Circulation

CARDIOVASCULAR CASE SERIES

In-Depth Evaluation of a Case of Presumed Myocarditis After the Second Dose of COVID-19 mRNA Vaccine

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INITIAL PRESENTATION

A 52-year-old man presented to the emergency department ≈90 min after the onset of substernal chest pain. Three days before presentation, he received his second dose of mRNA-1273 (Moderna) vaccine for coronavirus disease 2019 (COVID-19), and the next day had a severe reaction that he described as being the "worst he had ever felt." He had subjective high fevers, shaking chills, myalgias, and a headache. These symptoms largely resolved by the third day after vaccination except for a positional headache that was unusual for him. On the morning of hospitalization, he walked 3 to 4 miles and felt fine. Later that day, while in a meeting, he developed persistent midsternal chest discomfort without radiation, prompting him to seek evaluation in a university hospital emergency department. The pain subsided spontaneously after approximately 3 hours. He had no associated dyspnea, palpitations, dizziness, fever, chills, or myalgia.

The patient had a past medical history of hypertension, hypercholesterolemia, obstructive sleep apnea treated with an oral appliance, and minor elevations in liver function tests attributed to possible hepatic steatosis. A recent screening coronary artery calcium scan demonstrated coronary artery calcium at the 81st percentile for age and sex. The patient had no previous history of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. His medications included aspirin 81 mg, simvastatin 40 mg, ezetimibe 10 mg, and lisinopril 10 mg daily, and he took no supplements. He drank alcohol socially and denied use of tobacco and all recreational drugs.

On physical examination, the following vital signs were recorded: oral temperature 36.8°C, pulse 73/min, blood pressure 124/76, and respiratory rate 18/min, and his oxygen saturation was 100% on room air. Pulmonary and cardiac examinations were normal without a pericardial friction rub. The remainder of his physical examination was normal.

In the emergency department, his initial ECG showed sinus rhythm with left axis deviation and incomplete right bundle-branch block without ST or T wave changes (Figure 1A). His initial high-sensitivity cardiac troponin I was 2768 ng/L. Point-of-care echocardiogram showed normal left ventricular function and volumes, and no wall motion abnormalities. Urgent coronary angiography showed mild nonobstructive coronary artery disease with no stenoses or visible thrombus and no evidence of coronary embolism or dissection (Figure 1B and 1C).

His initial laboratory panel revealed normal white blood cells 6.3 × 10^9/L (76% polymorphonuclear leukocytes, 14% lymphocytes, 9% monocytes, 0.5% eosinophils, and 0.2% basophils), hemoglobin 14.9 g/L, and platelets 207 × 10^9/L. Chemistries were remarkable for glucose of 172 mg/dL, but creatinine 0.87 mg/dL and alanine aminotrans-

Key Words: COVID-19 ■ COVID-19 vaccine ■ myocarditis ■ vaccine

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The Data Supplement, podcast, and transcript are available with this article at https://www.ahajournals.org/doi/suppl/10.1161/CIRCULATIONAHA.121.056038.
For Sources of Funding and Disclosures, see page 497.

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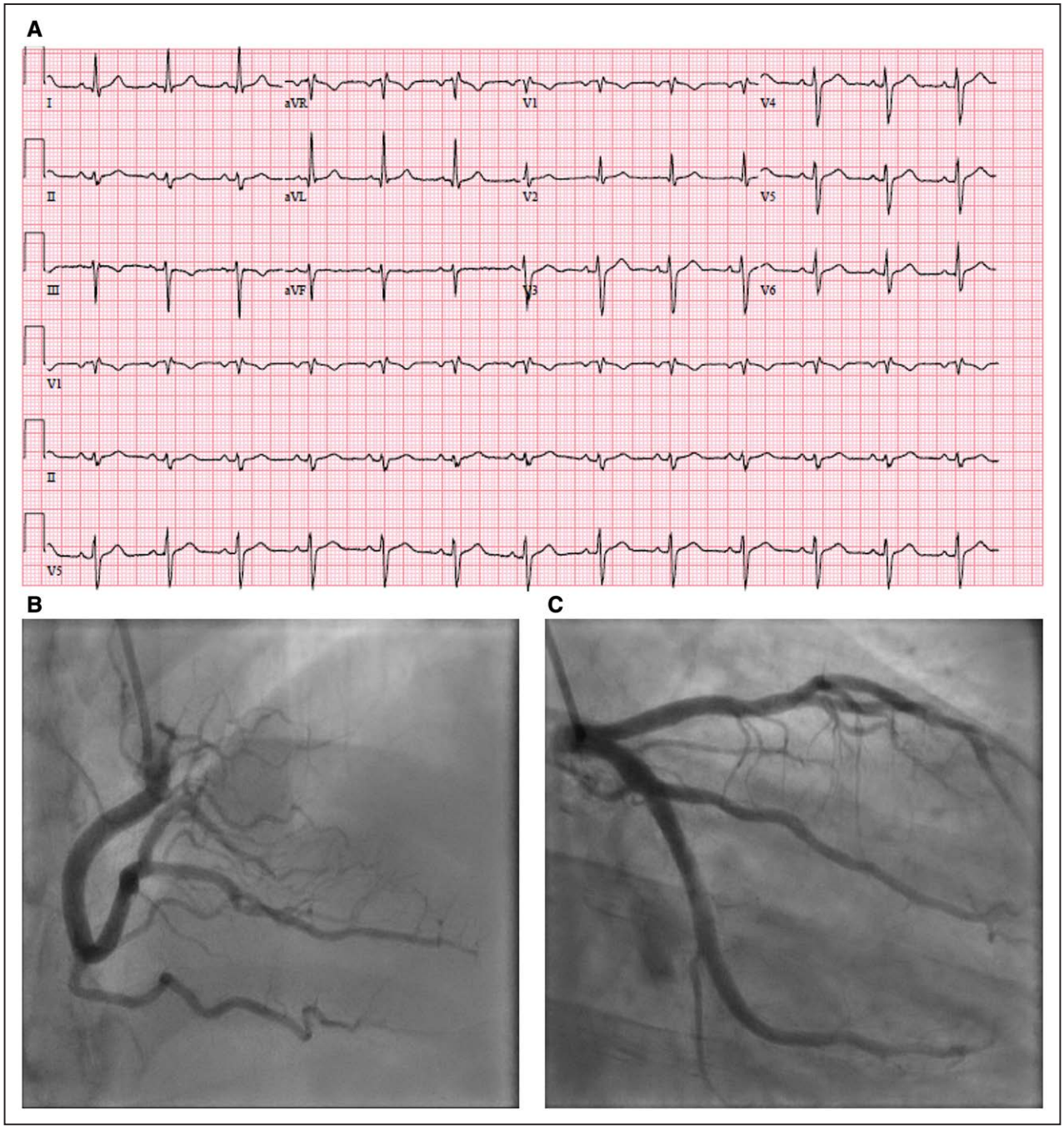

Figure 1. ECG and coronary angiogram.
A, The ECG on presentation to the emergency department. **B,** A posterior anterior cranial projection of a dominant right coronary artery and with no severe angiographic stenoses or flow-limiting lesions in the main vessel or its branches. **C,** A right anterior oblique caudal projection of a bifurcating left coronary artery and no severe angiographic stenoses or flow-limiting lesions in the main vessel or its branches.

ferase 58 U/L were consistent with his baseline. High-sensitivity cardiac troponin I peaked at 6770 ng/L at 7 hours after admission and remained elevated (551 ng/L) even after 4 days. In contrast, high-sensitivity cardiac troponin T and creatine kinase-MB biomarkers showed modest elevation (Table 1). C-reactive protein, erythrocyte sedimentation rate, and D-dimer were elevated in the first sample taken at the time of admission but resolved to near normal levels within 1 to 2 days. Antinuclear antibodies were negative.

