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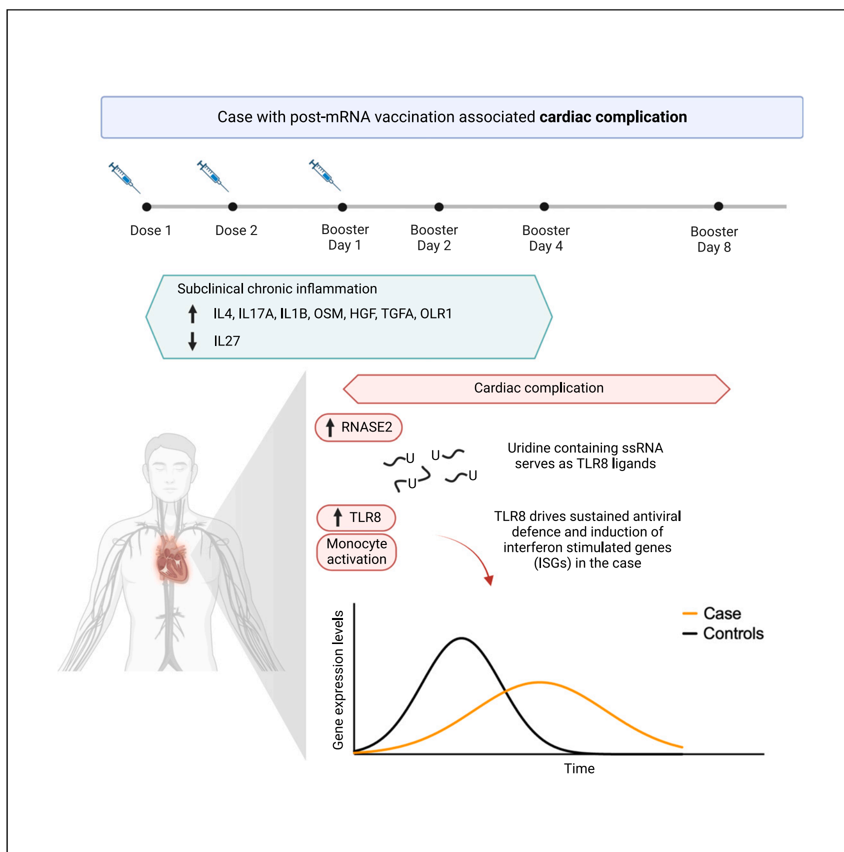
Case Report

RNase2 is a possible trigger of acute-on-chronic inflammation leading to mRNA vaccine-associated cardiac complication



Ong et al. show that the presence of subclinical chronic inflammation and elevated baseline expression of RNASE2 are possible triggers for post-vaccination cardiac complication. Their findings provide insights into the pathogenesis of cardiac complication and suggest the incorporation of additional nucleoside modifications in mRNA vaccines for an added safety margin.

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Highlights

Case of cardiac complication following COVID-19 mRNA booster vaccination

Pre-symptomatic cytokine levels in the case suggest subclinical chronic inflammation

Elevated baseline expression of RNASE2 and TLR8 drives acute-on-chronic inflammation

Further RNA chemistry studies may enhance mRNA vaccine safety

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Case Report

RNase2 is a possible trigger of acute-on-chronic inflammation leading to mRNA vaccine-associated cardiac complication

Eugenia Z. Ong,[1,2] Clara W.T. Koh,[1] Danny J.H. Tng,[1,3] Justin S.G. Ooi,[1] Jia Xin Yee,[1,2] Valerie S.Y. Chew,[1,2] Yan Shan Leong,[1,2] Kurugulasigamoney Gunasegaran,[4] Chin Pin Yeo,[5] Lynette L.E. Oon,[5] Jean X.Y. Sim,[3] Kuan Rong Chan,[1,*] Jenny G. Low,[1,2,3,*] and Eng Eong Ooi[1,2,6,7,*]

SUMMARY

Background: Post-mRNA vaccination-associated cardiac complication is a rare but life-threatening adverse event. Its risk has been well balanced by the benefit of vaccination-induced protection against severe COVID-19. As the rate of severe COVID-19 has consequently declined, future booster vaccination to sustain immunity, especially against infection with new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants, may encounter benefit-risk ratios that are less favorable than at the start of the COVID-19 vaccination campaign. Understanding the pathogenesis of rare but severe vaccine-associated adverse events to minimize its risk is thus urgent.

