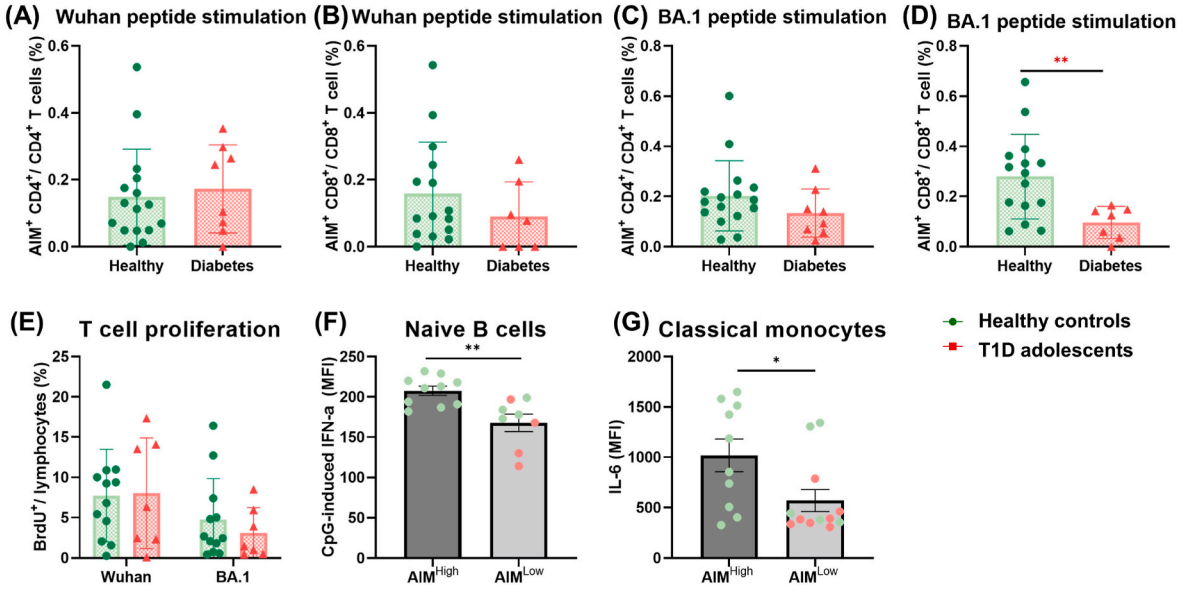

Fig. 5. S-specific T cell recall responses in healthy and T1D adolescents one year after the second BNT162b2 vaccination. (A–D) S-specific CD4$^+$ and CD8$^+$ T cells recall responses in healthy and T1D adolescents were quantified by the frequency of AIM expressing cells at TP5. The frequencies of AIM$^+$ CD4$^+$ T cells (A) and AIM$^+$ CD8$^+$ T cells (B) were determined after Wuhan spike peptide pools (BA.1 references) stimulation. The percentages of AIM$^+$ CD4$^+$ T cells (C) and AIM$^+$ CD8$^+$ T cells (D) were demonstrated after BA.1 peptide pools stimulation. (E) After Wuhan and BA.1 spike peptide pools stimulation, the S-specific T cell responses in healthy and T1D adolescents were analyzed using BrdU assay. (F–G) The AIM^{High} and the AIM^{Low} groups were divided based on the frequency of AIM $^+$ CD8$^+$ T cells of individuals after BA.1 peptide pools stimulation. The AIM^{High} group had frequencies above the median, while the AIM^{Low} had frequencies below the median. CpG-induced IFN-α expression of naïve B cells (F) and the first vaccine-induced IL-6 expression of CM (G) in the AIM^{High} and the AIM^{Low} groups were identified. Statistical analyses were evaluated using the Mann-Whitney *U* test. Error bars depict ±SD. *$P < 0.05$; **$P < 0.01$.

had lower CpG-induced IFN-a expression in naïve B cells, which included all T1D adolescents (Fig. 5F). Additionally, lower first vaccine-induced IL-6 expression in CM were also found in AIM^{low} group (Fig. 5G). Taken together, one year post-second vaccination, T1D adolescents exhibit lower frequency of cross-reactive $CD8^+$ T cell against BA.1, which may correlate with poor TLR9 responses in naïve B cells and diminished first vaccine-induced CM responses.