ADDITIONAL CLINICAL TESTING

A repeat echocardiogram performed on hospital day 2 revealed a normal ejection fraction without wall motion abnormalities and no valvular or pericardial abnormalities. Contrast-enhanced cardiac magnetic resonance imaging (MRI) with parametric mapping was performed on a 1.5T MRI scanner (Siemens Healthineers) on hospital day 3. Delayed contrast-enhanced phase-sensitive

 Circulation. 2021;144:487–498. DOI: 10.1161/CIRCULATIONAHA.121.056038

Table 1. Relevant Biochemical Parameters in the Case of Interest

Description	CI_{S1}	CI_{S2}	CI_{S3}	CI_{S4}	Reference range
cTnI HS (ng/L)	**6770**	**1596**	**1440**	**551**	<26
cTnT HS (V gen P) (ng/L)	n/a	n/a	n/a	**138**	≤15.0
BNP (pg/mL)	50	n/a	n/a	n/a	<100
CRP (mg/L)	**19.1**	**12.8**	**5.9**	n/a	≤5.0
ESR (mM/h)	**25**	**42**	n/a	n/a	0–15
CK-MB Index	**4.8**	**3.2**	n/a	n/a	0.0-3.0
Ferritin (ng/mL)	162	119	n/a	n/a	22–275
D-dimer (mg/L FEU)	**0.74**	0.57	n/a	n/a	≤0.59
IL-6 (pg/mL)	<2.0	<2.0	n/a	n/a	<2.0
Glucose (mg/dL)	**172**	113	107	**166**	70–139
AST (U/L)	49	n/a	n/a	n/a	10–50
ALT (U/L)	**58**	n/a	n/a	n/a	10–50

Abnormal results are shown in bold. CI_{S1}, CI_{S2}, CI_{S3}, and CI_{S4} indicate case of interest sample at day 1, day 2, day 3, and day 4 after symptom onset, respectively. ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; BNP, B-type natriuretic peptide; CK-MB, creatine kinase-MB; CRP, c-reactive protein; cTnI HS, high-sensitivity cardiac troponin I; cTnT HS, high-sensitivity cardiac troponin T; ESR, erythrocyte sedimentation rate; IL-6, interleukin 6; and n/a, not tested or not available.

images showed midmyocardial and subepicardial linear and nodular late gadolinium enhancement in the inferoseptal, inferolateral, anterolateral, and apical walls. The left ventricle showed mild dilatation and "low normal" left ventricular ejection fraction at 54%. The right ventricular ejection fraction was normal at 58%. Parametric mapping showed elevated T1 relaxation time and relative inhomogeneity and focal elevation of the T2 relaxation values (Figure 2). In addition, wall motion abnormalities with mild hypokinesis of the lateral and inferior apical walls were noted. These findings were consistent with myocarditis on the basis of the modified Lake Louise criteria.[1]

HOSPITAL COURSE

The chest discomfort in the patient had fully resolved within 3 hours after onset and did not recur. He reported feeling normal throughout the remainder of his 4-day hospital stay. Endomyocardial biopsy was not performed because of his resolution of symptoms, preserved left ventricular ejection fraction, and the absence of any hemodynamic or arrhythmic complications. The patient was treated with low-dose lisinopril and carvedilol, but no immunosuppressive or anti-inflammatory medications. At the time of discharge, the patient remained asymptomatic, and his high-sensitivity cardiac troponin T levels had fallen to 138 ng/L. His NT-proBNP (N-terminal pro-B-type natriuretic peptide) at discharge was <27 pg/mL.

POSTDISCHARGE COURSE

The patient has had no recurrent symptoms in >3 months since hospital discharge. Given the presumptive diagnosis of myocarditis, exercise has been restricted, and he has remained on β-blocker and angiotensin-converting enzyme inhibitor medications. Repeat high-sensitivity cardiac troponin I was 10 ng/L 4 days after discharge and undetectable (<5 ng/L) 2 weeks after discharge. Serial cardiac MRIs have been performed showing a gradual reduction in left ventricular volumes and reduction in the degree of late gadolinium enhancement abnormalities, with normalization of T1 relaxation time and decrease in the T2 relaxation time (Table 2).

EXPLORATORY STUDIES TO INVESTIGATE POTENTIAL PATHOLOGICAL MECHANISMS

Myopericarditis has been reported to the US national passive Vaccine Safety Surveillance System (VAERS) as a rare adverse event after vaccinations, with most reports associated with smallpox vaccination.[2] However, at the time of the patient's presentation, there were no reported cases of myocarditis caused by COVID-19 vaccination.

To explore potential mechanisms of myocardial injury in temporal association with vaccination in the present case, written informed consent was obtained for additional in-depth analysis of viral, cytokine, and autoimmune panels and subsequent research publication of the case. Samples from the patient of interest were compared with excess, remnant blood specimens that were available in the laboratory after routine clinical testing. Samples from the case of interest (CI) were collected on days 1 to 4 after symptom onset ($CI_{S1–S4}$) and were compared with 4 groups: naive unvaccinated (N_{UV}; n=8), unvaccinated patients hospitalized with COVID-19 (n=10), naive vac-