Methods: Here, we report a serendipitous finding of a case of cardiac complication following a third shot of COVID-19 mRNA vaccine. As this case was enrolled in a cohort study, pre-vaccination and pre-symptomatic blood samples were available for genomic and multiplex cytokine analyses.

Findings: These analyses revealed the presence of subclinical chronic inflammation, with an elevated expression of RNASE2 at pre-booster baseline as a possible trigger of an acute-on-chronic inflammation that resulted in the cardiac complication. RNASE2 encodes for the ribonuclease RNase2, which cleaves RNA at the 3′ side of uridine, which may thus remove the only Toll-like receptor (TLR)-avoidance safety feature of current mRNA vaccines.

Conclusions: These pre-booster and pre-symptomatic gene and cytokine expression data provide unique insights into the possible pathogenesis of vaccine-associated cardiac complication and suggest the incorporation of additional nucleoside modification for an added safety margin.

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CONTEXT AND SIGNIFICANCE

Cardiac complication is a rare but life-threatening adverse event following mRNA vaccination. Although inflammation has been found to be associated with such an adverse outcome, what triggers the inflammatory response is uncertain. Ong et al. found, in a participant in a cohort study, that high pre-booster baseline expression of RNASE2 in a background of subclinical chronic inflammation was the possible underpinning of cardiac complication. RNASE2 encodes for the ribonuclease RNase2, which cleaves RNA at the 3′ side of uridine, thus removing a TLR8-avoidance safety feature of mRNA vaccines. Activation of the TLR8 pathway triggered an acute-on-chronic inflammatory response that likely contributed to the post-booster cardiac event. Their finding suggests the incorporation of additional nucleoside modification for an added safety margin.

INTRODUCTION

Vaccines are useful tools in any public health program by eliciting adaptive immunity to prevent infectious diseases. They emphatically turned the tide against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the COVID-19 pandemic. Our immune system is, however, a double-edged sword. When over-activated, it can damage our tissues and organs, leading to autoimmune conditions. The development and application of any vaccine are thus based on unequivocal demonstration of benefits over risks of vaccination.

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The most widely applied COVID-19 vaccine has been the mRNA vaccine. When inoculated into vaccinees, mRNA is translated into the spike protein of SARS-CoV-2 to produce antibodies and CD4 and CD8 T cells soon after vaccination.[1] The elicited cellular immunity may be critical to prevent severe COVID-19, even against SARS-CoV-2 variants that escape vaccine-elicited neutralizing antibodies.[2] To better protect vulnerable individuals from COVID-19, booster mRNA vaccination using a more contemporaneous spike gene sequence has begun. Moreover, newer vaccines are also under development to expand the breadth of protection against other SARS-CoV-2-like Beta CoVs. However, as the majority of the global population is no longer immunologically naive against SARS-CoV-2 from either vaccination or infection, the risk of severe adverse events from mRNA vaccination, even if rare, may blunt the previously unequivocal benefit-risk ratio. Insights that can shape the development of even safer mRNA vaccines are thus highly desirable since mRNA vaccines will undoubtedly become a prominent feature in any public health toolbox.

Among the rare but severe adverse events following mRNA vaccination are those that affect the heart. The rate of post-mRNA vaccination myocarditis or pericarditis is estimated to be about 2.17 per 100,000 person days, with the risk being higher in younger males following the second vaccination.[3] These inflammatory conditions manifest symptomatically from 1 to 7 days after vaccination[3] and, although transient, can be both debilitating and alarming. That such events are rare makes it difficult for us to study the pathogenesis of post-vaccination cardiac complications. We hence believe that this case, a person who enrolled in our cohort of COVID-19 vaccinees, provides opportunistic insights into the susceptibility factors of post-mRNA vaccination cardiac complication.