4. Discussion

In the current study, we explored the immune activation and subsequent immunogenicity following Pfizer-BNT162b2 mRNA vaccination in adolescents with T1D and in healthy controls. Despite T1D adolescents displaying comparable antibody responses to both Wuhan strain and VOCs, distinct patterns of immune cell activation and gene expression were observed when compared with healthy controls. Furthermore, notwithstanding the robust antibody responses post-vaccination, there was a trend of reduced antibody activity correlating with elevated HbA1c levels. Additionally, longitudinal follow-up revealed deficient cross-protective $CD8^+$ T cell responses against the BA.1 strain in T1D patients.

We observed an impaired TLR9 response in B cells among T1D adolescents. However, the innate immune mechanism contributing to immunogenicity of BNT162b2 mRNA vaccine depends mostly on TLR3, TLR7/8, RIG-I, and MDA5-related pathways.[23,30] Therefore, we assume that the BNT162b2 mRNA vaccine may circumvent the impairment of TLR9 responses in T1D adolescents, and still evoke adequate antibody responses and cellular protection. In this study, we also found that CM and IM were strongly activated post-first vaccination, while NCM were more significantly activated in healthy controls post-second vaccination. The higher innate immune responses after second vaccination compared to first vaccination may be due to the trained immunity induced by the vaccination.[31] As a previous study of vaccinated individuals showed, NCM may contribute to the protection against severe COVID-19 disease,[32] our results highlight the importance of the second vaccination. Previous studies found that specific innate immune responses correlated with antibody responses to vaccine.[21,33] In the current study, we did not find any positive correlation between early innate immune activation and post-vaccination antibody level among BNT162b2 mRNA vaccinated T1D adolescents. However, we did find that early innate immune response, especially IL-6 expression in CM is correlated with S-specific T cell recall responses. This is compatible with previous findings that vaccine-associated increase of CM and IM results in a more robust antiviral response to stimulate adaptive immune responses.[34]

T1D patients have reported to be at a higher risk of severe COVID-19, due to impaired cellular response and decreased release of T-cell-specific factors.[35] However, there is no definite evidence of altered humoral response to COVID-19 vaccines or influenza vaccines in T1D patients, presenting as differences in the anti-viral antibody levels when compared with those in healthy controls after vaccination.[35–38] This study also support previous findings suggesting that antibody responses are not impaired in T1D patients. Even though previous studies indicated no association between humoral immune response and glycemic control among T1D individuals.[34,37,38] Our results suggested a negative correlation between antibody response and baseline HbA1c level before vaccination. Since the previous studies recruited mostly adult patient with a broad age range,[36,39,40] the differences may be due to the fact that we recruited patients from a more narrow age group, which might be more effective in revealing the correlation. T1D patients were found to have lower cytotoxic responses, as well as reduced T-cell-related cytokines secretion after vaccination,[35] which is also compatible with our result that they demonstrated weakened cross-reactive $CD8^+$ T cell responses.

A previous study identified that T1D patient exhibit significantly upregulated inflammatory response including IL-1 and IL-8 secretion.[41] These findings align with our observations of higher immune-related gene expression in both innate and adaptive immunity among T1D patients in the baseline condition, indicating a chronic anti-self-inflammatory response. Additionally, we observed impaired activation of B cells in response to TLR agonists among these T1D patients, similar to the phenomenon observed in the elderly population.[21] This suggests that the chronic active auto-inflammatory state in T1D may weaken immune responses to external stimuli including the vaccine antigens or pathogens, through mechanism such as immune cell exhaustion and disrupted immune tolerance.[42,43] Although T1D adolescents had lower up-regulated expression in innate immune-related pathways after first and second vaccination, including cytokine and dendritic cells differentiation pathways crucial for antigen-specific responses,[44] the lower up-regulation in adaptive immune-related pathways was only evident post-first vaccination in T1D adolescents. This underscores the significance of the second vaccination for these patients.