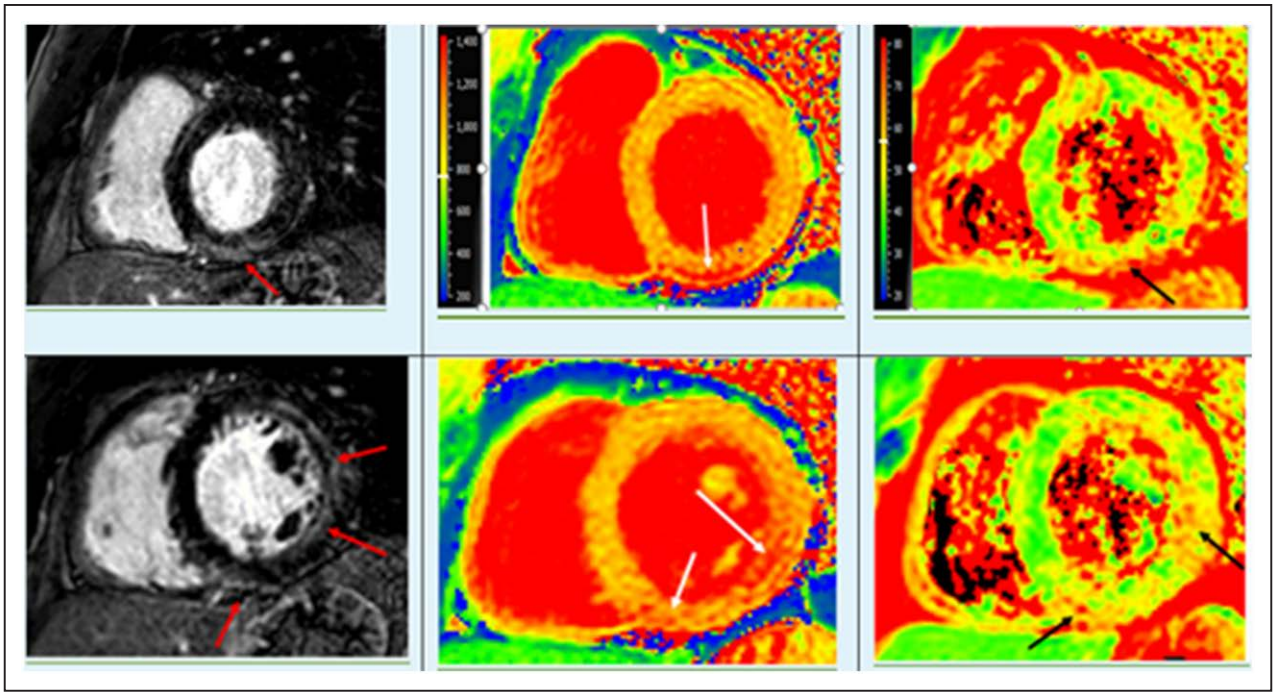

Figure 2. Phase-sensitive inversion-recovery cardiac magnetic resonance imaging.
Right, Short-axis views demonstrating linear and curvilinear delayed enhancement in the subepicardial inferior basal and mesocardial midventricular region, compatible with nonischemic pattern of delayed enhancement. **Middle**, A native T1 map showing globally increased T1 values (1054 ms), Local native myocardial T1 (short axis [SA] and 4 chamber [4CH] midwall) (965 ± 35) and specifically higher values in the regions of delayed enhancement. The color map shows relaxation times with normal relaxation time in green and increased relaxation time in red and orange. **Left**, Native T2 map with heterogenous relative increased T2 values within the same segments (arrows) (maximum T2 value was 65 ms) (local normal T2 values for our institution, 45–64 ms). Color scale shows time in milliseconds.

cinated (N_V; n=10), and age-matched controls receiving Moderna vaccine (N_M, n=2). N_V and N_M groups were tested ≈?2 weeks after receiving their second vaccine dose. The studies were performed as part of a biorepository protocol approved by the University of Texas Southwestern Institutional Review Board, and waiver of Institutional Review Board consent was obtained to use the remnant blood specimens. Detailed methods are provided in the Methods in the Data Supplement.

RESULTS OF EXPLORATORY STUDIES

Antibody response to viral antigens and SARS-CoV-2 nucleocapsid and spike proteins serum immunoglobulin (Ig) G antibodies against 18 different viral antigens and SARS-CoV-2 serology testing were measured using a custom developed proteome array and US Food and Drug Administration–approved standard assays, respectively, using the methods described in the Methods in the Data Supplement. These studies confirmed the absence of previous COVID-19 infection (negative reactivity for SARS-CoV-2 nucleocapsid IgG) (Figures 3 and 4). As expected, a clear immune response to the vaccine (SARS-CoV-2 spike as a component) was observed in the case of interest 5 and 6 days after the second dose of Moderna vaccine, which corresponds with the third and fourth day after symptom onset in the case of interest (CI_{S3} and CI_{S4}) (Figure 3). Comparison of the strength of vaccine-induced immune responses in the case of interest at the measured sampling period CI_{S2} and CI_{S4} with either N_V or N_M did not reveal abnormally elevated SARS-CoV-2 spike IgG or SARS-CoV-2 spike IgM levels (Figure 4). Low IgG serology reactivity was noted in the case patient samples ($CI_{S1–S4}$) for the other viral antigens, including cytomegalovirus,

Table 2. Left Ventricular Volume and Late Gadolinium Enhancement Abnormalities in the Case of Interest

Variable	Left ventricular volume	Late gadolinium enhancement	T1 relaxation time (ms)	T2 relaxation time (ms)
Hospitalization	196 mL	+++	1054	50–64
2 wk after discharge	163 mL	++	1015	43–52
12 wk after discharge	138 mL	++	969	42–47

+++ indicates high; ++, moderate; normal native myocardial T1 (short axis [SA] and 4 chamber [4CH] midwall): 965±35; and normal T2 values: 40–64 ms.

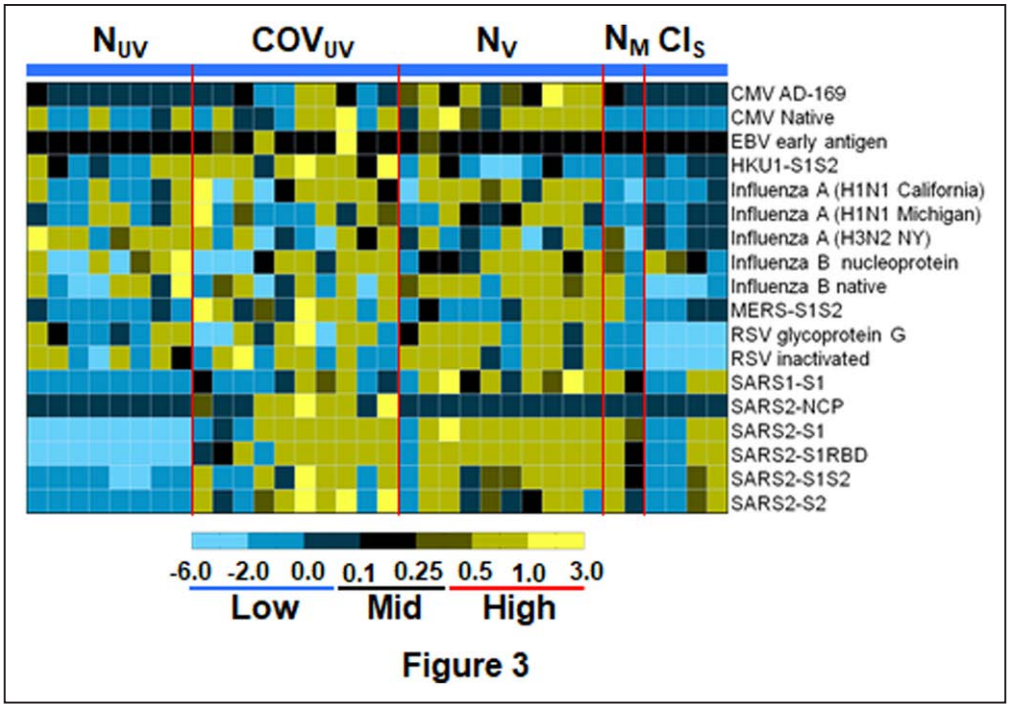

Figure 3. Antibody profile to viral antigens in the case of interest as compared with naive vaccinated, naive unvaccinated, and COVID-19 unvaccinated patients.