RESULTS

The subject in this case is a fit and healthy 42-year-old male with neither underlying illness nor allergy history. He received the first two primary series doses of BNT162b2 vaccination 8 months prior to the booster. The side effects reported following the first two doses of BNT162b2 were injection site tenderness and mild myalgia. He was well on day 1 when he was boosted with mRNA-1273. On day 2, he began experiencing shortness of breath upon exertion that continued to worsen until day 4, where he was unable to ambulate continuously without intermittent rest. Onset of exertion-induced chest pain occurred on day 5. When he presented to the cardiac unit on day 6, his electrocardiogram showed ST elevation in anterior leads with T wave inversion in V2, which is significant (Table S1). Full blood count was normal except for elevated eosinophils. He received an intravenous loading dose of 300 mg aspirin for suspicion of acute myocardial ischemia, although plasma levels of cardiac enzymes and troponin-T turned out normal. PCR for known etiologies of myocarditis—human herpes virus 6 (HHV6), cytomegalovirus (CMV), parvovirus B19, adenovirus, and enterovirus—yielded negative findings. He also tested negative for anti-nucleocapsid protein (NP) antibodies on day 1, ruling out the possibility of post-COVID-19 cardiac complication. His symptoms resolved by day 8; a cardiac echocardiography performed on this day was also unremarkable. He was discharged on day 8 without further intervention and subsequently returned to regular daily activities a week later. His clinical symptoms and course are summarized in Figure 1A, and clinical details are provided in Table S1.

As he had enrolled with written informed consent in our cohort, blood samples were collected before and at various time points after vaccination as previously described.[1] As pre-vaccination baseline factors influence susceptibility to post-vaccination adverse events,[4,5] we compared blood specimens from the subject against 18 controls from our cohort to identify host differences that may explain the observed cardiac complication.

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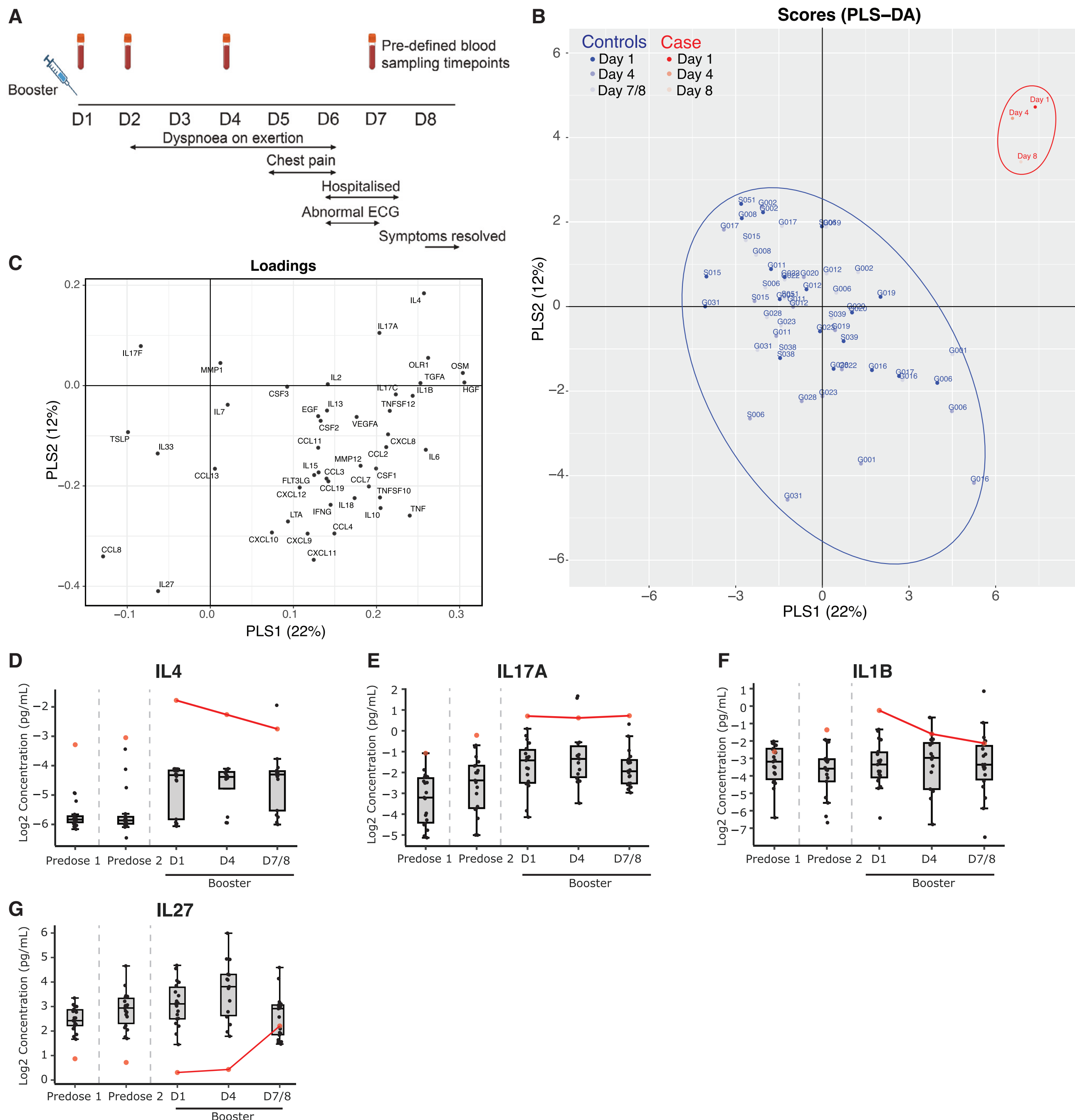