This study pioneers the exploration of immune cell activation and gene activation post-vaccination in a susceptible population, shedding light on the complex interplay between T1D and immune response to vaccinations. Being a real-world clinical study, our study had several limitations. First, due to the limited blood volume drawn from adolescents, we focused on immune cells abundant in PBMCs, excluding lower quantity cells like pDCs (0.3–0.5 %). Second, our patients exhibited relatively good glucose control, potentially obscuring the influence of blood sugar on post-vaccination response. Third, bulk RNA-sequencing may not reflect the specific cellular responses, causing discrepancies between flow cytometry and RNA-seq data. Lastly, the study was hindered by a relatively small sample size. Despite this limitation, our study verifies the immunological effectiveness of a COVID-19 mRNA vaccine in T1D adolescents. This study lays the groundwork for improving the vaccine immunogenicity for T1D patients.

CRediT authorship contribution statement

Ching-Fen Shen: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Pei-De Chang:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Yen-Yin Chou:** Resources, Methodology, Data curation. **Shih-Wei Wang:** Resources, Methodology, Data curation. **Yu-Wen Pan:** Resources, Methodology, Data curation. **Chih-An Chen:** Resources, Methodology, Data curation. **Ching-Wei Lin:** Resources, Methodology, Data curation. **Bo-Yang Tsai:** Visualization, Methodology, Formal analysis, Data curation. **Pei-Jane Tsai:** Visualization, Methodology, Formal analysis, Data curation. **Ching-Chuan Liu:** Visualization, Supervision, Methodology, Formal analysis, Data curation. **Chao-Min Cheng:** Validation, Supervision, Methodology, Formal analysis, Data curation. **Wen-Chien Ko:** Validation, Supervision, Methodology, Formal analysis, Data curation. **Chi-Chang Shieh:** Writing – review & editing, Validation, Supervision, Project administration, Conceptualization.

Ethics statement

Samples were obtained with written informed consent under a study protocol approved by the Institutional Review Board of National Cheng Kung University Hospital (No. A-BR-110-051).

Consent for publication

Not applicable.

Availability of data and materials

The relevant data and its supplemental data can be found in the article or obtained from the corresponding author upon request.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgments

We would like to thank all participants for their regular blood donations. We also extend our gratitude to Meng-Che Tsai, Hui-Feng Lee, Hui-Chin Shih, Yi-Chen Fu, Li-Chun Wang, Tzu-Chi Shen, Chu-Han Hsieh, Po-Ta Chen, and Wei-Yun Huang for their assistance with clinical recruitment and expert laboratory support. Our sincere thanks go to Luigi Notarangelo and Helen Su for their valuable discussions. We are grateful to Chung-I Li, Ph.D., from the Department of Statistics for providing statistical consulting services. Additionally, we acknowledge the technical services provided by the Biosafety Level 3 Core Laboratory at the National Core Facility for Biopharmaceuticals, National Science and Technology Council, Taiwan. Finally, during the preparation of this work, the authors utilized ChatGPT for grammar checking. This study was supported by grants from Taiwan's National Science and Technology Council (110-2923-B-006-001-MY4, 111-2321-B-006-009, 113-2321-B-006-007, 113-2622-E-007-019, 113-2314-B-006-102-MY3).

Abbreviations

T1D Type 1 diabetes
COVID-19 Severe coronavirus disease 2019
SARS-CoV-2 severe acute respiratory syndrome coronavirus 2
TLR Toll-like receptor
PRR Pattern recognition receptor
NK cell Natural killer cell
CM Classical monocytes
IM Intermediate monocytes
NCM Non-classical monocytes
USM B cell Unswitched memory B cell
SwMe B cell Switch memory B cell
IFN-γ Interferon-gamma
IL-6 Interleukin-6
IL-10 Interleukin-10
IFN-α2b Interferon alpha-2b
RBD Receptor binding domain
S Spike protein
Nab Neutralizing antibody
DEG Differential expression gene
GSEA Gene set enrichment analysis
VOCs Variants of concern
HbA1c Glycated hemoglobin
ISGs Interferon-stimulating genes

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2024.12.009.

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