The heatmap shows immunoglobulin G reactivity expressed in terms of row z-score for a respective antigen across different patient samples. Each antigen is organized into rows color-coded by virus, for serum specimens organized into columns classified as naive unvaccinated (N_{UV}, 8 samples), (COV_{UV}, 10 samples), naive vaccinated (N_V, 10 samples), naive Moderna vaccinated controls (N_M, 2 samples), and case of interest samples (CI_S, collections at day 1, day 2, day 3, and day 4 after symptom onset: S1, S2, S3, and S4 in the respective order). Reactivity is represented by color (light blue=low, black=mid, ellow=high). The heatmap has normalized row z-score values, a typical scaling method that helps better visualization of analytes with varying trends in the expression/reactivity between samples. Although a normalized row z-score can better represent the nonrandomness of directionality within a dataset, a negative z-score does not indicate a complete absence of expression/reactivity. A negative z-score means comparatively a lower raw scores/absolute expression. CMV indicates cytomegalovirus; COVID-19, coronavirus disease 2019; EBV, Epstein-Barr virus; and RSV, respiratory syncytial virus.

Epstein-Barr virus, influenza A, and respiratory syncytial virus compared with vaccinated control samples (Figure 3). It is interesting that, although anti-spike antibody levels were higher than the manufacturer-recommended positive threshold, the SARS spike protein antibody levels were either lower or just comparable in the case com-

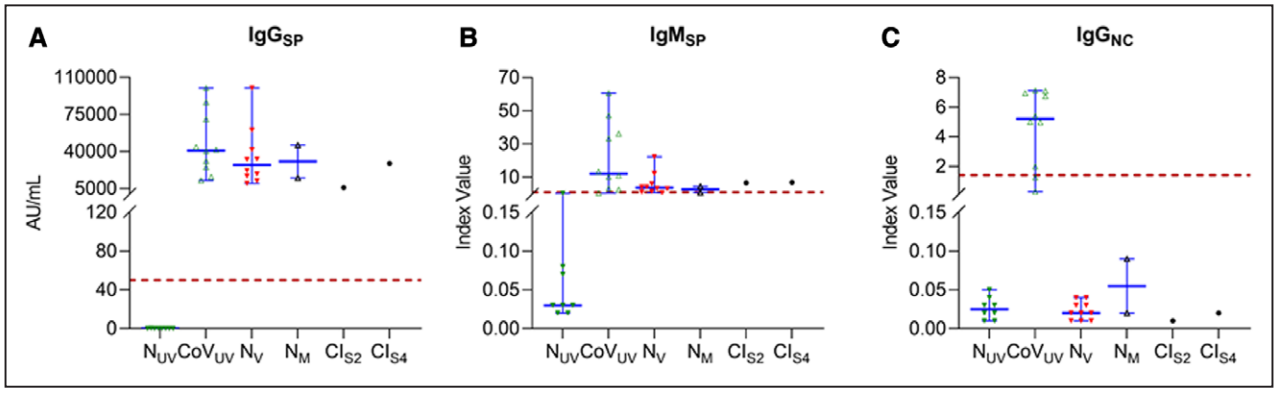

Figure 4. SARS-CoV-2–related antibody status in the case of interest as compared with naive vaccinated, naive unvaccinated, and COVID-19–unvaccinated patients.

Comparison of SARS-CoV2–related antibody response in the case of interest with naive vaccinated, naive unvaccinated, and COVID unvaccinated patients. **A**, Evaluation of spike-specific IgG antibody response. **B**, Comparison of spike-specific IgM levels. **C**, Comparison of nucleocapsid-specific antibody response. For **A** through **C**, all the patient samples in the N_V group were immunized with Pfizer vaccine. AU indicates arbitrary units; CI_{S2}, case of interest sampled at day 2 after symptom onset; CI_{S4}, case of interest sampled at day 4 after symptom onset; COVID-19, coronavirus disease 2019; COV_{UV}, COVID-19 unvaccinated; Ig, immunoglobulin; N_M, age- and vaccine (Moderna)–matched naive (positive controls for case of interest); N_{UV}, naive unvaccinated; N_V, naive vaccinated; SARS-CoV2, severe acute respiratory syndrome coronavirus 2; and SP, spike. Dashed brown line indicates the manufacturer-recommended positive threshold of the respective antibody assays used.

pared with N_V (Figure 4). This may be partly explained by a difference in the timing of blood sampling after immunization among the vaccinated controls (2 weeks) versus case patient (5–6 days) for assessing antibody response. Concurrent clinical evaluation for known infectious causes of acute myocarditis, including multiple SARS-CoV-2 nasopharyngeal polymerase chain reaction tests and Food and Drug Administration–approved multiplex respiratory viral polymerase chain reaction panels and serologies, were all negative with 2 exceptions. An IgG antibody for *Mycoplasma pneumoniae* was positive but IgM antibody was negative, consistent with previous exposure and not acute infection. In addition, an IgG titer of 1:320 was reported for coxsackie B virus 4, but IgM antibody titers were negative. However, convalescent serum antibody testing 3 weeks later revealed a titer of 1:160, consistent with remote and not acute or recent infection.

Genetic Testing

Given that inherited cardiomyopathy may present clinically as myocarditis,[3] a panel test for variants in 121 genes potentially linked to cardiomyopathy was performed (Invitae, San Francisco, CA). No pathogenic variants and 1 intronic variant of unknown significance (heterozygous, ACTN2, c2367+5G>A) were identified, suggesting that the known gene variants are not the cause of myocarditis in the case patient.