Figure 1. Differential pre- and post-vaccination levels of inflammatory and anti-inflammatory cytokines in the subject compared with controls

(A) Outline of clinical symptoms and course.

(B) Partial least squares-discriminant analysis (PLS-DA) score plot showing clear clustering between subject and controls across all time points after booster mRNA vaccination. Components 1 and 2 account for 22% and 12% of the variance, with R2Y = 0.949, Q2Y = 0.802, and root mean square error of estimation (RMSEE) = 0.066. Measurements taken at days 1, 4, and 7/8.

(C) Loading biplot of the PLS-DA model.

(D–G) Expression levels of IL-4, IL-17A, IL-1B, and IL-27 in serum prior to doses 1 (pre-dose 1) and 2 (pre-dose 2), prior to booster (day 1), and at days 4 and 7/8 post-booster. Red lines and dots indicate data trends and points for subject. Box and whisker plots show the mean (center line of boxes), 25th and 75th percentiles (lower and upper boundary of boxes), and minimum and maximum values in the controls. n = 19 for controls and n = 1 for subject. See also Table S1 and Figure S1.

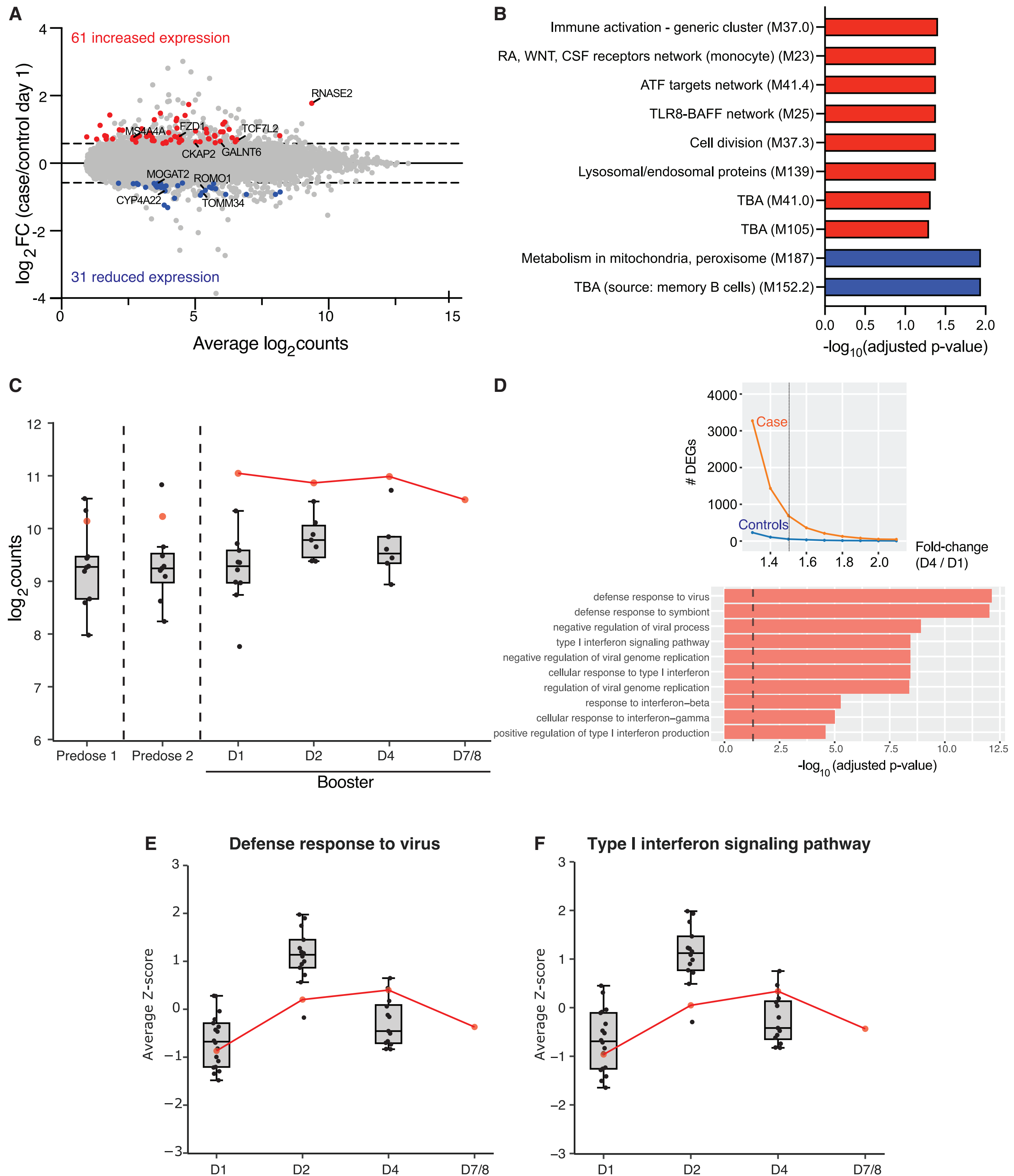

Figure 2. High expression of RNASE2 during booster vaccination was associated with cardiac complication

(A) MA plot showing average expression value of transcripts and log2 fold change (log2FC) of subject compared with controls at day 1. Genes with *Z* score >3 are colored, where red and blue indicate significant increase and decrease in expression, respectively, in the subject at day 1. Highlighted genes are those involved in either immune activation, Wnt signaling, cell division, or mitochondria metabolism pathways. Dotted line indicates FC >1.5.
(B) Enriched GOBP terms for transcripts with increased (red) or reduced (blue) expression in subject compared with controls at day 1.

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Figure 2. *Continued*

(C) RNASE2 expression prior to doses 1 (pre-dose 1) and 2 (pre-dose 2), prior to booster (day 1), and at days 2, 4, and 8 post-booster.

(D) Number of differentially expressed genes (DEGs) in subject and controls (*Z* score > 3) across different FC cutoffs. Based on 1.5 FC, the top 10 GOBP pathways induced in the subject at day 4 (vs. day 1) are displayed. Dotted line indicates adjusted p value <0.05.

(E and F) Average *Z* score values of transcripts involved in pathways of defense response to virus and type I interferon responses in the subject and controls. The genes used for *Z* score calculations are shown in Figures S2O and S2P. Number of controls used for pre-dose 1, pre-dose 2, and booster day 1 are 11, 10, and 18, respectively. All box and whisker plots show the mean (center line of boxes), 25th and 75th percentiles (lower and upper boundary of boxes), and minimum and maximum values in the controls.

See also Figure S2.

The controls were of similar age and also received booster vaccination but without cardiac complication. They also completed the per-protocol blood sampling schedule.

Plasma cytokine levels measured with the Olink Target 48 Cytokine panel were compared between the subject and controls using partial least squares-discriminant analysis (PLS-DA), which segregated the subject from controls at all 3 measured time points (Figure 1B). Plasma levels of 45 cytokines discriminated the subject from controls (Figure 1C), among which are the Th2 cytokines interleukin-4 (IL-4), the pro-inflammatory IL-17A, and IL1B and the inflammation-induced growth factors hepatocyte growth factor (HGF), oncostatin-m (OSM), and transforming growth factor α (TGF-α), as well as the scavenger receptor oxidized low-density lipoprotein receptor 1 (OLR1). These proteins were elevated in the plasma at baseline and at day 4 post-vaccination (Figures 1D–1F and S1A–S1D). Interestingly, the inhibitory IL-27 was low in the subject compared with controls until his symptoms resolved on day 8 post-vaccination (Figure 1G). Retrospective analysis of the pre-dose-1 and -2 blood samples also showed elevated pro-inflammatory and Th2 cytokines, with repressed expression of IL-27, collectively suggesting an underlying chronic but asymptomatic inflammation.

As the underlying inflammation did not trigger cardiac complication following the first two doses, we turned to genomic analysis to identify mRNA transcripts in our subject. We identified 92 genes in which the expression showed 1.5-fold difference and 3 standard deviations (SDs) or more from the mean of the controls to determine outliers that may explain this rare adverse event (Figure 2A). Pathway analysis of the 61 genes with elevated expression clustered into innate immune pathways, while the 31 genes were in the metabolic pathway (Figure 2B), which is consistent with an activated immune system.[6] Furthermore, gene set enrichment analysis using blood transcription modules found monocyte and immune activation signatures being most positively enriched in the subject compared with controls (Figure S2A). Other immune cells, such as neutrophils, were also positively enriched, although not to the levels seen in monocytes; eosinophil was not flagged in this analysis. Critically, this was unique to the sample taken before booster but not before doses 1 and 2 (Figures S2B and S2C). Likewise, RNASE2, which is known to be expressed in activated myeloid-derived mononuclear cells and eosinophils,[7] was identified as a prominent outlier in the pre-vaccination gene expression data (Figure 2A). Strikingly, RNASE2 expression level was also an outlier in the subject compared with controls after booster vaccination when the cardiac complication arose; expression of RNASE2 was within the SDs of the controls before the first two doses of mRNA vaccine (Figure 2C).

RNASE2 encodes the ribonuclease RNase2, which has been known to be an endogenous Toll-like receptor 2 (TLR2) ligand that drives Th2 response.[8] Recently, RNase2 was also found to be an integral part of endosomal synthetic RNA sensing; RNase2 cooperates with RNase T2 to cleave RNA on either side of uridine to form TLR8 ligands.[9] Consistent with this recent report, we found heightened expression of TLR8, but not other TLRs, at pre-booster baseline and on day 4 post-booster vaccination (Figures S2D–S2M).

Correspondingly, the largest differences in host response to booster vaccination between the subject and controls occurred on day 4 (Figures 2D and S2N), where expression of anti-viral defense as well as interferon response genes, which were elevated from baseline on day 2, albeit not to the levels of controls, was sustained through to day 4 (Figures 2E, 2F, S2O, and S2P). Transcripts of these genes in the subject were reduced to levels comparable to baseline on day 8, when the subject was discharged from the hospital.

DISCUSSION

Although post-vaccination cardiac complications are alarming and potentially life threatening, they remain poorly understood because of their rarity. That this subject had both pre-vaccination and post-vaccination blood samples that preceded the peak of symptoms thus provides a unique glimpse into the plausible pathogenic underpinnings of such complications.