Screening of Cytokine Response

Although the vaccine-induced immune response is chiefly linked to protective immunity, an exaggerated and unwarranted immune reaction could potentially heighten inflammation and augment the risk of immunopathology. We measured a panel of 48 cytokines and chemokines in the case patient using fluorescent bead-based Bio-Plex Pro Human Cytokine Screening Panel, per the manufacturer's instructions (Bio-Rad, CA), as described in the Methods in the Data Supplement. Cytokine levels in the case patient were N_V or N_M (Figure 5). The trend of cytokine changes in the case of interest along with the control groups is shown in Table 3. To aid efficient interpretation of this data, we considered as abnormal only the analytes with ≥2.0-fold increase (bold) or a ≥2.0 decrease (bold and italics) in CI_{S1-S4} versus both N_M and N_{UV} groups. The N_{UV} comparison provides a reference interval to interpret the case patient's cytokine results. Given the inclusion of 2 comparators N_M and N_{UV}, if the 2-fold change is in 1 direction versus 1 comparator and in the opposite direction for another comparator, then those cytokine changes are indicated in italics. This analysis revealed in the case patient elevated levels of 4 cytokines (IL-1ra, IL-5, IL-16, and MIG), diminished levels of 1 cytokine LIF (leukemia inhibitory factor), and 3 other cytokines (IL-10, MIF, and VEGF) with bidirectional pattern (increase or decrease) relative to the comparators, N_M or N_{UV} (Table 3). Although statistical inference is not possible because of the single case patient, and the clinical relevance of the magnitude of difference seen is not clear, some of the following changes are of potential interest. The level of IL-1ra (IL-1 receptor antagonist) in the first sample from the case patient after symptom onset (CI_{S1}; 1174 pg/mL) was comparable with levels in patients with active COVID-19 infection (unvaccinated patients hospitalized with COVID-19; 1183 pg/mL). Generation of IL-1ra could be a compensatory counterattacking mechanism to limit excessive inflammation. In support of this notion, it has been documented that treatment with IL-1ra rescues myocarditis-associated end-stage heart failure.[4] Around the time of symptom onset, the case patient also displayed elevated levels of other cytokines, IL-5, IL-16, and MIG (CXCL9), which play inflammatory roles in either myocarditis or related cardiac complications in humans or in experimental animal models.[5–8] In contrast, relative to N_M or N_{UV}, the first sample of the case patient (CI_{S1}) showed a decrease in the levels of cytokine LIF, which provides cellular stability and ensures survival of cardiomyocytes during stress.[9] The other 3 cytokines, VEGF, IL-10, and MIF, did not reveal a unidirectional regulatory pattern with comparators (N_M and N_{UV}); however, each spiked above the N_{UV} reference group and has been individually implicated in immune vasculitis.[10–12] Additional clinical laboratory assessment of IL-1β, IL-2, and IL-6 cytokines revealed normal levels of these cytokines (data not shown).

It should be emphasized that these cytokine analyses are exploratory and limited by the absence of baseline measurements in the case patient before vaccination. Although this empirical evidence cannot identify a specific cytokine candidate or signature, this approach represents a first step of searching for such a cytokine signature in COVID-19 vaccine–associated myocarditis and may provide important insights for subsequent studies in larger numbers of patients.

Autoantibodies

Immunizations with adverse effects typically induce disproportionate autoantibody generation.[13,14] Thus, we next investigated whether the COVID-19 mRNA vaccine and the associated nonviral acute myocarditis seen in the patient of interest may be a consequence of an autoimmune response, using a proteome array printed with HuProtTM version 3.1 arrays (CDI Laboratories, Mayaguez, PR) comprised of ≈19,500 unique full-length human proteins (Methods in the Data Supplement).

Analyses for potentially informative autoantibodies were clustered into 3 separate subpanels representing common, COVID-specific, and CI_S-specific groups for both IgM and IgG classes of circulating autoantibodies (Figure 6A and 6B). In the common subpanel, the case patient was characterized by higher levels of 2 IgM autoantibodies (CRK and UNC45B) (Figure 6A) and 6 IgG autoantibodies (IL-

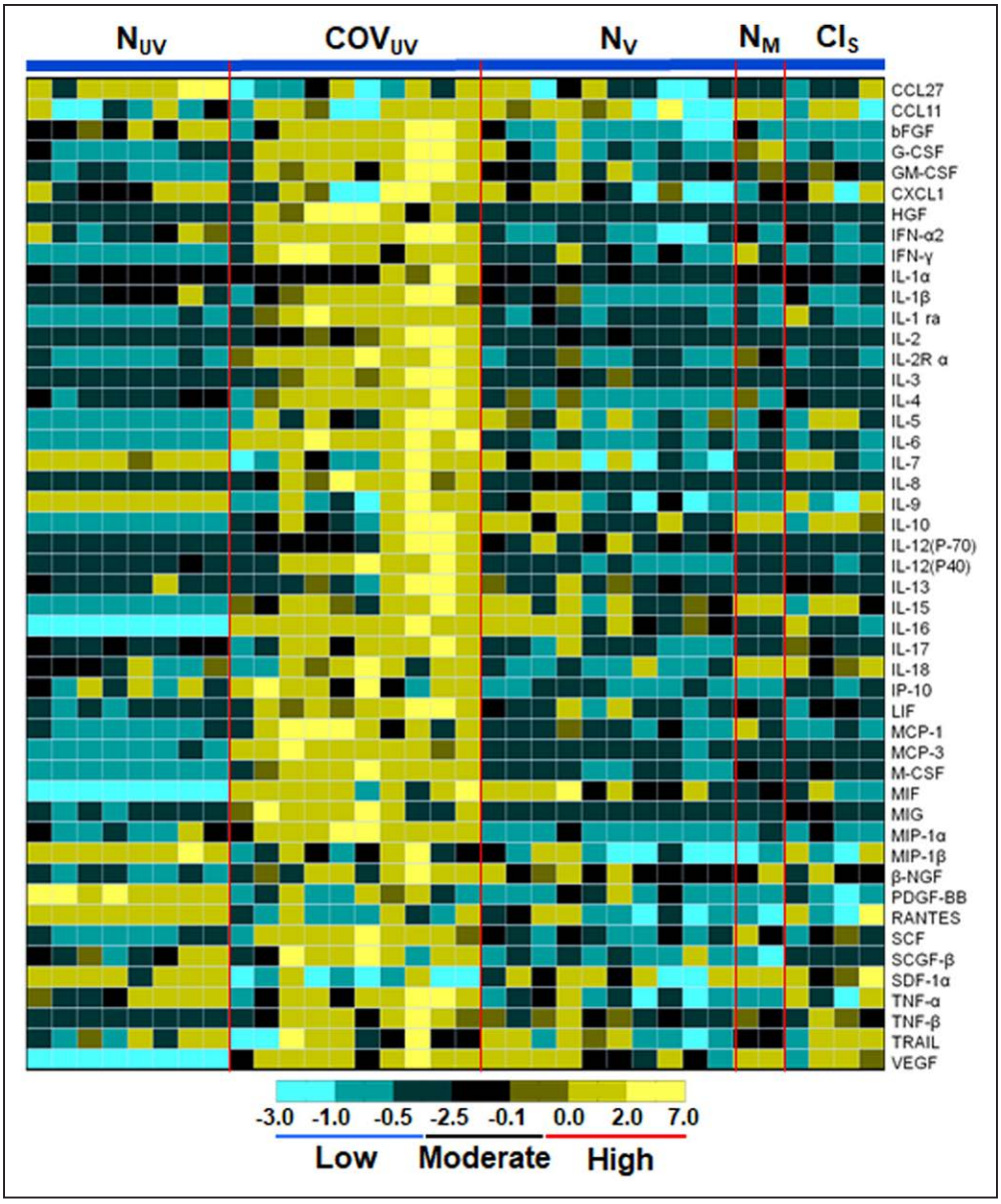

Figure 5. Cytokine profile in the case of interest as compared with naive vaccinated, naive unvaccinated, and COVID-19–unvaccinated patients.

The heatmap shows reactivity expressed in terms of row z-score for a respective antigen across different patient samples. Each row in the graphics represent a cytokine for serum specimens organized into columns classified as naive unvaccinated (N_{UV}, 8 samples), COVID-19 unvaccinated (COV_{UV}, 10 samples), naive vaccinated (N_V, 10 samples), naive Moderna vaccinated controls (N_M, 2 samples), and case of interest samples (CI_S, 4 different collection days at day 1, day 2, day 3, and day 4 after symptom onset: S1, S2, S3, and S4 in the respective order). The reactivity intensity ranges from turquoise (low) to black (moderate) or yellow (high). For the groups N_M and CI_S, each patient sample was run in duplicates that were averaged and represented. Some of the samples that displayed values below the least detection range were arbitrarily assigned a lowest value. COVID-19 indicates coronavirus disease 2019.

10, KCNK5, PARP1, VCL, AKAP5, and IFNγ) compared with the patient with active COVID-19 and N_{UV} controls (Figure 6B), suggesting potential specific associations with myocarditis. Autoantibodies against IL-10 and IFNγ have been detected in patients with life-threatening COVID-19, and previous reports indicate a cardioprotective effect for these cytokines in humans and rodents.[15–17] IgM autoantibodies against several common antigens, including TNNC1 (troponin C1) and IL-1RN, were elevated in both the case patient and the patient with COVID-19, which is expected given the presence of cardiac injury and inflammation present in both disease scenarios.

In the CI_S-specific cluster, the case patient (CI_S) had a pronounced excess of 3 IgM (CCDC97, CDK6, and EPHX2) and 21 IgG (AK1, CIRBP, CKM, CTGF, CXCL16, DGKZ, DNAI1, DNAI2, GDI1, HIP1R, HSPA9, IFT122, JUNB, KIF6, PQBP1, SF3A2, SH3GL2, STAMBP, THBD, TSEN34, and XXYLT1) specific autoantibodies compared with N_{UV} or unvaccinated patients hospitalized with COVID-19 (Figure 6A and 6B). Out of this

Table 3. Cytokine Profile in Naive Unvaccinated, COVID-19 Unvaccinated, Naive Vaccinated, and the Case of Interest

Cytokine	N_{UV}	COV_{UV}	N_V	N_M	CIS_1	CIS_2	CIS_3	CIS_4
CCL27	1168	581	621	634	542	668	610	803
CCL11	76	120	107	150	72	117	120	40
bFGF	46	67	32	37	36	32	31	28
G-CSF	127	331	134	222	122	150	122	95
GM-CSF	1.1	5.7	3.1	3.9	2.3	4.2	3.7	2.1
CXCL1	662	671	641	627	647	669	535	678
HGF	521	2687	363	316	396	372	331	725
IFN-α2	11	20	7	8	11	8	7	9
IFNγ	15	96	30	48	17	25	24	10
IL-1α	15	33	13	15	19	19	13	15
IL-1β	1.6	3.0	1.2	1.2	1.9	1.3	1.2	1.5
IL-1ra	183	1183	295	297	**1174**†	308	235	181
IL-2	5.7	14.2	6.6	6.0	7.5	7.5	5.7	4.1
IL-2Rα	47	189	57	100	40	63	62	43
IL-3	0.01	0.72	0.14	0.09	0.16	0.08	0.16	0.01
IL-4	0.9	1.7	0.8	1.0	1.1	0.9	1.0	0.9
IL-5	0.0	52.7	29.5	17.3	1.7	**84.5**†	**69.2**†	25.6
IL-6	0.8	18.9	3.0	3.3	2.6	4.4	5.2	1.8
IL-7	14	11	9	8	17	14	7	4
IL-8	8	63	13	13	8	15	10	9
IL-9	311	253	221	190	284	207	112	284
IL10	2	11	8	13	*4*‡	17	15	10
IL-12 (P-70)	2.8	4.8	4.6	3.0	3.2	2.8	1.9	1.9
IL-12 (P-40)	121	389	95	103	147	112	81	112
IL-13	1.9	2.9	2.0	1.9	2.6	2.8	2.4	2.0
IL-15	0.0	356.6	248.7	289.2	0.0	453.0	370.6	224.2
IL-16	3	389	261	193	**607**†	199	169	132
IL-17	10	16	7	9	12	11	7	9
IL-18	42	59	20	120	58	47	54	74
IP10	930	1759	378	302	794	607	521	718
LIF	21	57	22	29	***2****‡	32	33	14
MCP1	35	194	57	85	25	43	50	23
MCP3	0.01	21.45	1.66	2.16	3.31	2.20	1.66	1.66
M-CSF	13.4	69.8	18.0	23.5	19.8	29.7	22.0	18.7
MIF	460	5348	4210	2809	2702	5270	2053	*1223*‡
MIG	280	2955	409	407	**941**†	**1342**†	**918**†	501
MIP-1α	2.2	6.3	1.6	2.1	2.0	2.7	2.0	2.1
MIP-1β	221	173	155	141	229	159	97	228
β-NGF	1.4	5.6	4.2	4.6	3.0	5.7	4.0	4.1
PDGF-BB	4550	1168	842	387	1168	225	89	375
RANTES	13776	7002	7027	4784	14831	5225	2311	20040
SCF	67	199	80	125	48	111	115	81
SCGFβ	116351	188150	84747	70200	94735	109163	95983	107049
SDF1α	944	535	781	1077	843	759	784	1493
TNFα	102	119	80	71	105	84	56	104
TNFβ	0.0	11.0	5.0	7.7	1.5	11.5	9.4	5.3
TRAIL	44	45	42	45	37	54	52	57
VEGF	44	490	343	447	*149*‡	622	549	345

Presented are the median pg/mL values for the groups, N_{UV} (n=8), N_V (n=10), and COV_{UV} (n=10), average pg/mL values for N_M (n=2), and individual pg/mL values for the groups CI_{S1-S4} (n=1 in each group). To help efficiently interpret these data, we considered only the analytes with a fold change of ≥2.0 in CI_{S1-S4} versus both N_M and naive unvaccinated healthy control (N_{UV}). CI_{S1}, CI_{S2}, CI_{S3}, and CI_{S4} indicate case of interest sample at day 1, day 2, day 3, and day 4, respectively, after symptom onset; COVID-19, coronavirus disease 2019; COV_{UV}, COVID-19 unvaccinated; n/a, not tested or not available; N_M, age- and vaccine (Moderna)–matched naive (positive controls for case of interest); and N_V, naive vaccinated.