Our findings suggest that the cardiac complication arose as a confluence of several factors. Firstly, our subject was receiving his third dose of mRNA vaccine that induced anamnestic B and T cell responses. Secondly, high pre-booster levels of pro-inflammatory and Th2 cytokines in our subject suggest the possibility of undetected autoimmune activity. This notion is supported by the finding of an elevated inflammation-induced scavenger receptor (OLR1),[10] growth factors for the repair of stromal tissue (HGF), and epithelial barriers (OSM and TGF-α).[11,12] Interestingly, OLR1 is better known for its role in atherosclerosis,[13,14] although clinical investigations did not suggest ischemic heart disease as a cause of the cardiac event. Instead, OLR1 also functions to support B cell responses,[10,15] which is supported by the high baseline IL-4 level in our subject. In addition, IL-17A is closely linked to autoimmune conditions,[16] and its role in the development of cardiac complications in our subject is further suggested by the resolution of symptoms on day 7 when the plasma level of its antagonist, IL-27, rose.

Finally, while both anamnestic response and baseline inflammation may have elevated the risk of cardiac complications, mRNA vaccination in a person with high expression of RNASE2 may have been the trigger. The mRNA vaccine incorporates pseudouridine to avoid overstimulating RNA sensors in cells.[17] When highly expressed in antigen-presenting cells, RNASE2 may remove the only immunomodulatory feature of mRNA vaccine as the encoded enzyme cleaves RNA on the 3′ side of uridine, resulting in an increased abundance of TLR8 ligands.[9] That downstream cellular defense and interferon pathways were more elevated and prolonged in the subject further supports this mechanism as the trigger of the pathogenic process.

This case presents an exceptional opportunity to glimpse the baseline factors that may explain the pathogenesis of post-vaccination cardiac complications. Our findings suggest that a confluence of factors gave rise to the cardiac complication, which underscores the rarity of such adverse events. While the findings of monocyte activation at pre-vaccination, as well as elevated TLR8-driven anti-viral defense and interferon-stimulated gene expression after vaccination, suggest that RNASE2 expression in monocytes may play a more important role than eosinophils, future studies that confirm this notion would be useful.

In conclusion, our findings suggest that cardiac complication is a confluence of factors, with endosomal RNA processing through RNase2 to stimulate TLR8 as a trigger of an acute-on-chronic inflammatory outcome. Perhaps additional nucleoside

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modifications would be useful to prevent such rare over-stimulation of RNA sensors for added vaccine safety.

Limitations of the study

The key limitation in our study is that our findings are based on a single case and that susceptibility to mRNA vaccination-associated cardiac complications may be heterogeneous. Finally, clarifying the immune subset-specific expression of RNASE2 may permit a better mechanistic understanding of post-vaccination cardiac complication.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.medj.2023.04.001.

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AUTHOR CONTRIBUTIONS

D.J.H.T., J.X.Y.S., and J.G.L. led the clinical portion of this study and enrolled the subjects. E.Z.O., J.X.Y., V.S.Y.C., and Y.S.L. conducted the gene expression and cytokine profiling and managed sample processing and archiving. K.G., C.P.Y., and L.L.E.O. conducted additional clinical investigations. E.Z.O., C.W.T.K., J.S.G.O., and K.R.C. analyzed the data. E.E.O. conceived the study. E.Z.O., K.R.C., J.G.L., and E.E.O. wrote the manuscript. E.Z.O., K.R.C., J.G.L., and E.E.O. had unrestricted access to all of the data. All authors agreed to submit the manuscript, read and approved the final draft, and take full responsibility for its content, including the accuracy of the data.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Blood from vaccinated healthcare workers	This study	N.A.
Critical commercial assays		
Tempus™ Blood RNA Tube	Applied Biosystems	Cat# 4342792
Tempus™ Spin RNA Isolation Kit	Invitrogen	Cat# 4380204
Olink Target 48 Cytokine	Olink AB	Cat# 93200
Agilent RNA 6000 Pico Kit	Agilent	5067–1513
GeneChip Human Gene 2.0 ST Array	Applied Biosystems	902499
GeneChip WT PLUS Reagent Kit	Applied Biosystems	902280
GeneChip Hybridization, Wash, and Stain Kit	Applied Biosystems	900720
Deposited data		
Raw data for microarray profiling of case and controls	This paper	Array Express: E-MTAB-12829
Software and algorithms		
GeneChip Command Console Software	Thermo Fisher Scientific	https://www.thermofisher.com/sg/en/home/life-science/microarray-analysis/microarray-analysis-instruments-software-services/microarray-analysis-software/affymetrix-genechip-command-console-software.html
Olink NPX Signature v1.6	Olink AB	https://olink.com/products-services/data-analysis-products/olink-npx-signature/
Partek Genomics Suite v7.21	Partek Inc.	https://www.partek.com/partek-genomics-suite/
Graphpad Prism v9.4	Graphpad	https://www.graphpad.com/features
R software	The R Project for Statistical Computing	https://www.r-project.org

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Eng Eong Ooi (engeong.ooi@duke-nus.edu.sg).