*≥2.0-fold decrease (bold and italics); †≥2.0-fold increase (bold); ‡>2.0-fold change (italics) in either direction of comparison with N_M or N_{UV}.

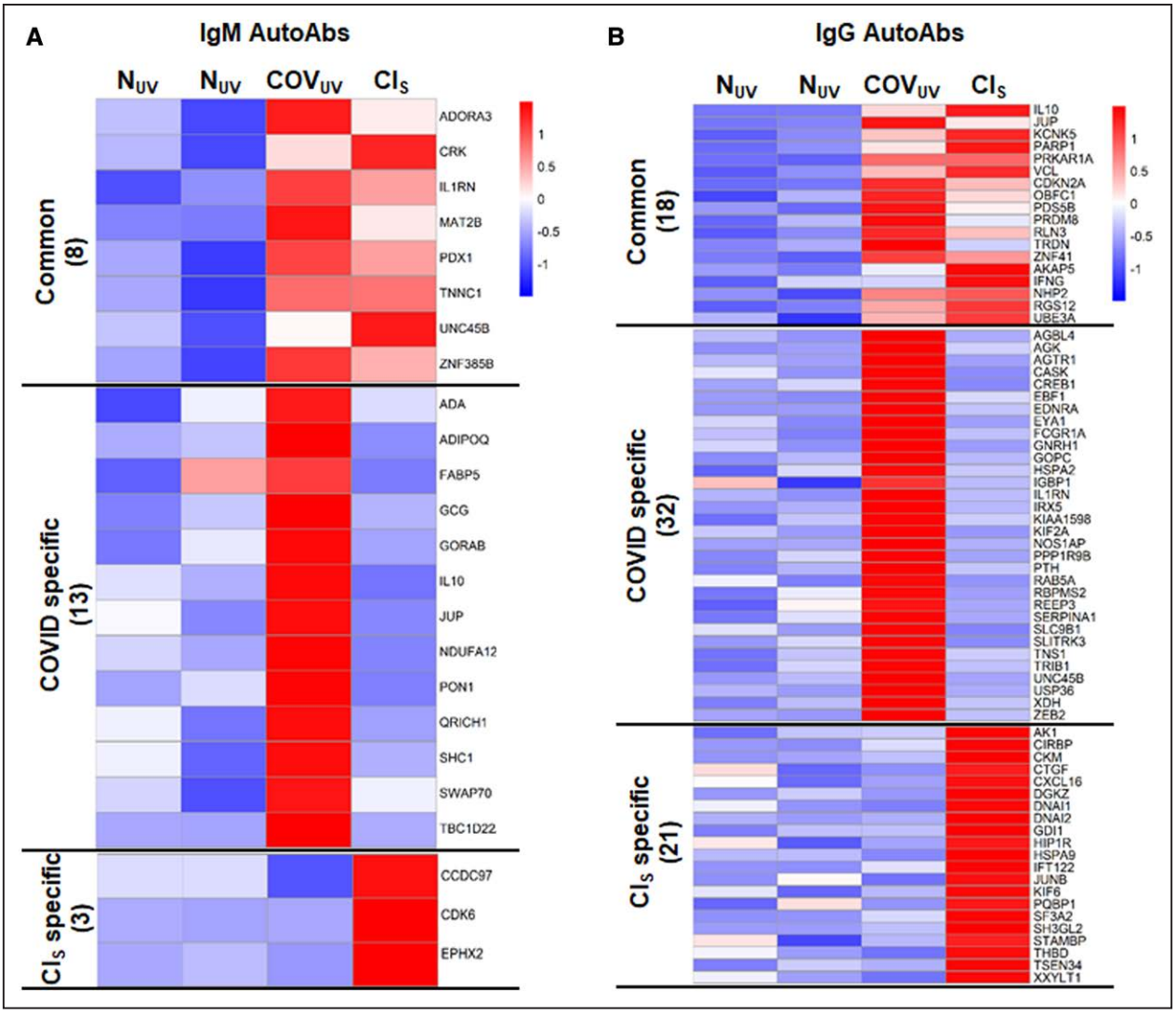

Figure 6. Antibody profiles to self-antigens in the case patient relative to unvaccinated naive and COVID-19 patient samples. The heatmap shows the Phenolyzer-prioritized candidate proteins involved in cardiac disease expressed in terms of mean of the individual signal intensities from the duplicate samples that were corrected for background intensity followed by variance stabilizing normalization (VSN). Each row in the graphics represent the analytes for serum specimens organized into columns classified as naive unvaccinated (N_{UV}, 2 samples), COVID-19 unvaccinated (COV_{UV}, 1 sample) and case of interest samples (CI_S; day 1 sample after symptom onset). The reactivity intensity ranges from blue (low) to white (moderate) or red (high). Horizontal black lines segregate the subpanel cluster where autoantibodies are altered either commonly in both COV_{UV} and CI_S or only in COV^{UV} or CI_S relative to N_{UV}. **A**, IgM-specific autoantibody changes. **B**, IgG-specific autoantibody changes. Abs indicates antibodies; COVID-19, coronavirus disease 2019; and Ig, immunoglobulin.

list, CXCL16 protein has been shown to increase in acute versus chronic myocarditis and suggested to be a novel biomarker for inflammatory cardiomyopathy.[18] Likewise, elevated levels of connective tissue growth factor (CTGF/CCN2) have been elevated in fibrotic and tissue injury in heart failure.[19] In addition, CIRBP/CIRP (cold-inducible RNA binding protein), a known cardiac electrophysiological regulator, has been shown to govern ventricular and atrial repolarization on cellular stress.[20] The absence of antibody levels exceeding the prespecified criteria that cross-react with cardiac myosin and first and second extracellular loops of the β-adrenergic receptor is notable given previous reports of these autoantibodies in viral myocarditis.[21,22] Whether the specific autoantibodies identified in the case patient play a role in disease progression or resolution needs to be determined by assessing their dynamics over longer-term follow-up in studies with larger sample sizes.

This exploratory autoantibody analysis encompasses changes in autoantibodies against both intracellular and extracellular proteins/targets (Figure 6). However, it should be noted that autoantibodies against extracellular proteins/targets are more plausibly linked to disease pathology, in part because of their easy accessibility for binding.[23] Also, autoantibodies to extracellular proteins are frequently reported to mimic genetic diseases for the same target protein or pathway with corresponding gain or loss of function.[24] When autoantibodies against

intracellular proteins are linked to a pathogenesis, it is often indirect, because the clonal expansion and the related process of recognition of antigens through the B-cell receptor occur in extracellular space with the support of extracellular proteins.[25] Nevertheless, the autoimmune reactivity seen in the patient with respect to these self-antigens need not necessarily be pathogenic and could also be a part of the normal healing process of the inflamed myocardium. Future studies that specifically characterize the function and origin of these autoantibodies will be essential to understand their potential role in vaccine-associated myocarditis. It is ideal for such future studies to include a baseline sample from the same patient and also age-, sex-, and vaccine type–matched controls.

Immune Cell Subsets

Last, we assessed alterations in immune cell subsets in the patient of interest by enumerating T, B, and natural killer (NK) lymphocytes and investigating immunophenotypic aberrancy. This was performed using Becton Dickinson's Multitest 6-color lymphocyte subsetting reagent with Becton Dickinson Trucount and additional immunophenotyping (CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD13, CD14, CD16, CD19, CD20, CD22, CD25, CD31, CD34, CD38, CD45, CD45RA, CD62L, CD64, CD123, CD127, CD197, HLA-DR, TCRαβ, TCRγδ) by flow cytometry on a Becton Dickinson FACSCanto. Table 4 demonstrates that a naive vaccinated subject had a frequency of $CD3_{neg}CD16_{pos}CD56_{pos}$ NK cells within the normal reference range. In contrast, the case patient had a nearly 2-fold increase in the frequency of $CD3_{neg}CD16_{pos}CD56_{pos}$ NK cells from the upper limit of the reference interval. This may indicate activation of a distinct subset of NK cells ($CD3_{neg}CD16_{pos}CD56_{pos}$) that have been documented to be the most abundant (10% peripheral blood lymphocytes) and efficient cytotoxic effectors that kill their target cells by secreting cytoplasmic proteins.[26] Although the changes in the absolute NK cell number are intriguing at present, the surge may have either contributed to the pathology or the disease resolution process. Low absolute $CD3_{neg}CD16_{pos}CD56_{pos}$ NK cell counts have been shown to correlate with orbital myositis, and the levels of these cells normalized with improvement in the disease activity.[24] In addition, the patient showed a marginal increase in percentage and absolute count of NK lymphocytes by about 17% relative to the N_M sample. This finding of high NK cell fraction in the case patient with resolved myocarditis contrasts with the recent multicenter IPAC study (Investigation of Pregnancy-Associated Cardiomyopathy), which showed reduced levels of NK cells in peripartum cardiomyopathy, which normalized over time postpartum.[27] Although the significance of this finding in the current patient with nonviral myocarditis is unclear, NK cells are known to play a cardioprotective role in viral myocarditis and autoimmune myocarditis by limiting viral replication and through modulation and inhibition of cardiodestructive activity by eosinophils, respectively.[28] No other disease-driving immunophenotypic aberrancies were noted in the patient.

Table 4. Enumeration of T, B, and NK Lymphocyte Subsets in the Case of Interest Relative to Age- and Vaccine-Matched Naive Subject

Description	CI_{S3}	N_M	Reference range
Absolute lymphocyte count	**2732**	2321	1312–2660
$CD3_{pos}{}^{ABS}$	1669	**1763**	1402–1626
$CD3_{pos}CD4_{pos}{}^{ABS}$	1231	1109	564–1320
$CD3_{pos}CD8_{pos}{}^{ABS}$	455	601	314–931
CD4/CD8 ratio	2.7	1.8	0.97–3.71
$CD3_{neg}CD16_{pos}CD56_{pos}{}^{ABS}$	**791**	190	27–483
$CD3_{neg}CD19_{pos}{}^{ABS}$	182	343	64–452

Abnormal results are shown in bold. ^{ABS} indicates absolute cell count; CI_{S3}, case of interest sample at day 3 after symptom onset; and N_M, age- and vaccine-matched naive subject.

DISCUSSION

This case describes a 52-year-old previously healthy man who presented with an acute myocarditis-like illness 3 days after the administration of the second dose of Moderna's COVID-19 vaccine. Although endomyocardial biopsy was not performed, the clinical and cardiac MRI features were consistent with myocarditis, as was the resolution of symptoms and gradual improvement in cardiac MRI findings. The case does not prove a causal association between the vaccine and the observed myocarditis-like syndrome. However, ischemic injury and other potential causes of acute myocardial injury were excluded, as were other potential infectious causes of myocarditis, and there was no evidence of systemic autoimmune disease.

The US Centers for Disease Control and Prevention have received reports of possible cases of vaccine-associated myocarditis through the VAERS reporting system, and anecdotal cases have been recently been reported in the lay media. Moreover, 2 case series in this issue of *Circulation* report similar presentations of myocarditis-like illness 2 to 4 days after COVID-19 vaccination.[29,30] However, the link between COVID-19 vaccination and myocarditis remains circumstantial, and a mechanism has not been established. This case represents one of the first reports of possible mRNA-based COVID-19 vaccine–associated myocarditis reported in the medical literature, with in-depth clinical and translational investigation and comparison with different control groups.

Although multiple cytokines and autoantibodies with plausible links to myocarditis or cardiac pathogenesis appeared to differ in the case patient compared with controls, a specific signature was not identified. There

was an increase in numbers of a specific subset of NK cells and increased expression of several autoantibodies compared with controls. T helper 17 cells–related IL-17–enriched immune signature has been implicated in the development of myocarditis and its associated transition of fibrosis to heart failure.[31] It is interesting that such upregulation of IL-17 levels was not observed in our patient. The lack of evidence for upregulation of this cytokine, combined with the increased NK cell numbers observed in the case patient, could suggest a distinct vaccine-associated immunophenotype with a high likelihood for rapid recovery. However, it is not clear whether the observed differences reflect a potential (causal) pathological immune response or rather appropriate healing responses to myocardial inflammation. These differences may also be chance findings, given the exploratory nature of our investigations and large numbers of tests performed in few patients. Additional studies in larger numbers of individuals are needed to explore potential mechanisms, including prospective studies with biospecimen collection before and after vaccination.

Clinicians should be aware that myocarditis may be present in patients exhibiting cardiac signs and symptoms 2 to 4 days after COVID-19 vaccination. However, we emphasize that this report of a rare potential vaccine-related adverse event does not change the highly favorable risk/benefit of COVID-19 vaccination, including in patients with underlying heart disease or cardiomyopathy. Additional surveillance of such adverse events after COVID-19 vaccination will help to identify whether there are subgroups who are at higher risk for this vaccine-related effect, and if so, whether additional precautions are necessary.

ARTICLE INFORMATION

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Acknowledgments

The authors thank the Abbott Diagnostics Division in Illinois for providing SARS-CoV-2–specific antibody test kits for clinical research.

Sources of Funding

Dr Muthukumar has received grant support from Abbott and Roche Diagnostics. The autoantibody assay was funded through the Alfred W. Harris, MD, Professorship in Cardiology awarded to Dr Mammen. Dr de Lemos has received grant support from Abbott Diagnostics and Roche Diagnostics.

Disclosures

Dr de Lemos has received consulting income from Siemens Health Care Diagnostics, Ortho Clinical Diagnostics, and Quidel, Inc. The other authors report no conflicts.

Supplemental Materials

Expanded Methods

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