Materials availability

This study did not generate new reagents.

Data and code availability

- Raw data for microarray profiling of case and controls have been deposited at Array Express and are publicly available as of the date of publication. Accession number is listed in the key resources table.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

METHOD DETAILS

The case and controls in this study were healthcare workers who were receiving their COVID-19 vaccination and invited to participate in a study defining the correlates of immunogenicity and adverse events of RNA vaccines. This study was approved by the SingHealth Centralized Institutional Review Board (CIRB/F 2021½014). Following written informed consent, whole blood and serum samples were collected prior to vaccination (pre-doses 1 and 2, and pre-booster) and at indicated time points following vaccination. Additional clinical investigations were conducted for the

case upon admission for cardiac complication. This included performing an electrocardiogram (ECG), measurements for cardiac biomarkers, renal and liver panel, full blood count, PCR for known viral etiologies of myocarditis, and testing levels of anti-SARS-CoV-2 nucleoprotein antibodies.

Gene expression profiling

Blood was collected in Tempus Blood RNA tubes. RNA isolation from whole blood was performed using the Tempus Spin RNA Isolation Kit (ThermoFisher Scientific) according to manufacturer's protocol. Total RNA concentration and quality check was done using the Varioskan Lux and RNA 6000 Pico Kit on the Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA) at the Duke-NUS Genome Biology Core Facility. Microarray was performed using Affymetrix GeneChip Human Gene 2.0 ST Array at the Duke-NUS Genome Biology Core Facility. Fragmented, labeled single-stranded cDNA were prepared from 200ng of total RNA according to manufacturer's protocol using the GeneChip WT PLUS Reagent Kit (Affymetrix). Samples were hybridized on the GeneChip Human Gene 2.0 ST Array (Affymetrix Inc., Santa Clara, CA) for 16 h at 45°C. GeneChips were washed and stained using the GeneChip Fluidics Station 450 (Affymetrix Inc., Santa Clara, CA). Scanning was done using the GeneChip Scanner 3000 7G (Affymetrix Inc., Santa Clara, CA) and Affymetrix GeneChip Command Console Software (AGCC) to produce.CEL intensity files.

Olink proteomic profiling

Serum protein levels were measured using Olink proximity extension assay (PEA) Target 48 cytokine panel. Samples were first incubated with proximity antibody pairs tagged with unique DNA oligonucleotides. The antibody pairs come into proximity upon binding to target proteins, resulting in hybridization and extension of DNA oligonucleotides. This created an antigen-specific double stranded DNA barcode that was amplified and quantified using multiplex high throughput PCR on the Olink Signature Q100.

QUANTIFICATION AND STATISTICAL ANALYSIS

The Partek Genomics Suite was used to analyze the transcriptomic differences between case and controls. PLS-DA multivariate analysis was performed using the ropls R package, with the case and controls used to specify the clusters. Boxplots and MA-plots were plotted using GraphPad Prism (version 9.4.1) software and the ggplot2 R package. Comparisons between case and controls were determined using Z-scores, where *Z* score values above 3 are considered as outlier values, and thus, differentially expressed. For pathway analysis, differentially expressed genes were used as input data and analyzed against the Gene Ontology (GO) Biological Processes database using the Enrichr tool.[18] The significant genes contributing to the top enriched terms within the pathways, namely antiviral defense as well as interferon response pathways, were then further analyzed to tabulate the normalized expression levels. The *Z* score values for these significant genes were calculated and the mean value was tabulated for the individual subjects. To identify the blood transcription modules (BTMs) involved, gene expression fold-change of case against controls were ranked, and analyzed by gene set enrichment analysis (GSEA)[19] using BTMs as gene sets in the analysis.[20]